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# CONTENTS

## Original Articles

BEZMİÂLEM VAKIF ÜNİVERSİTESİ

BEZMİÂLEM science

# CONTENTS

BEZMİÂLEM VAKIF ÜNİVERSİTESİ

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# EDITORIAL

**Dear Colleagues;**

We are proud and happy to meet you in the second issue of Bezmialem Science this year. First of all, I would like to thank our authors, referees and editorial board who contributed to our journal with their articles. Bezmialem Foundation University is a thematic university in the field of health. Among the foundation universities having faculty of medicine, we have the pride of being in the higher rankings in the URAP (University Ranking by Academic Performance) compared with many universities established before us. We continue our efforts to get a good place among the journals indexed abroad as in our country with your support.

In this issue; the article entitled " The Effects of Camellia sinensis Extract on Proliferation, Apoptosis and Oxidative Stress in Insulinoma INS-1 Cells" by KACAR et al., the article entitled "Aberrant Methylation Profile and Microsatellit Instability In Turkish Sporadic Colorectal Carcinoma" by EKMEKCI et al., the article entitled "The Role of PET/CT to Detect Bone Marrow Involvement in Hodgkin Lymphoma" by ESER et al., and the article entitled " Extra-Parenchymal Chest HRCT Findings of Patients With Systemic Sclerosis at The Time of Initial Diagnosis" by UFUK et al. are the articles that are prominent.

Welcome to our new editors who have joined our editorial board from different disciplines and our new scientists who have joined our scientific board and I wish them success in their duties.

Goodbye until we meet again in our next issue.

**Prof. Dr. Adem AKCAKAYA**

**Editor-in-Chief**

Original Article

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Bezmialem Science 2019;7(2):80-5

# The Effects of *Camellia sinensis* Extract on Proliferation, Apoptosis and Oxidative Stress in Insulinoma INS-1 Cells

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## ABSTRACT

**Objective:** Green tea (*Camellia sinensis*) is one of the most consumed beverages in the world. *C. sinensis* consumption may prevent tumor and other diseases. Insulinomas are pancreatic islet cell tumors. In present study, the effects of the extract from *C. sinensis* were investigated in rat insulinoma INS-1 cells.

**Methods:** *C. sinensis* leaves were collected in Rize, Turkey. The leaves were brewed in tap water for 20 and 40 minutes at 80°C. INS-1 cells were treated with different doses (1-5 mg/mL) of *C. sinensis* extract for 24 h. Cell viability, proliferation, death were examined. The malondialdehyde, glutathione and protein carbonyl levels were measured in INS-1 cells given *C. sinensis* extract.

**Results:** The cell viability and the percentage of proliferating cell nuclear antigen immunopositive cells decreased, while the percentage of TUNEL positive cells increased in INS-1 cells treated with the extract from *C. sinensis* according to control group. While malondialdehyde and protein carbonyl levels in INS-1 cells treated with *C. sinensis* extract decreased, glutathione levels increased according to control group.

**Conclusion:** The results indicate that the extracts from *C. sinensis* inhibited proliferation and caused apoptosis in INS-1 cell. *C. sinensis* may be natural agent for supporting the treatment of pancreatic tumors.

**Keywords:** *Camellia sinensis* extract, proliferation, apoptosis, oxidative stress, INS-1 cells

## Introduction

The use of primary beta cells in biochemistry and molecular investigations is limited due to difficulty in obtaining pancreatic endocrine tissue. For this reason, many investigators prefer to study insulin-releasing cell lines, which maintain normal insulin secretion. The most commonly used insulin-releasing cell lines are RIN, HIT, beta TC, MIN6 and INS-1 cells (1). MIN6 and INS-1 cells reflect the physiological situation of beta cells in the best way when stimulated by glucose (2). Although the behavior of these cell lines does not exactly mimic the physiology of the primary beta cells, it is highly valuable for studying the molecular events underlying beta cell function and dysfunction (1). INS-1 exhibits several important characteristics of pancreatic beta cells (2).

There are various types of herbal teas including black and green tea. Green tea (*Camellia sinensis*) is one of the most consumed beverages in the world. People living in different regions have a variety of ways to brew tea (3,4). The previous studies with different brands of tea demonstrated that brewing time is important to increase its antioxidant capacity, total phenolic and catechin content (5). Furthermore, the protective effects of *C. sinensis* were demonstrated in different types of cancers such as breast, ovarian, prostate and pancreatic cancers. The importance of anti-cancer activity of tea was highlighted (6-9)

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In the current study, the effects of the extract from *C. sinensis* on cytotoxicity, proliferation, apoptosis and oxidative stress status were investigated in rat insulinoma INS-1 cells.

## Methods

### Extraction of *C. sinensis*

*C. sinensis* leaves were collected in Rize, Turkey as first harvest in May the leaves were dried, and a classic brewing method was applied to the green tea leaves to prepare the extract. The water used as solvent. Two hundred fifty mL water was used for the brew of 5 g of *C. sinensis* leaves. The dried leaves were brewed in tap water for 20, 40 and 60 minutes at 80°C and extracts were obtained. And then, the extracts were cooled at room temperature, and the supernatants were removed after centrifugation at 4000 g for 15 minutes. So, the extract was obtained from *C. sinensis. In vitro* study was not approved.

### Cell Culture

Insulinoma INS-1 cell line was a generous gift from Prof. Dr. Claes B. Wollheim (University Medical Center, Geneva). INS-1 cells were cultured in a humidified atmosphere containing 5% $CO_2$/95% humidified air at 37°C in Roswell Park Memorial Institute (RPMI) 1640 (Invitrogen) containing 10 mM HEPES (Sigma), 50 mM 2-mercaptoethanol (Biorad), 1 μM sodium pyruvate (Sigma), 5% fetal bovine serum (Gibco), 100 IU/mL penicillin and 100 μg/mL streptomycin (Gibco).

The INS-1 cells which were treated with brewed *C. sinensis* leaves in water for 20, 40 and 60 minutes were named as group I, II and III, respectively. Also, the untreated INS-1 cells were named as control group.

### Cell Viability Assay

The effects on cell viability of *C. sinensis* extract determined by using 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) (Applichem). INS-1 cells were seeded, and different doses (1-5 mg/mL) of *C. sinensis* were applied to INS-1 cells for 24 hours. The final concentration of the *C. sinensis* extract was 1-5 mg/mL. After that, MTT was added and incubated for 2 hours. The medium was removed from cells and isopropanol was added to solve formazan (10). Absorbance values were measured at 540 nm. Cell viability % rate was calculated. IC50 value of *C. sinensis* was 4.535 mg/mL for group I, 4,121 mg/mL for group II, 4.041 mg/mL for group III.

### Cell Proliferation Assay

Cell proliferation assay was performed by PCNA immune marking. On 24-well plates, $25x10^4$ cells/well were seeded and grown on coverslip. Then it was incubated in 5% $CO_2$/95% humidified air at 37°C until the cells proliferated. Three and 4 mg/mL final concentration of *C. sinensis* extract were applied to the cells for 24 hours. The cells were washed with phosphate buffered saline (PBS), and fixed with methanol for 5 minimum (min) at -20°C. The cells were incubated with blocking solution for 20 min at room temperature to avoid non-specific immunostaining. PCNA antibody (Neomarkers, USA; dilution 1:300, overnight at 4°C) was used for immunocytochemical labeling by streptavidin-biotin-peroxidase technique in INS-1 cells. After that, the cells were washed with PBS, and biotinylated secondary antibodies and streptavidin, biotinylated horseradish peroxidase (Invitrogen, USA) were applied to INS-1 cells. AEC (Invitrogen, USA) was used to reveal immunopositive cells. The cells were mounted in glycerin gelatin and preserved at 4°C until microscopic examination. The percentage of immunopositive cells were calculated according to following formulas: (The number of immunpositive cells /total cells) x 100.

### Cell Death Assay

Cell death assay was determined with TUNEL method by using the *In situ* Cell Death Detection Kit (Millipore, USA). On 24-well plates, $25x10^4$ cells/wells were seeded and grown on coverslip. Then it was incubated in 5% $CO_2$/95% humidified air at 37°C until the cells proliferated. Three and 4 mg/mL final concentrations of *C. sinensis* extract were applied to the cells for 24 hours. Then the cells were treated with equilibration buffer for 2 min at room temperature. Terminal deoxynucleotidyl transferase (TdT) enzyme and reaction buffer were mixed. It was treated for 1 hour at 37°C. After that, the cells were incubated with the anti-digoxigenin conjugate for 30 min at room temperature. The diaminobenzidine was used to reveal immunopositive cells. The percentage of immunopositive cells was calculated according to the following formulas: (The number of apoptotic cells / total cells) x 100.

### Glutathione, Protein Carbonyl and Malondialdehyde Assay

On 6-well plates, $10^6$ cells/wells were seeded and incubated in 5% $CO_2$/95% humidified air at 37°C for determination of biochemical analysis. After incubation, 0.25% tripsin-EDTA was added the cells. It was removed and centrifuged at 1500 rpm for 5 minutes. Lysis buffer (Cell Signaling) was added on pellet. The cells were kept on ice for 5 minutes, and sonicated with ultrasonic water bath. They were centrifuged at 14000 g for 10 minutes. Supernatants were collected and the levels of malondialdehyde (MDA), glutathione (GSH), protein carbonyl (PCO), and protein content were measured by Ledwozyw's (11), Beutler's (12), Reznick and Packer's (13), and Lowry's methods (14), respectively.

### Statistical Analysis

The image was captured using an Olympus BX - 50 microscope. Minimum 10 fields were selected randomly and immunopositive cells were counted in each field. Microscopic results were analyzed using One-Way variance (ANOVA), followed by Tukey's multiple comparisons test by using GraphPad Prism version 5.0 computer package for immunohistochemistry. Biochemical results carried out using SPSS software (SPSS, version 21.0). The statistical analysis was performed for statistical significance using ANOVA, followed by Tukey's post-hoc test. A $p<0.05$ was considered significant.

## Results

### The Cell Viability % of INS-1 Cells Decreased with *Camellia sinensis* Extract

We determined cell viability percentage of INS-1 cells treated with the extract obtained from *C. sinensis* by MTT assay. INS-1 cells were treated with 1-5 mg/mL extract for 3 groups. For group I, the cell viability % significantly decreased in INS-1 cells given *C. sinensis* extract at 3 mg/mL ($p<0.05$), 4 mg/mL ($p<0.001$) and 5 mg/mL ($p<0.0001$) concentrations compared with the control group. The cell viability % significantly decreased in the group II at 3 mg/mL ($p<0.001$), 4 mg/mL ($p<0.0001$) and 5 mg/mL ($p<0.0001$) concentrations of the *C. sinensis* extract compared with the control group. Similarly, for group III, the cell viability % significantly decreased in INS-1 cells given 2 mg/mL ($p<0.001$), 3 mg/mL ($p<0.0001$), 4 mg/mL ($p<0.0001$) and 5 mg/mL ($p<0.0001$) concentrations of *C. sinensis* extract compared with the control group (Figure 1). The results of group II and III were similar for 3 mg/mL, 4 mg/mL and 5 mg/mL doses of extract. Therefore, we studied group I and II brewing *C. sinensis* leaves in tap water for 20 and 40 minutes, respectively in later experiment.

### INS-1 Cell Proliferation was Inhibited by Given *C. sinensis* Extract

PCNA antibody was examined as a proliferation marker. The percentage of PCNA immunopositive cells significantly decreased in group I at both 3 mg/mL and 4 mg/mL concentrations of the extract, as well as group II given 4 mg/mL extract from *C. sinensis* compared with the control group ($p<0.001$, $p<0.05$, and $p<0.001$, respectively). Besides, PCNA immunopositive cells significantly decreased in group II given 4 mg/mL extract from *C. sinensis* compared with the group II given 3 mg/mL extract ($p<0.05$) (Figure 2).

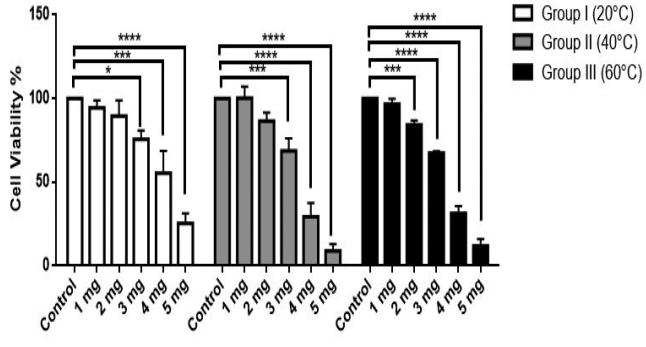


**Figure 1.** The cell viability % in INS-1 cells with *Camellia sinensis* extract. *p<0.05, ***p<0.001, ****p<0.0001

### The Number of Apoptotic Cells Increased after Treating INS-1 Cells with *C. sinensis* Extract

TUNEL method was examined for the cell death detection. The percentage of TUNEL positive cells increased in group II given 4 mg/mL extract from *C. sinensis* compared with the control group ($p<0.001$). Besides, TUNEL positive cells increased in group II given 4 mg/mL extract from C. sinensis compared with group II given 3 mg/mL extract from C. sinensis ($p<0.01$) (Figure 3).

### Glutathione, Protein Carbonyl and Malondialdehyde Levels Significantly Changed in INS-1 Cells Given *C. sinensis* Extract

GSH levels showed a significantly increase in group I given 3 and 4 mg/mL extract from *C. sinensis* compared with the control group ($p<0.001$ and $p<0.001$). GSH levels increased in group II given 3 mg/mL extract from *C. sinensis* compared with the control group ($p<0.001$), while GSH levels in group II given 4 mg/mL extract from *C. sinensis* were lower than both control group ($p<0.001$) and group I given 3 mg/mL extract from *C. sinensis* ($p<0.001$) (Table 1).

PCO levels significantly increased in group I given 3 mg/mL extract from *C. sinensis* ($p<0.001$), while PCO levels decreased in group I given 4 mg/mL extract from *C. sinensis* compared with the control group ($p<0.001$). Besides, PCO levels decreased in group II given both 3 and 4 mg/mL doses of *C. sinensis* extract compared with the control group ($p<0.001$ and $p<0.001$) (Table 1).

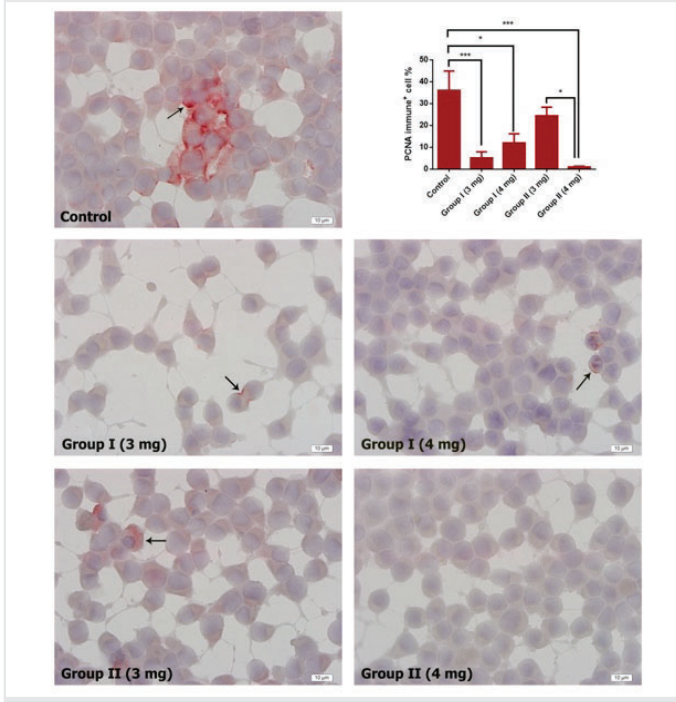


**Figure 2.** Proliferating cell nuclear antigen immunopositive cell number % in INS-1 cells with *Camellia sinensis* extract. Ò: Proliferating cell nuclear antigen immunopositive cells, *p<0.05, **p<0.001

**Table 1.** Glutathione, protein carbonyl, and malondialdehyde levels in INS-1 cells with *Camellia sinensis* extract

| | GSH | | PCO | | MDA | |
|---|---|---|---|---|---|---|
| | 3 mg/mL | 4 mg/mL | 3 mg/mL | 4 mg/mL | 3 mg/mL | 4 mg/mL |
| Control | 5.43±0.00 | 5.43±0.00 | 1.94±0.00 | 1.94±0.00 | 2.33±0.05 | 2.33±0.05 |
| Group I | 6.25±0.00[a] | 5.95±0.05[a] | 2.86±0.05[a] | 1.79±0.00[a] | 1.46±0.00[a] | 1.01±0.00[a] |
| Group II | 7.89±0.05[a,b] | 4.69±0.00[a,b] | 0.95±0.00[a,b] | 1.72±0.00[a,b] | 1.88±0.00[a,b] | 1.98±0.00[a,b] |
| $P_{ANOVA}$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | < 0.001 |

* Means ± SEM, [a]p<0.001 versus control group, [b]p<0.001 versus group I, GSH: Glutathione, PCO: Protein carbonyl, MDA: Malondialdehyde

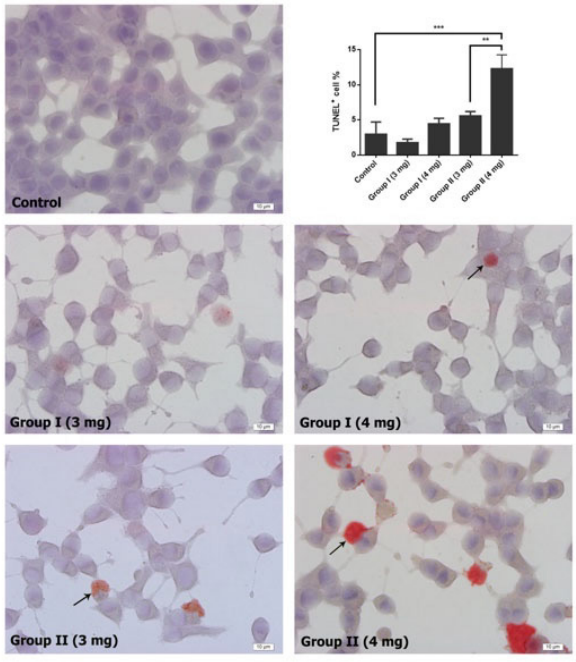


**Figure 3.** TUNEL positive cell number % in INS-1 cells with *Camellia sinensis* extract. Ò : TUNEL immunopositive cells, **p<0.01, ***p<0.001

MDA levels showed a significant decrease in both group I and II given 3 mg/mL and 4 mg/mL concentration of *C. sinensis* extract compared with the control group (p<0.001 and p<0.001). Furthermore, MDA levels were higher in group II given 4 mg/mL extract from *C. sinensis* compared with group II given 3 mg/mL extract (p<0.001) (Table 1).

## Discussion

Green tea (*C. sinensis*) is widely use in the world. There are phytochemicals and functional components in this species of tea. These components have significant benefits on human health. Therefore, consumption of tea at effective levels is important (15). *C. sinensis* contains polyphenols which are related with antioxidant activity. Brewed green tea contains high levels of catechins (16). Catechin levels are important factors indicating the quality of the tea (17). Catechins and caffeine were shown in green tea in several studies (18-20). There are five catechins including catechin, (2)-epicatechin, (2)-epicatechin gallate, (2)-epigallocatechin and (2)-epigallocatechin gallate. Different types of tea may have different caffeine and catechin contents (21). Oxidative damage can be cured with phytochemicals found in tea in diseases such as cancer (15,22). Healing properties of *C. sinensis* have been investigated in many types of cancers such as metastatic breast (6), human ovarian (7), lung (23), and prostate cancers (8). The effects of *C. sinensis* were investigated in pancreatic carcinoma cells (9,24). However, there is no investigation of cell viability in insulinoma INS-1 cells. We applied extract of *C. sinensis* at different doses (1-5 mg/mL) and brewing times (20, 40 and 60 minutes) at 80°C to the cells to investigate killing effect of *C. sinensis* in insulinoma INS-1 cells. The brewing time is important to increase the antioxidant capacity. Therefore, we continued to study with groups I (20 minutes) and II (40 minutes), due to similar results in groups II and III (60 minutes). Furthermore, 3 mg/mL and 4 mg/mL concentration of the extract were considered as effective doses in these groups.

In a previous study, the authors showed that green tea inhibited proliferation and also induced apoptosis in 4TI metastatic breast cancer cells (6) and in A549 lung carcinoma cells (23). These effects have not been determined in insulinoma INS-1 cell line. Our results indicated that extract from *C. sinensis* inhibited proliferation, and caused apoptosis in insulinoma INS-1 cell. It was observed that the increase of brewing time changed cell proliferation and death. The increase in brewing time reduced the cell proliferation, and elevated apoptosis in INS-1 cells.

The authors have shown in a study that green tea can regulate the antioxidant capacity in people exposed to oxidative stress (25). Besides, *C. sinensis* decreased oxidative stress in patients with prostate cancer (26). In a study, it was suggested that green tea showed protective effects through its antioxidant properties against oxidative stress and DNA damages, (27). Furthermore,

antioxidant capacity in *C. sinensis* was increased depending on the duration of brewing. Our data showed a decrease in the levels of MDA and PCO that are known as markers of oxidative stress in insulinoma INS-1 cells treated with extract of *C. sinensis*. Besides, glutathione is an important antioxidant and protects cells against oxidative stress. According to our results, extract of *C. sinensis* increased the glutathione levels. We hypothesized that *C. sinensis* has a positive protective effect on insulinoma cells.

Our aim was to prevent the proliferation of tumor cells and to induce cell death. We observed both inhibition of cell proliferation, and increase in apoptotic cells. We have also shown that the oxidative stress may cause decreased MDA, PCO levels and increased GSH levels in insulinoma cells. So, we can say that *C. sinensis* may be natural agent to support the treatment of various carcinomas.

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**Ethics Committee Approval:** *In vitro* study was not approved.

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**Peer-review:** Externally and internally peer-reviewed.

**Authorship Contributions**

Concept: A.K.K., M.E., I.S., Z.M.C., Design: M.E., Z.M.C., Data Collection or Processing: M.E., Z.M.C., Analysis or Interpretation: A.K.K., M.E., I.S., Z.M.C., Literature Search: A.K.K., I.S., Writing: A.K.K.

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Original Article

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# Aberrant Methylation Profile and Microsatellit Instability in Turkish Sporadic Colorectal Carcinoma

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## ABSTRACT

**Objective:** Genomic DNA obtained from paraffin blocks of the intended colorectal cancer cases was evaluated for promoting colorectal cancer by investigating the promoter methylation of 6 different gene promoter regions and microsatellite instability.

**Methods:** DNA was isolated from the paraffin tissue of 76 sporadic colorectal cancer patients by cross sections from the areas determined to be tumorous. The methylation specific PCR (MS-PCR) method was used for these DNA samples fo rmethlylation studies in promoter region of six different *APC, hMLH1, p16INK4A, p15, p73* and *DAPK1* genes. In the same samples, the presence of microsatellite instability (MSI).

**Results:** The fequencey of methylation was 24% for hMLH1, 31.5% for APC, 19.6% for DAPK1, 42.8% for p16, 30% for p15, 17% for p73. We calculated a methylation index (MI=ratio between the number of genes methylated and the number of genes analyzed). MI was ranged from 0-0.83, with an average of 0.271 corresponding to 1.6 genes /sample and median was 0.225 and there were 15 samples which doesn't methylated in any loci. We analysed MSI in C-kit (21%), hMSH2 (18%) and APC (15%), microsatellite region.

**Conclusion:** We observed APC methylation was common then the other population for Turkish patient. P16 was the most commenest methylated loci among the 6 of gene and it seems storongly related with female patients. P73 was releated with left colorectal cancer and additionally it was related with the MSI.

**Keywords:** Epigenetic, promoter methylation, colorectal cancer, microsatellite instability, methylation specific-PCR

## Introduction

Colorectal cancer (CRC) is the third most common cancer type and cause of death in the world and it is estimated that more than 1.3 million new diagnosed individuals are present in the world and CRC may cause more than 700 000 deaths a year (1). The incidence varies all over the world. It was reported that India had the lowest incidence rate. On the other hand, highest incidence rates are present in developed countries (2,3).

Data from immigrants show that there are also international differences. Incidence of CRC has changed very quickly in Italy, Japan, poor regions of China, and Polynesianmales in Hawaii. Furthermore, it was highly sensitive to environment, suggesting that colon cancer is, in part, highly susceptible to environmental changes (2,3). The incidence of migrants and their descendants quickly reached to the rates in countries (4,5). The internationally stated rate of 20 times more frequent observation can be explained by diet habits and environmental differences in large section (2). However, it has been known that CRC is common in some families (6-8). As a result, CRC is considered as a disease associated with genetic and environmental factors.

Despite the rapid and major advances in human cancer biology, no significant increase has been observed in the overall life span and survival rates of these patients over the past three decades.

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Many genetic alterations leading to cancer development have been identified and technical developments have been added, and molecular profiling has been established. The formation of a molecular profile is in its broadest sense and the identification of genetic abnormalities occurs within the cell. Moreover, occurrence of events is in proteins or protein networks. These developments are promising to increase the life span and rates of the cases with diagnostic and prognostic approaches (9).

CRC develop due to accumulation of genetic and epigenetic changes. The development of CRC is assumed to primarily start with alteration of Wnt and (Transforming growth factor) TGF-Beta signal pathways and than activation of KRAS followed by the inactivation of APC and TP53 (10-12). Large group of CRC (80-90%) occurs in this order. In the remaining 10-20%; mismatch repair system does not fulfill its function adequately which results in mutations and microsatellite instability (MSI) (10-12). Another genetic classification model of CRC describes two subtypes: Chromosomal instability (CIN) type and (MSI) type (13). First group is consisted of multiple chromosomal alterations together with P53 mutations and MSI, CpG island methylator phenotype (CIMP) on a genome wide level and BRAF mutations are observed in the second group (13,14). For CRC development, DNA methylation is accepted as third underlying mechanism after CIN and MSI. Changing of gene expression due to DNA methylation may contribute CRC initiation and development (15-17). It is very well known that genome wide epigenetic alterations play important role not only in CRC but also in most of the cancers. Recently, genome wide transcriptomic studies have revealed five distinct molecular mechanisms in CRC which result in changes in genetic expression pattern (18-20).

Latest discoveries also revealed that tumor location may also be important in CRC development (left vs right side CRC). Underlying molecular mechanism of right side colon cancer development seems more relevant with MSI, CIMP and frequently BRAF mutations, and TP53 mutations are rarely seen in CRC of right side. And for left sided CRC, copy number alterations and TP53 mutations are more frequent and left sided CRC is more relevant with MSI phenotype. CIMP is observed lower in left sided CRC (21,22).

Methylation profiles of CRC are widely studied to investigate and identify any molecular marker for early detection, any prognosis-related marker, and markers for diseases classification, therapeutic target, and also for risk assesment (23-26). Early genome -wide DNA hypomethylation has been observed in CRC and LINE-1 (long interspersed nucleotide element 1) hypomethylation has been found connected with increased cancer related mortality and overall mortality in patients with CRC (27-29).

To understand methylation profile and MSI in Turkish CRC samples, we have used methylation specific -PCR technique to examine six differentially methylated tumor-suppressor genes and we analyzed three microsatellite loci for MSI. Methylation of the APC, hMLH1, p16INK4A, p15, p73 and DAPK1 promoters were performed using methylation-specific PCR in 76 sporadic CRCs.We performed these analyses on sporadic CRC samples gathered from their paraffin embedded tissue blocks. We also performed MSI analysis of same samples and together with methylation profile we performed statistical analysis based on avaliable proporties of patient.

## Methods

### Patients and Specimens

The samples were surgical resection specimens of consecutive patients with CRC at Istanbul University, Faculyt of Medicine hospital in Turkey. This study was a retrospective study that is why we could not take informed consent from the patients. This study was conducted in accordance with the ethical principle of Helsinki Declaration. Paraffin block samples that were stored along side were used. Data such as age, tumor type and tumor stage were used. Inclusion criteria were having resection of CRC and being available of tissue block of the primary tumor. Exclussion criteria were having history or pathologic evidence of familial adenomatous polyposis or idiopathic inflammatory bowel disease, or family history of hereditary non-polyposis colorectal cancer syndrome. Thus, blocks of 76 patients were selected from pathology department of Istanbul Faculty of Medicine in 2001 through 2004. While we were chosing patients from avaliable data, we aimed to select equal number of patients in terms ofsex, age, histological characterization, tumor localization and stage.

Both tumor tissue and non-neoplastic control tissue were collected from patients. Each formalin-fixed paraffin embedded (FFPE) resection specimen was microdissected and DNA extracted as reported previously (30).

### Microdissection from FFPE Samples and DNA İsolation

10-15 tissue sections of a 10-micrometer-thick paraffin block taken from tumorogeneous and non-tumorogeneous distal part of the same patient were used.

First, paraffin was removed using xylene from the samples and 100% ethyl alcohol was added and the samples were precipitated. Then, tissue digestion buffer solution which includes proteinase K were added to lyse cells. Phenol/chloroform mixture (1/1 ratio) was added and centrifuged at 14.000 rpm for 3 minutes. The supernatant was taken and this process was repeated once more. The supernatant was taken up in new tubes. Fifty μL of 5M ammonium acetate was added to each of the tubes. After adding the chilled 100% ethyl alcohol, it was left at -20°C overnight. The next day, samples were centrifuged at 14.000 rpm for 30 minutes, then the supernatant was taken and left for drying. Samples were allowed to stand for 10-15 minutes at 55°C, then 50 μL of sterile $H_2O$ was added and they were allowed to stand at room temperature for at least 1 hour before measuring optical densities. Obtained DNA samples were maintained at -20°C until analyses were done.

### Sodium Bisulfite Treatment of DNA

DNA treated with sodium bisulfite as previously described (30). Briefly, 5μg of DNA were denaturated at 42°C for 30 min with 0.4M NaOH, incubated in 10mM hydroquinone, 3M (Sodium

Bisulfite, Sigma, St Louis, USA) at 55°C for 16 hrs and purified with the (GeneClean III kit, Bio 101, Vista, USA). Prior to ethanol precipitation, DNA was desulfonated in 0.4M NaOH for 15 min at 37°C. DNA was resuspended in water and stored at -80°C.

Preparation of positive controls used in methylation (*In Vitro* methylation).

(Sss I enzyme, New England BioLabs, Ipswich, USA), which methylates cytosines in the presence of CpG dinucleotide through whole genome, was used to generate positive controls. This process also uses S-Adenyl Methionine (SAM, New England BioLabs, Ipswich, USA), as the methyl donor group.

**Methylation-Specific PCR**

Bisulfite-treated DNA was used in methylation-specific PCR (MSP) reactions for 6 genes. Table 1 shows the sequences of the primers used, specific for the methylated (M) and unmethylated (U) forms. And Table 2 presents the gene regions examined for methylation and the lengths of the regions amplified in the PCR. The amplification reactions contained 25 pmoles of each primer, 200 μM dNTP, 1U (Hotstart Taq polymerase and 1x Q buffer, Qiagen, Valencia, USA) with variable amounts of $MgCl_2$ and cycling conditions are shown in Figure 1. All MS-PCR products were run in a 4% agarose gel.

**Assay for Microsatellite Instability (MSI)**

MSI was determined by fluorescently labeled PCR amplification kit for this purpose ("HNPCC Microsatellite Instability Test", Roche, Berlin, Germany). Normally, it consists of 5 markers from panel described by the American Cancer Association, International HNPCC colobaration group and German Cancer Research group. Although with this kit 5 markers were provided, we could manage to analyze only 3 loci: BAT25 (c-kit), D5S346 (APC) and D17S250 (hMSH2). Primer sequences are given in Table 3.

**Statiscal Analysis**

To test methylation and MSI of one locus and its relationship with other loci in terms of methylation status and to make comparisons with outcome, we used Fisher's Exact test and if the sample size was smaller than 2, then we used Pearson test. Comparison of methylation status and nonparametric outcome were tested by the nonparametric Mann-Whitney U Test. All tests were done by (SPSS software 10.0, Chicago, IL, USA) for windows and $p<0.05$ was considered statistically significant with only 2- sided results of the tests.

## Results

We analyzed the methylation status of 6 genes in 76 patients with sporadic CRC (27-86 years, median age 58.8 years). We analyzed MSI in neoplastic tissue and we compared it with normal peripheral mucosa in the same patient group. Since we were particularly interested in the correlations of methylation of tumor suppressor genes previously confirmed to be methylated in CRC, we prioritized our selection of genes to include APC

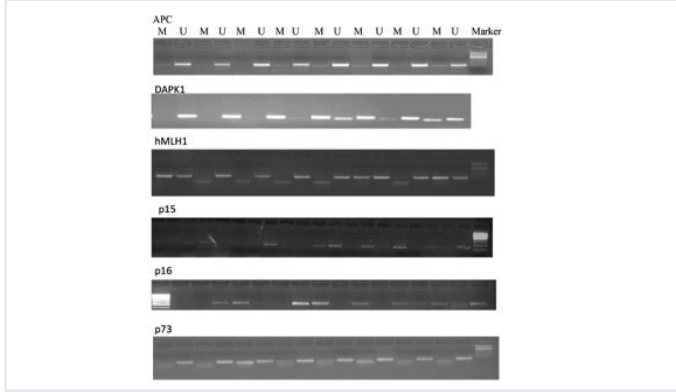


**Figure 1.** Agarose gel electrophoresis of polymerase chain reaction products

**Table 1.** Methylation and unmethylation of specific primers for each locus

| | |
|---|---|
| DAPK1-M-F: | 5' GGA TAG TCG GAT CGA GTT AAC GTC 3' |
| DAPK1-M-R: | 5' CCC TCC CAA ACG CCG A 3' |
| DAPK1-U-F: | 5' GGA GGA TAG TTG GAT TGA GTT AAT GTT 3' |
| DAPK1-U-R: | 5' CAA ATC CCT CCC AAA CAC CAA 3' |
| **p73** | |
| p73 M-F | 5' GGA CGT AGC GAA ATC GGG GTT C 3' |
| p73 M-R | 5' ACC CCG AAC ATC GAC GTC CG 3' |
| p73 U-F | 5' AGG GAT GTA GTG AAA TTG GGG TTT3' |
| p73 U-R | 5' ATC ACA ACC CCA AAC ATC AAC ATC CA3' |
| **APC** | |
| APC M-F | 5' T C G A G A A C G C G A G C G A T T C G 3' |
| APC M-R | 5' G A C C A A T C C A A C C G A A A C G A 3' |
| APC U-F | 5' T T G A G A A T G T G A G T G A T TT G A 3' |
| APC U-R | 5' A A C C A A T C C A A C C A AAA C A A 3' |
| **P15** | |
| p15 M-F | 5' G T T C C A G A A C C G G C G C T A C A A G T G 3' |
| p15 M-R | 5' G C G T G C CC G A G C T C A G T C CC A G T T 3' |
| p15 U-F | 5' G T T C C A G A A C C G G C G C T A C A A G T G 3' |
| p15 U-R | 5' G C G T G C CC G A G C T C A G T C CC A G T T 3' |
| **P16** | |
| p16 M-F | 5' T T A T T A G A G GG T G GGG C G G A T C G C 3' |
| p16 M-R | 5' C C A C C T A AA T C G A C C T C C G A C C G 3' |
| p16 U-F | 5' T T A T T A G A G GG T G GGG T G G A T T G T 3' |
| p16 U-R | 5' C C A C C T A AA T C A A C C T C C A A C C A 3' |
| **hMLH1** | |
| hMLH1M-F | 5' TTC GCGTG TAT TTT TAG GTC GGT C 3' |
| hMLH1M-R | 5' CGA CAC AAC TCC TAC AAC GAC CG 3' |
| hMLH1U-F | 5' TTA TGA GTA TTT GTG TGT ATT TTT AGG TTG GTT 3' |
| hMLH1U-R | 5' CAC TAA CAA CAC AAC TCC TAC AAC AAC CA 3' |

**Table 2.** The gene regions examined for methylation and the lengths of the regions amplified in the polymerase chain reaction

| Gen | Gen Bank No. | Location of primers according to transcription start point | PCR product length (bp) |
|---|---|---|---|
| APC | U02509 | -17 147 / -17 050 | 108 |
| | | -17 153 / -17 046 | 97 |
| DAPK | NM_004938 | -332 / -229 | 103 |
| | | -332 / -234 | 98 |
| HMLH1 | AB017806 | -526 / -661 | 125 |
| | | -531 / -655 | 124 |
| p15INK4B | NM_004936 | -318 /-188 | 130 |
| | | -312 / -187 | 125 |
| p16INK4A | NM_000077 | -80 / +46 | 126 |
| | | -80 / +44 | 124 |
| P73 | AB031234 | -1585 / -1505 | 80 |
| | | -1588 / - 1510 | 78 |

PCR: Polymerase chain reaction, APC: Adenomatous polyposis cair, DAPK1: Death-associated protein kinase

**Table 3.** Primers used in determination of microsatellite instability

| Name of Loci | Chromosomal localization | Primer sequences | PCR product size and label |
|---|---|---|---|
| BAT25 (c-kit) | 4q12-4q12 | F. 5' TCGCCTCCAAGAATGTAAGT 3'<br>R. 5' TCTGCATTTTAACTATGGCTC 3' | 110-130bp florasan 6-FAM |
| D5S346 (APC) | 5q21- q22 | F. 5'ACTCACTCTAGTGATAAATCGGG 3'<br>R.5'AGCAGATAAGACAGTATTACTAGTT3' | 100-130bp florasan HEX |
| D17S250 (hMSH2) | 17q11.2- q12 | F. 5' CAGGAAGAATCAAATAGACAAT 3'<br>R.5'GTGCTGGCCATATATATATTTAAACC3' | 200- 230bp florasan 6-FAM |

PCR: Polymerase chain reaction, APC: Adenomatous polyposis cair

**Table 4.** Number of samples and their stages and their distribution according to the TNM classification based on the world health organization

| Stages | | |
|---|---|---|
| Stage | Number of sample | Percentage (%) |
| Stage I | 8 | 10.5 |
| Stage II | 29 | 38.2 |
| Stage III | 32 | 42.1 |
| Stage IV | 7 | 9.2 |

and hMLH1. Since our patients with CRC were derived from a distinct geographical and ethnic back ground, we also wanted to revisit the pattern of methylation and included 4 genes (DAP-Kinase, p15, p16 and p73).

Among 76 samples, distrubition of tumor localization was as follows: 26 (34.2%) rectum, 18 (23.7%) sigmoid colon, 13 (17.1%) right colon, 8 (10.7%) left flexura and left colon, 7 (9.2%) recto sigmoid, 3 (3.9%) transvers colon and 1 (1.3%) all colon cancers. Histologic and morphologic classifications were done according to the WHO's TNM classification. Most of our sample group were in stage II and III and only 7 samples were in stage IV. Table 4 represents the number of patients and their stages according to the TNM classification.

There was mucinous area in 22 samples but only 8 (10.5%) of them were diagnosed as having mucinous adenocarcinoma. In 14 (18.4%) samples, tumors developed from tubulo-villous adenomas. In 19 (25%) samples, cripriform area was present. Pathologic investigation showed necrotic area in 28 and desmoplastic area in 17 samples. Additionally, there was blood vessel invasion in 13 samples, lenfatic invasion in 34 samples and perinoural invasion in 35 samples. There was no pathological tumor evidence in either proximal or distal surgical border in all samples.

**Table 5.** The number and percentage of methylated samples for each locus

| | hMLH1 (%) | APC (%) | P16 (%) | P15 (%) | P73 (%) | Dap-Kinase (%) |
|---|---|---|---|---|---|---|
| Number of methylated samples/number of analyzed samples | 18/73 (%24) | 23/73 (%31.5) | 30/70 (%42.8) | 20/64 (%30) | 12/71 (%17) | 10/51 (%19.6) |

APC: Adenomatous polyposis cair

**Table 6.** Relation of methylated loci with other methylated loci

| | hMLH1 | APC | p16 | p15 | p73 |
|---|---|---|---|---|---|
| DAP-kin | p=0.816 | p=1.00 | p=0.17 | p=0.128 | p=0.519 |
| p73 | p=0.431 | p=0.317 | p=0.392 | p=0.97 | |
| p15 | p=0.523 | p=0.099 | p=0.117 | | |
| p16 | p=0.558 | p=0.62 | | | |
| APC | p=0.68 | | | | |

DAP: Days after pollination, APC: Adenomatous polyposis cair

Methylation specific PCR (MSP) was used to determine methylation of p15, p16, p73, APC, DAPK1 and hMLH1. MSP reactions for each gene were first standardized using *in vitro* methylated DNA (IVM), which yielded a positive product with primers for the methylated form. Under our experimental MSP conditions, a consistent absence of an amplification product for the methylated form was observed in 15-20 different DNA samples obtained from peripheral blood mononuclear cells from healthy individuals. This indicated that methylation was tumor specific. Methylation was very common in CRC. In 15 samples (19.8%) there was no detectable methylation among those six loci. The frequency of methylation of each gene varied notably. The frequency of methylation is shown in Table 5.

The frequency of methylation was 24% for hMLH1, 31.5% for APC, 19.6% for DAP-Kinase, 42.8% for p16, 30% for p15, and 17% for p73. To quantify the extent of methylation in our series of CRC, we calculated a methylation index (MI=ratio between the number of genes methylated and the number of genes analyzed) based on the 6 genes that demonstrated some degree of methylation. MI ranged brtween 0-0.83, with an average of 0.271 corresponding to 1.6 genes/sample and median was 0.225 and when we removed 15 samples which was not methylated in any loci, the average of MI was 0.337 (represent 2.13 genes/sample) and median was 0.33.

For analyzing whether the coexistence of interrelationship between two different loci, we performed two-sided Fisher's exact test to understand whether one locus was methylated or un-methylated and whether methylation status of one locus was affected by another locus test results were shown in Table 6.

We also performed MSI analysis for 3 loci by ABI Prisim 310 sequencher (Applied Biosystem, Foster City, CA, USA) in these group of samples. We compared tumor tissue samples with normal mucosal specimen for the same patients' samples. We analyzed MSI in C-kit, hMSH2 and APC micro satellite region. Among these 3 regions; for c-kit locus MSI was positive in 14 of 67 samples, for hMSH2 locus MSI was positive in 12 of 66 and for APC locus, 10 of 67 analyzed samples. In all samples; there was one locus MSI in 18 patients, 2 loci MSI in 10 patients and 3 loci MSI in 4 samples.

All the analyzed 3 loci of MSI and methylation of 6 genes were compared statistically with avaliable data (age, sex, tumor localization, tumor cell differentiation, histologic charecterization of tumor cell) of patients by SPSS 10.0 for Windows software. We observed p73 methylation was significantly related with left colon cancer (p=0.003, 2-sided), p16 methylation was seen in females more than males (p=0.029, 2-sided), p15 methylation was seen more related with the well differentiated tumor cells (p=0.028, 2-sided). Also, APC methylation was correlated with stage I tumor (p=0.05, 2-sided) and stage I tumor was related with rectum cancer (p=0.017). Age and methylation index were compared with the other parameters by nonparametric Mann-Withney U Test (SPSS for Windows software). According to test results, MSI in APC loci was positively corelated with methylation index (p=0.029), MSI in APC kit and c-kit loci wererelated with age (p=0.032 and p=0.011, respectively) and for both loci, instability was increasing with age. Among those 6 loci which we performed methylation analysis, only p73 methylation was related with age (p=0.017) and inverse relation was present between age and left colorectal cancer (p=0.015). MI was observed higher with stage I (p=0.006) and stage III (p=0.03) disease.

In the same patient group, MSI studies were performed using DNA samples isolated from paraffin blocks of tumor and peripheral mucosa. DNA was used from same patient group but additionaly we used paraffin blocks from distal healty region which wasalso used for MSI comparison.

The MSI kit was used for this aim and it was used to study microsatellite instability of three regions. These regions were microsatellites present in the C-kit, hMSH2 and APC regions. Microsatellite unstable tumors are tumors in which, a new allele is observed, which is not observed in healthy tissue samples, or a decrease in the number of alleles are analyzed when normal and tumor tissues are compared.

Instability was determined in 14 of the 67 samples for the C-Kit microsatellite locus from 3 regions examined for MSI, 12 of 66 samples for the MSH2 microsatellite locus, and 10 for the APC microsatellite locus. Eighteen samples had MSI in at least one locus. MSI was observed in 2 loci in 10 specimens, 3 loci and 1 locus in 4 specimens.

## Discussion

We investigated the methylation status of 6 gene regions using the methylation-specific PCR method from paraffin block samples of 76 patients with sporadic colorectal cancer. The most frequent methylation was observed in the *p16* gene region (42.8%), followed by the methylation ratios in APC, p15, hMLH1, DAP kinase and p73 regions, respectively.

In similar studies, p16 methylation was found to be 10% (10/65, 39% of sporadic) (31), 28% (27/97) in Americans, 27% (13/48) in Jordanian society and 10% (7/68) in another study. We observed *p16* gene methylation more frequent in females than in males in our study population (p=0.023). Although *p16* gene methylation was more associated with mucinous carcinomas than other gene regions, there was no statistical significance (p=0.058).

Pehlivan et al. (32) from Turkey studied on metastatic colon cancer samples and MSI-H group. KRAS mutations are common and found in 42.1% of their samples. They have observed P16 methylation in only one sample out of 17 samples and it was in MSI-H group.

The methylation rate for p15 with the same chromosomal location was 69%, 39% (44/64) for sporadic and 30% (20/64) for our study group (31). On the other hand studies from United States did not identify any methylation on this region (33). In our study group, *p15* gene methylation was associated with well-differentiated tumor cells (p=0.009).

There are three members of the *p53* gene family (*p53*, *p63*, *p73*) and transcriptional activation is specific to the sequences of gene products (34-36). Functionally, overexpression of the *p73* gene has been reported to activate the targets of p53, such as cell cycle inhibitor p21 (34-35). However, there are also significant differences between the genes that *p53* and *p73* activate. P53 and p73 proteins are involved in the development of the organism in response to DNA damage. It is known that p73 is involved in p53-like response to cell stress. Overexpression of P73 triggers apoptosis (34-37). These and similar observations suggest that p73 is a tumor suppressor gene that affects p73 expression in some tumor types. Different studies have shown that p73 broad methylation is present in different proportions and in different populations. Xu et al. (31) found p73 methylation in 63% (41/65) of the patients, whereas in our group this rate was 17%. Very strong correlation was found between left colon cancer and *p73* gene methylation (p=0.003), and we observed that MSH2 was related with MSI, so it was related with p73 methylation (p=0.017). In addition, we found that the age was related with p73 methylation (p=0.017, Mann-Witney U test) .

Apopitosis-associated protein kinase (Dap-kinase1) is involved in different apoptosis systems as a calcium/calmodilin-dependent serine/thironine kinase. However, the mechanism of triggering apoptosis is not completely understood (38). We determined in our study group that the methylation rate was 19.1% in the DAPK1 region. Xu et al. (31) did not identify methylation in 39 sporadic cases. Satoh et al. investigated DAPK1 methylation in a series of 9 colorectal cancers. Pehlivan et al. (32) worked on Turkish CRC samples and searched for 5 genes related with methylation and 2 of them were the same with the ones in our study (DAPK1 and p16). DAPK1 methylation was found in 53% of of CRC samples and 29% of methylation was in healty part of CRC. They concluded that DAPK1 was not directly related with cancer formation but it was considered as an important factor for sensitivity (32). Mutation and methylation were found in MSI -H group. In our study group, there was no association between DAPK1 methylation, any features of the disease and methylation of other gene regions.

Loss of function in the *APC* gene results decrease in its product, a tumor suppressor protein, that it is known as the initiator. This causes development of multi-step cancer in the large intestine (39). APC protein is directly related to β-catenin signaling proteins (40). APC proteins are located in the microvessel network *in vivo*, whereas *in vitro* they cause polymerizations of tubules into microtubules (41). Early-occurring mutant proteins lose ability to suppress the β-catenin/Tcf signaling pathway in regions close to the carboxy end of the *APC* gene (42).

We observed APC methylation in 31.5% of our case group. There are reports in the literature that the rates of APC methylation are relatively low (8%) (31). Esteller et al. (43) showed the methylation of the APC region in 18% of 108 colorectal cancer cases examined. In another study, the methylation of the APC promoter was shown to be 28% of 137 colorectal cancer cases. Loss of heterozygosity and immunohistochemical staining of APC methylation are important in loss of gene expression and in the formation of second hit in the development of colorectal cancer (44).

Homozygous deletions of *p16* and *p14* genes are frequently observed in lung and breast cancers, which are not encountered in colorectal cancers (45). In contrast, methylation of p16 and p14 is more common in colorectal cancers (46). APC promoter methylation is a similar condition, although methylation in gastric cancers is a more common condition

Statistical analysis of the patient group showed that APC methylation was significantly associated only with the stage 1 disease group. This result was evaluated in the same direction as the rate limiting effect of the *APC* gene in the literature.

It is known that MSI, as a genetic finding, is an alternative pathway in the development of cancer after the detection of HNPCC and sporadic colorectal cancers (47). In our samples, there was a significant correlation between microsatellite instability in the APC region and hMLH1 methylation (p=0.032) in the three regions where we performed the MSI study.

HMLH1 methylation was not statistically related to MSI in the c-kit region (p=0.054, 2-sided), and there was no significant correlation with MSI in the MSH2 region. We hypothesized that *hMSH2* gene methylation may be significantly related to MSI in this region. It is reported that MLH1 and MSH2 mutations are not associated with low levels of MSI (48). It has been suggested that the methylation state in DNA repair genes which are not involved in the MMR mechanism may be the cause of the MSI (49). Interestingly, we found that only methylation in the *p73* gene region was significantly associated with MSI in the MSH2 region (p=0.017, 2-sided), while other methylation regions were not significantly associated with MSI screening.

Furukawa and colleagues examined hMLH1 methylation in three regions and found to be associated with MSI-H, proximal colon cancer involvement with a type of methylation, termed type 1, which contained more than 80% of the CpG island. The methylation of the PTEN promoter region was highly related with increased MSIand was claimed to be a second hit in the HMLH1 methylation (50). In our study group, the rate of HMLH1 methylation was 24% and it was found to be compatible with the literature (18%). Our samples were consisted of sporadic patients and there was no clinical relationship except HMLH1 methylation and MSI regions.

Co-methylation of some important tumor suppressor genes have been identified in some studies and it was defined as methylator phenotype of the relevant cancer. In this study, a similar relationship for CRC was not demonstrated for the 6 regions in which we studied. The most striking aspect of our results, could be summarized as, the association of p73 methylation with left colon involvement, correlation with MSI, and increasedmethylation with age. More frequent observation of p16 methylation in female gender and the fact that p15 methylation was associated with well differentiated cancers were the original results of our study for Turkish society.

## Conclusion

We observed that APC methylation was more common in Turkish population than other populations. P16 was the most common methylated locus among the 6 gene regions and it seemed strongly related with female patients. P73 was releated with left colorectal cancer and additionally it was related with the MSI.

### Ethics

**Ethics Committee Approval:** This study was a retrospective study that is why we could not take informed consent from the patients.

**Informed Consent:** This study was a retrospective study that is why we could not take informed consent from the patients.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Concept: C.G.E., M.G., F.D., Design: C.G.E., M.G., U.Ö., Data Collection or Processing: C.G.E., M.G., Analysis or Interpretation: C.G.E., U.Ö., Literature Search: C.G.E., Writing: C.G.E.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# The Role of PET/CT to Detect Bone Marrow Involvement in Hodgkin Lymphoma

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## ABSTRACT

**Objective:** Bone marrow involvement (BMI) is a significant component of staging of Hodgkin's lymphoma (HL). Unilateral bone marrow biopsy (BMB) from dorsal iliac bone is the standard method to determine BMI. Positron emission tomography/computerized tomography (PET/CT) is recommended as a complimentary technique to determine BMI and to evaluate response to treatment. The aim of this study is to determine whether PET/CT can replace BMB to detect BMI in patients with HL.

**Methods:** A total of 159 patients diagnosed as having HL were evaluated retrospectively. One hundred and four patients who met the criteria were included in the study. BMB and PET/CT were performed on all patients during initial staging.

**Results:** Of the 104 patients, 44 (42.3%) and 17 (16.3%) had BMI in PET/CT and BMB respectively. All patients who had BMI in BMB also had involvement in PET/CT. BMB did not detect BMI in 27 patients who had BMI in PET/CT. All 27 patients had partial or complete remission on PET/CT performed at the end of treatment. This finding was regarded as a relative indicator for BMI. PET/CT had a sensitivity and negative predictive value of 100%, specificity of 68.9% and accuracy rate of 74% to determine BMI.

**Conclusion:** We observed that PET/CT and BMB were compatible to determine BMI in patients diagnosed with HL. Unilateral BMB may result false negative especially in cases with focal involvement. Therefore, PET/CT should be considered as a complimentary technique to determine BMI. Staging should be reevaluated in patients who have BMI in PET/CT and a negative BMB treatment should be planned accordingly.

**Keywords:** Hodgkin's lymphoma, positron emission tomography, bone marrow biopsy, bone marrow involvement

## Introduction

Hodgkin's lymphoma (HL) comprises 12% of all lymphomas and 1% of all malignancies (1). It is possible to achieve cure with modern first line treatment modalities in about 90% of patients. However treatment fails in 10% of early stage and 30% of advanced stage disease. Cure rates are around 50% in relapsed disease, in extra-nodal disease and in patients relapsing early after treatment of advanced stage disease (2-5). Correct staging is necessary for an effective treatment plan (6,7). Bone marrow involvement (BMI) is one of the most important prognostic factors in lymphomas. BMI corresponds to stage IV disease according to the Ann Arbor staging system (8). Staging of HL is based on the Cotswold's modification of the Ann-Arbor system (8,9) and commonly includes computerized tomography (CT) and bone marrow biopsy (9). BMI is detected in 5-14% of patients with HL (10,11). Unilateral bone marrow biopsy (BMB) performed from dorsal iliac crest is considered as standard and performed routinely during staging (12,13). However main disadvantages of BMB are its interventional nature and it allows only a limited region of bone marrow to be investigated. A previous study reported 80% false negativity of a unilateral iliac crest biopsy compared with a bilateral biopsy in patients with lymphoma (14). Imaging

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techniques may be used to detect bone marrow involvement, CT may be able to show bone lesions or late occurring bone changes; however its sensitivity in early stage disease is low (7). Magnetic resonance imaging (MRI) is a sensitive technique although it is not used widely in routine practice. F-18 fluorodeoxyglucose (FDG) positron emission tomography/computerized (PET/CT) has been used for staging and evaluating response in patients with lymphoma. A number of studies have shown that PET/CT is useful to evaluate bone marrow (6,7,15). It has been shown that PET/CT and BMB are 78-82% compatible, thus PET/CT is recommended as a complimentary technique (14-17).

The aim of this study is to compare BMB and FDG-PET/CT to detect BMI and to determine whether FDG-PET/CT can replace BMB in patients with HL.

## Methods

Patients over 18 years old who had been diagnosed as having HL between January 2007 and January 2015 in our University Hospital were evaluated retrospectively. Patients who did not have bone marrow biopsy and PET/CT performed simultaneously at diagnosis were excluded from the study. All patients had BMB and PET/CT performed at diagnosis. Age, sex, date of diagnosis, type of PET/CT involvement (focal, diffuse), $SUV_{max\text{- values}}$, BMI, stage of disease, risk score, B symptoms, date of last follow up, treatments, and PET/CT results before and after treatment were recorded.

Administration of PET/CT was carried out locally on dedicated machines in accordance with at the manufacturer guidelines Marmara University Pendik Training and Research Hospital. During the evaluation of PET/CT, bone marrow activity greater than that of liver was considered positive for BMI by HL unless definitively explained by an alternate cause. Involvement was evaluated as diffuse or focal (1,2,3 and>3 areas).

All bone marrow samples were obtained unilaterally from dorsal iliac crest and each was 15-20 mm in length. Samples were evaluated by an expert hematopathologist.

Since our study was conducted retrospectively, it was not feasible to get informed consent from the patients, however it was approved by the Instutional ethical review board. Marmara University Faculty of Medicine Clinical Research Ethics Committee, Approval no: 09.2013.0242.

### Statistical Analysis

BMB was considered as standard reference to determine BMI. Results that were compatible with BMB and PET/CT were regarded as true positive and true negative initially. Cases that were PET/CT positive and BMB negative initially were evaluated with PET/CT during and at the end of treatment. Sensitivity, specificity, negative and positive predictive values were calculated. PASW 18.0 for Windows software was used for the statistical analysis. Descriptive statistics were expressed as average, standard deviation, median, percentile 25 (Q1), percentile 75 (Q3), minimum and maximum. Conformity of the variables to the normal distribution was analyzed with visual (histogram and probability diagram) and analytical (Kolmogorov-Simirnov/ Shapiro-Wilktests) methods. The paired group comparison was conducted by the Mann-Whitney U test for the diagnosis age, $SUV_{max}$, tracking duration and phase, the IPS and PET phase escalation which were not normally distributed. The chi-square test was used for the paired comparison between the categorical variable, and the Fisher exact test was used where the chi-square condition was unable to be provided. Cases where type-1 error was below 5% were regarded as statistically significant.

## Results

A total of 159 patients who had been diagnosed as having HL between January 2007 and January 2015 in Marmara University Medical Faculty were evaluated retrospectively. Fifty-five patients who did not have bone marrow biopsy and PET/CT performed simultaneously at diagnosis were excluded from the study. A total of 104 patients were evaluated.

Both PET/CT and BMB were performed for initial staging in a total of 104 newly diagnosed patients with HL (45 females and 59 males). Median age was 32 (18-80) years and 94.2% of the patients were diagnosed as having classical HL (58.3% nodular sclerosis, 28.2% mixed cellularity, 5.8% lymphocyte rich, 1.9% lymphocyte depleted), and the remaining 5.8% was diagnosed as having nodular lymphocyte predominant type. One patient had stage I, 36 patients had stage II, 37 patients had stage III and 30 patients had stage IV disease. According to IPS risk score; 67 patients had low, 17 intermediate and 18 high risk. Clinical characteristics of patients are summarized in Table 1.

BMI was detected in 44 patients (42.3%) with PET/CT and in 17 patients (16.3%) with BMB. Out of 44 patients who had BMI in PET/CT, 27 did not have BMI in BMB, however all 17 patients who had BMI in BMB also had involvement in PET/CT (Table 2). Two patients with stage II and 17 patients with stage III disease were regarded as stage IV after restaging with PET/CT

Disease stage and higher IPS risk were statistically significant in patients who were PET positive for BMI compared to PET negative patients ($p<0.001$ and $p<0.001$, respectively).

Disease stage and higher IPS risk were statistically significant in patients who had BMI in bone marrow biopsy compared to patients who were bone marrow biopsy negative ($p<0.001$ and $p<0.001$, respectively).

PET/CT was negative for BMI in 60 patients. All of these patients were also negative for BMI in BMB. Remaining 44 patients were PET/CT positive. There were not any uncertain cases since FDG values greater than liver was considered as positive. Involvement was evaluated as focal or diffuse. Focal group was classified as 1, 2, 3, and >3 areas. According to this classification 3 patients had focal 1, 4 patients had focal 2, 6 patients had focal 3 and

1 patient had focal >3 areas of involvement with a total of 28 patients having focal involvement. Sixteen patients had diffuse involvement. PET/CT positive, BMB negative patients had a median $SUV_{max\text{-}value}$ of 5.1 while PET/CT positive, BMB positive patients had 6.8 which was not statistically significant.

**Table 1.** Demographic features of patients

| | | |
|---|---|---|
| Sex, n (%) | Female | 45 (43.3) |
| | Male | 59 (56.7) |
| Age at diagnosis, median (Q1-Q3) | | 32.00 (24.50-46.00) |
| Disease subtype, n (%) | Nodular sclerosis | 60 (58.3) |
| | Mixed cellularity | 29 (28.2) |
| | Lymphocyte rich | 6 (5.8) |
| | Lymphocyte depleted | 2 (1.9) |
| | Nodular lymphocyte predominant | 6 (5.8) |
| Stage, n (%) | 1A | 1 (1.0) |
| | 2A | 21 (20.2) |
| | 2B | 15 (14.4) |
| | 3A | 12 (11.5) |
| | 3B | 25 (24.0) |
| | 4A | 7 (6.7) |
| | 4B | 23 (22.1) |
| IPS, n (%) | Low risk | 69 (66.3) |
| | Intermediate risk | 17 (16.3) |
| | High risk | 18 (17.3) |

IPS: Intrusion prevention system

**Table 2.** Bone marrow involvement

| | | n (%) |
|---|---|---|
| Bone marrow involvement in PET/CT | | 44 (42.3) |
| Bone marrow involvement in bone marrow biopsy | | 17 (16.3) |
| PET/CT -Bone marrow biopsy | PET/CT (+) Bone marrow biopsy (-) | 27 (61.4) |
| | PET/CT (+) Bone marrow biopsy (+) | 17 (38.6) |

PET/CT: Positron emission tomography/computerized tomography

When evaluating involvement type in PET/CT, it was observed that as the areas of focal involvement increased, the rate of involvement in BMB also increased. BMB was positive in all cases with diffuse involvement. Increase in disease stage in patients who had BMI in PET/CT was statistically significantly higher thanpatients who were negative for ($p<0.001$). All patients who had BMI in PET/CT at diagnosis had partial or complete regression after treatment. Twenty-two (81.5%) of PET/CT positive cases had complete regression and 5 (18.5%) had partial regression, a finding that was regarded as a sign of BMI (Table 3).

Analysis revealed a sensitivity of 100% for PET/CT to detect BMI, while specificity was found to be 68.97 % (Table 4).

**Table 3.** Comparison of bone marrow biopsy positive and negative groups within the positron emission tomography/computerized tomography positive patients

| | | PET/CT positive n=44 | | |
|---|---|---|---|---|
| | | BMB (-) n=27 | BMB (+) n=17 | |
| $SUV_{max,\ value,\ median}$ (Q1-Q3) | | 5.10 (3.60-11.10) | 6.80 (4.00-9.80) | 0.571 |
| Considering PET/CT, increase in stage, n (%) | - | 19 (70.4) | 0 (0.0) | <0.001 |
| Involvement type, n (%) | Focal 1 area | 3 (11.1) | 0 (0.0) | -* |
| | Focal 2 area | 3 (11.1) | 1 (5.9) | |
| | Focal 3 area | 3 (11.1) | 3 (17.6) | |
| | Focal >3 areas | 10 (37.0) | 5 (29.4) | |
| | Diffuse | 8 (29.6) | 8 (47.1) | |
| Diffuse–Focal Groups, n (%) | Focal | 19 (70.4) | 9 (52.9) | 0.242 |
| | Diffuse | 8 (29.6) | 8 (47.1) | |
| Regression of PET after treatment, n (%) | Complete regression | 22 (81.5) | 16 (94.1) | -* |
| | Partial regression | 5 (18.5) | 1 (5.9) | |

*Analysis was not performed due to low patient numbers, PET/CT: Positron emission tomography, computerized tomography

**Table 4.** Positron emission tomography-bone marrow biopsy sensitivity, specificity and positive-negative predictive values

| | Value | Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| Sensitivity | 100.00 % | 80.49 % | 100.00 % |
| Spesificity | 68.97 % | 58.14 % | 78.45 % |
| Positive predictive value | 38.64 % | 24.36 % | 54.50 % |
| Negative predictive value | 100.00 % | 94.04 % | 100.00 % |
| Accuracy rate | 74.04 % | 64.52 % | 82.14 % |

## Discussion

Anatomical extensiveness and to a lesser degree histological subtype of the disease are factors that define prognosis and guide treatment in HL. Aim of treatment is to achieve cure with minimal complications. Therefore, correct staging is necessary to make an effective treatment plan. Evaluating BMI is an important step in staging. BMI is detected in about 10% of patients with HL (10). In our study, BMI was detected in 16.3% of patients, which was higher than previously reported. Standard procedure to evaluate BMI is to perform unilateral BMB. BMI may be focal or diffuse. Blind biopsy samples may reveal false negative results if performed from uninvolved areas of bone marrow and BMI should be checked with other techniques. Positivity of bilateral biopsy is 10-50% which is higher than unilateral biopsy. Therefore, standard BMB does not reflect a correct evaluation of bone marrow (18). A negative BMB does not rule out BMI if imaging techniques show abnormalities. BMI is nodular in 65% of patients with HL on MRI. This explains a negative BMB when BMI is in other areas (19). It has been suggested that PET/CT could replace blind BMB or could be used as a guide to find the correct area for BMB. For patients with a high risk of BMI, FDG may be decisive. Since PET/CT detects a greater area than BMB, if there is not any FDG uptake, then there is no BMI (15). Therefore it is more sensitive than CT and bone scintigraphy to detect BMI. PET/CT may reveal early BMI in patients with a negative CT scan (6,7,10).

For this reason, true positivity should be determined. PET/CT results are evaluated in 3 reference levels in PET/CT positive cases. First of all, if BMB is positive, this is a verification at the highest level. Secondly, skeletal PET/CT findings of multifocal pattern (3 or more skeletal lesions): This pattern is typical for HL and may rarely be seen in other diseases (6). Thirdly, complete or near complete regression in PET/CT after chemotherapy is an intermediate level of verification, since FDG uptake may be observed in inflammatory and traumatic lesions as well. In our study, twenty-seven patients who had BMI in PET/CT but not in BMB were re-evaluated after treatment. Partial or complete regression of BMI was regarded as a clue for true positivity.

PET/CT should not be regarded as positive when FDG uptake is diffuse, homogeneous and symmetrical in axial and appendicular skeleton unless the FDG uptake is overt. It is regarded as bone marrow activation when FDG uptake of bone marrow is greater than liver. PET/CT may result false positive when there is a concomitant systemic disease or granulocyte colony stimulating factor use. Such cases and benign increases due to inflammatory reactions may be interpreted as false positivity, however BMI cannot be ruled out (7,20).

Most studies include a heterogeneous population of Non Hodgkin's and HL. This results in a variety of FDG uptake due to different subtypes (18). In our research, solely patients with HL were evaluated.

In a study conducted by Cetin et al. (21), 61 patients with HL were analyzed. Although, concordant results were revealed between PET/CT and BMB in 52, there were 2 patients with positive BMB while PET/CT was negative. On the other hand, 7 patients had negative BMB, while they were positive on PET/CT (21). In contrast, our study did not reveal any false negative cases.

Moulin-Romsee et al. (7). reported 83 patients diagnosed as having HL. Seven patients had BMI with BMB. Eighteen patients had BMI with PET/CT. Out of 11 patients that were PET/CT positive and BMB negative, 2 had stage IV disease due to lung and liver involvement. Remaining 9 patients were considered as having disseminated disease and treated accordingly (22). In our study, 2 patients with stage II and 17 patients with stage III disease initially were regarded as having stage IV disease after detecting BMI with PET/CT and treatment plans were re-evaluated.

We showed that sensitivity and negative predictive value of PET/CT were 100%. Specificity was 68.9% and accuracy rate was 74 %. These findings were similar to the data published by Pakos et al. (18). In a study by Anjum Bashir Khan et al. (23), 130 patients diagnosed as having lymphoma were evaluated. PET/CT and BMB proved BMI in 35 (27%) patients. PET/CT detected BMI in 33 (94%) of these patients, while BMB detected in 14 (40%) patients (23). Sensitivity and specificity of PET/CT to detect BMI was 94% and 100%, respectively. Sensitivity of BMB was found to be 40% and specificity was 100%. Accuracy rate was 98.5% for PET/CT and 84% for BMB. We observed similar results for sensitivity and specificity in our current study.

Although, our study confirmed studies previously performed, the most important limitation of our study was the relative small number of study population and its retrospective nature. Therefore, prospective studies including larger patient populations are necessary.

## Conclusion

FDG-PET/CT increased the sensitivity of detecting BMI compared to conventional staging. In patients who have BMI

according to PET/CT but not according to BMB, staging should be re-evaluated and treatment should be planned accordingly. Absence of false positive results in PET/CT and the observation of regression in FDG uptake after treatment in all patients may be an indicator to show PET/CT could replace BMB to determine BMI.

**Ethics**

**Ethics Committee Approval:** Marmara University Faculty of Medicine Clinical Research Ethics Committee, Approval no: 09.2013.0242.

**Informed Consent:** Since our study was conducted retrospectively, it was not feasible to get informed consent from the patients, however it was approved by the instutional ethical review board.

**Peer Review:** Externally peer-reviewed.

**Authorship Contributions**

Concept: A.E., F.P.T., Design: A.E., A.T.T., Data Collection or Processing: A.E., Analysis or Interpretation: A.E., F.P.T., Literature Search: A.E., A.S., Writing: A.E., A.S.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# Extra-Parenchymal Chest HRCT Findings in Patients with Systemic Sclerosis at the Time of Initial Diagnosis

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## ABSTRACT

**Objective:** In systemic sclerosis (SS) patients who are receiving treatment, findings of extra-parenchymal chest involvement (such as thymic abnormality, mediastinal lymph node enlargement) have been previously examined. However, these findings may be affected by medical treatment. Our aim was to evaluate the extra-parenchymal chest high-resolution computed tomography (HRCT) findings of patients with SS at the time of initial diagnosis.

**Methods:** We retrospectively analyzed medical database of patients with SS. Chest HRCT images within 1 month after initial diagnosis of these patients were re-evaluated for the presence of distal esophageal dilatation, thymic hyperplasia, mediastinal lymph node enlargement, pleural or pericardial abnormalities (effusion or thickening). Intergroup comparisons were performed using independent t-test or a Mann-Whitney U test. To detect the relationship between continuous variables, Spearman's correlation coefficients and univariate correlations were used.

**Results:** A total of 51 patients (45 women and 6 men, mean age + STD; 49.2 years+13.9) with SS were included in the study. Esophageal dilatation (88.2%) was the most common finding. Six patients (11.8%) had thymic enlargement. Mediastinal lymph node enlargement (11.8%) and pleural abnormalities (11.8%) were significantly more common in patients with SS diagnosed at late age ($p<0.05$).

**Conclusion:** The extra-parenchymal findings are common in patients with SS at the time of initial diagnosis. Esophageal dilatation is usually present at the time of diagnosis in most patients with SS regardless of age.

**Keywords:** Systemic sclerosis, scleroderma, high-resolution computed tomography, thymus, lymph node

## Introduction

Systemic sclerosis (SS), also known as scleroderma, is an autoimmune collagen vascular disease and it causes abnormal collagen growth in tissues such as skin, lung, heart and digestive tract. SS is usually characterized by swelling in the fingertips, joint pain and excessive tension and hardness of skin (1). Chest high-resolution computed tomography (HRCT) significantly increases the diagnostic sensitivity of lung involvement in SS (1-4). Also, mediastinal lymph node enlargement (MLNE), pleural disease or thymic enlargement may be seen in patients with SS at any time during the illness (5-10). However, the extra-parenchymal findings of SS may change with medical treatment. For example, thymus gland size decreases in response to use of corticosteroids or immunosuppressants. However, the risk of MLNE and pleural disease (pleural effusion or thickening) increases because of the increased frequency of infection in patients using corticosteroids or immunosuppressants (11,12). To the best of our knowledge, there is no study that evaluates extra-parenchymal chest CT findings in newly diagnosed patients with SS. Therefore, our aim was to evaluate the extra-parenchymal chest HRCT findings in newly diagnosed patients with SS and compare with literature.

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We also aimed to demonstrate the relationship between the age at initial diagnosis and extra-parenchymal HRCT findings.

## Methods

This single-center retrospective study was approved by our institutional local ethics committee. This single-center retrospective study received approval from Pamukkale University, Medical Ethics Committee (03.10.2017/13).

## Patients

The patients diagnosed as having SS according to the criteria of the American Rheumatism Association 1980 (13) or ACR/EULAR 2013 classification criteria (14) for SS, between January 2009 and August 2017 were retrospectively analyzed.

The patients with SS who underwent HRCT scan within one month after the initial diagnosis were included in the study. Having respiratory symptoms was indication for performing HRCT. Patients with history of malignancy, esophageal-gastric surgery or smoking and patients with additional rheumatologic disease (such as rheumatoid arthritis, Sjögren syndrome) or patients with overlap syndromes (such as SS and Sjögren syndrome overlap syndrome) were excluded from the study. Patients without HRCT screening within one month after initial diagnosis were not included in the study.

### High-Resolution Computed Tomography (HRCT) Scanning Protocol and Radiological Evaluation

All HRCT scans were obtained in the supine position with using a multi-detector CT system (Brilliance 16; Philips Healthcare, Best, the Netherlands) with full inspiration and the following parameters: Kv, 140; mAs, 280; detector collimation, 16x0.8/0.4 mm; slice thickness, 1 mm; slice interval, 10 mm; field of view (FOV), 35 cm and matrix, 512x512. Expiratory scans at least three selected levels (aortic arch, 2 cm above the diaphragm and tracheal carina) were obtained.

All HRCT images of the patients with SS were evaluated by two radiologists by consensus. Axial HRCT images of the chest were evaluated in the lung window for the presence of distal esophageal dilatation (with a maximum esophageal diameter ≥9 mm). Also HRCT images were evaluated in the mediastinal window for the presence of thymic hyperplasia (TH) (increased thymic thickness with ≥13 mm), MLNE (MLNE) (increased short-axis diameter with ≥10 mm), pleural or pericardial abnormalities (effusion or thickening).

### Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 19.0: SPSS Inc. IBM Corp, Chicago, IL, USA). Continuous and categorical data were reported as mean ± standart deviation (SD), frequency and percentage. Intergroup comparisons were performed using independent t-test or a Mann-Whitney U test for continuous variables. Spearman's correlation coefficients and univariate correlations were used to detect the relationship between continuous variables. $P<0.05$ was considered to indicate statistical significance.

## Results

Medical database analysis revealed a total of 101 patients who were newly diagnosed as having SS between January 2009 and August 2017. Among these patients, 4 patients with history of malignancy (2 breast, 1 lung, 1 rectum cancer), 9 patients with additional rheumatic disease or overlap syndromes (4 Sjögren syndrome, 2 rheumatoid arthritis, 2 dermatomyositis, 1 systemic lupus erythematosus) and 9 patients with smoking history were excluded from the study. In addition, 26 patients with >1-month interval between imaging and initial diagnosis, 2 patients with severe artifacts on HRCT. Totally, 51 patients [88.2% (n=45) females, 11.8% (n=6) males]; whose mean age ± STD was 49.2 years ± 13.9 (range 18-71 years) and who met the appropriate conditions were included in the study. The median time between SS diagnosis and HRCT examination was 8 days (range: 1-23 days).

The HRCT findings (thymic enlargement, MLNE, esophageal dilatation, pleural-pericardial abnormalities) and frequencies are shown in Table 1 and 2. Also, correlations of variables are shown in Table 3. The HRCT images of the patients are shown in Figures 1-4.

**Table 1.** High-resolution computed tomography findings in patients with systemic sclerosis

| HRCT Finding | Yes | No |
|---|---|---|
| | n (%) | n (%) |
| Thymic enlargement | 6 (11.8%) | 45 (88.2%) |
| MLNE | 6 (11.8%) | 45 (88.2%) |
| Esophagus dilatation | 45 (88.2%) | 6 (11.8%) |
| Pleural abnormalities | 6 (11.8%) | 45 (88.2%) |
| Pericardial abnormalities | 4 (7.8%) | 47 (92.2%) |

HRCT: High-resolution computed tomography, MLNE: Mediastinal lymph node enlargement, n: Number of patients

Four patients had pericardial effusion (7.8%). Pericardial thickening was not seen in any patient. Six patients (11.8%) had pleural abnormality, including effusion (n=4), and focal pleural thickening (n=2).

There was no significant difference between male and female patients in terms of thymic enlargement (p=0.699), MLNE (p=0.351), esophagus dilatation (p=0.699), pleural (p=0.699) and pericardial abnormalities (p=0.457).

**Table 2.** Comparison of high-resolution computed tomography findings in patients with systemic sclerosis with mean age of the patients

| HRCT Finding | Yes | No | p value |
|---|---|---|---|
| | Mean Age | Mean Age | |
| Thymic enlargement | 44 years | 49.9 years | 0.441 |
| MLNE | 61.3 years | 48.6 years | 0.021 |
| Esophagus dilatation | 50.2 years | 41.8 years | 0.166 |
| Pleural abnormalities | 64.8 years | 47.1 years | 0.002 |
| Pericardial abnormalities | 62 years | 48.1 years | 0.054 |

HRCT: High-resolution computed tomography, MLNE: Mediastinal lymph node enlargement, n: Number of patients, p<0.05 means statistical significance

**Table 3.** Correlations of variables

| HRCT Finding | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1. Thymic enlargement | 1 | -0.13 | 0.13 | -0,13 | -0,11 |
| 2. MLNE | -0.13 | 1 | 0.13 | 0.62** | 0.35* |
| 3. Esophageal dilatation | 0.13 | 0.13 | 1 | 0.13 | 0.11 |
| 4. Pleural abnormality | -0.13 | 0.62** | 0.13 | 1 | 0.35* |
| 5. Pericardial abnormality | -0.11 | 0.35* | 0.11 | 0.35* | 1 |

*p<0.05, **p<0.01, MLNE: Mediastinal lymph node enlargement, HRCT: High-resolution computed tomography

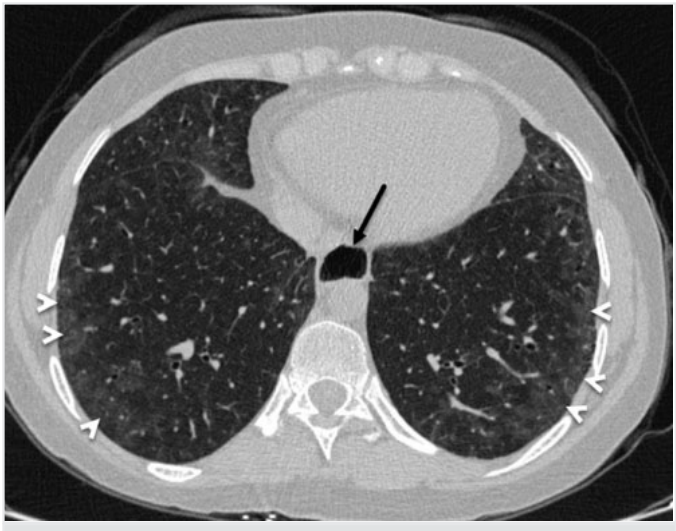

**Figure 1.** A 42-year-old female with systemic sclerosis. Axial high-resolution computed tomography image obtained during deep inspiration shows ground-glass opacity areas (arrow heads). Also, distal esophageal dilatation is seen (black arrow)


**Figure 2.** A 56-year-old female with systemic sclerosis. Axial high-resolution computed tomography image shows right para-tracheal lymph node enlargement with a 14 mm short axis


**Figure 3.** A 48-year-old female with systemic sclerosis. Axial high-resolution computed tomography image obtained during deep inspiration shows pericardial effusion with a 9 mm thickness

**Figure 4.** A 36-year-old male with systemic sclerosis. Axial high-resolution computed tomography image shows thymic enlargement with a 21 mm thickness (white arrows)

## Discussion

According to our results, esophageal dilatation (88.2%) is frequently found in SS patients with respiratory symptoms, at the time of initial diagnosis. MLNE and pleural abnormalities are significantly more common in patients with SS diagnosis at the late age.

Although the actual role of thymic abnormalities on imaging studies in patients with SS is still unclear, the possible involvement of the immune-mediated SS pathogenesis at an early stage may be presumed (7,8,14). When the increased thymic thickness was accepted as a threshold value of greater than 13 mm, Colaci et al. (15) reported that the frequency of abnormalities in thymus size was 9.5% (19 of 200 patients) in patients with SS with a mean of 5-year (0-38 years) disease duration. Ferri et al. (7) found that, radiological thymic enlargement were correlated with SS disease duration and thymic enlargement more common in patients with short disease duration (≤5 years or >5 years; p=0.007). They detected thymic enlargement in 9 of 34 cases (26%). We found that thymic enlargement was present in 11.8% (6 of 51) of patients with SS, at the time of initial diagnosis. In addition, studies showed that patients with SS with thymic enlargement were found at a younger age. For this reason, it has been argued that thymic enlargement is due to insufficient involution of the thymus gland (16-18). In our study, although the mean age of cases with thymic enlargement was lower than that of without thymic enlargement, there was no statistically significant difference between the age of initial diagnosis and presence of thymic enlargement (44 vs 49.9 years; p=0.441). This may be due to a small number of cases in our study. In our study, unlike previous studies, the HRCT findings of newly diagnosed patients with SS were evaluated. We suggest that the duration of the disease and the medications may cause thymic abnormalities. There is a need for more extensive studies in order to explain this situation.

MLNE has been reported to be a common finding in patients with SS (9). In patients with SS, Santos et al. (5) demonstrated that, MLNE was present in 5 of 23 patients (21%), Yamakawa et al. (17) found that MLNE was seen in 20% (n=40), and Farrokh et al. (18) found that 30% (n=40) of patients with SS had MLNE on HRCT. Wechsler et al. (19) and Garber et al. (20) found that MLNE was present in %50 and 32% of patients with SS, respectively. In those studies, examining the prevalence of MLNE, the duration of the disease and medical treatment were not considered (5,9,17-20). In our study, MLNE was present in 11.8% of newly diagnosed SS patients (n=51). The mean age of the patients with MLNE was significantly higher than those without MLNE (p=0.021). It is more likely that elderly patients have more SS disease duration and risk of infectious lung diseases than younger ones.

Another common finding in patients with SS is esophageal involvement. Esophageal involvement is common in SS and it can occur from 58 to 80% in different studies (9,21,22). Vonk et al. (21) reported that, esophageal dilation in patients with interstitial lung disease suggests a diagnosis of SS with a high sensitivity and specificity on HRCT (63% and 88%, respectively). In our study, we demonstrated that esophageal dilation is a frequent HRCT finding in patients with SS at the time of initial diagnosis, with a rate of 88.2% (45/51).

It has been reported that pleural abnormalities are rarely present in patients with SS. Pleural abnormalities may be seen as, diffuse or focal pleural thickening, pleural effusion, and pseudo-plaques (22). Farrokh et al. (18) reported that pleural abnormality was present in 30% of patients (12 of 40) with SS and they found 4 patients had pleural effusion, 8 patients had pleural thickening. Yamakawa et al. (17) found that, pleural abnormality (thickening or effusion) was present in 10% of patients with SS (n=40). Pleural effusion in patients with SS is an uncommon finding that requires a complete evaluation to exclude different causes of pleural effusion such as heart failure, side effects of medication and pneumonia (23). Similar to Yamakawa et al. (17), we also

detected that 11.8% of SS patients had pleural abnormalities on HRCT. We also found that, as expected, the age of the patients with pleural abnormality was significantly higher than the age of the patients without pleural abnormality (p=0.002). This may be due to the fact that elderly patients are more likely to develop empyema or hemothorax than younger patients. Also, pleural abnormality may occur due to long disease duration or heart failure in the elderly.

This study has some limitations. First, the HRCT images were evaluated by two observers by consensus so the differences between the observers could not be assessed. Second, it was a retrospective study that involved a relatively small number of cases. Third, there was also sampling bias since only patients with positive respiratory symptoms were included in the study. So, we could not evaluate the extra-parenchymal chest CT findings in patients without respiratory symptoms. Finally, we did not have histopathological findings and we could not make a radiological-histopathological comparison.

## Conclusion

Despite these limitations, our study suggests that esophageal dilatation is frequently found in patients with SS with respiratory symptoms at the time of initial diagnosis. MLNE and pleural effusion are more common in newly diagnosed patients with SS at the late age, probably due to long disease duration and advanced age.

### Ethics

**Ethics Committee Approval:** This single-center retrospective study received approval from Pamukkale University, Medical Ethics Committee (03.10.2017/13).

**Informed Consent:** Written informed consent was not obtained from participants due to retrospective nature of study.

**Peer review:** Externally peer-reviewed.

### Authorship Contributions

Concept: F.U., P.Ç., Design: F.U., E.S., Data Collection or Processing: F.U., Analysis or Interpretation: F.U., Literature Search: E.S., P.Ç., Writing: F.U., B.Y.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# *In vitro* Antifungal Activities of Fluconazole, *Camellia sinensis* and *Cydonia oblonga* Leaf Extracts Against *Candida* Species Isolated from Blood Cultures

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## ABSTRACT

**Objective:** In this study we investigated *in vitro* antifungal activity of fluconazole, green tea (*Camellia sinensis*) and quince leaf (*Cydonia oblonga*) extracts in *Candida strains* isolated from blood cultures.

**Methods:** Fifty *Candida* spp were included to study. *Camellia sinensis* and *Cydonia oblonga* leaves collected from Rize and Erzurum/Tortum regions were prepared as an extract. Then stock solutions of the extracts and fluconazole powder obtained from the manufacturer were prepared for use in the Broth Microdilution (BMD) method according to Clinical and Laboratory Standards Institute (CLSI) M27 A3 recommendation. Fluconazole minimum inhibitory concentration (MIC) values obtained from BMD method were evaluated according to CLSI M27 A4 break points. MIC values obtained for *Camellia sinensis* and *Cydonia oblonga* were interpreted according to fluconazole break points since there is no break points in the literature.

**Results:** Of the 50 *Candida* spp. included in the study, 37 (74%) were *C. albicans* while 13 (26%) were non-albicans species. The most common isolated non-albicans species was *C. parapsilosis* (14%). For all tested *Candida* strains $MIC_{50}$ and $MIC_{90}$ values for fluconazole were found to be 0.125 µg/mL- 0.25 µg/mL while they were determined as 0.125 µg/mL-64 µg/mL and 0.125 µg/mL-0.125 µg/mL for green tea and quince leaves, respectively.

**Conclusion:** According to the low MIC values, it is seen that green tea and quince leaf are *in vitro* effective against *Candida species*. However, further extensive *in vitro* and *in vivo* studies should be done on the possibility that green tea and quince leaf can be used alternatively in the treatment of invasive *Candida* infections.

**Keywords:** Antifungal activity, *Candida*, fluconazole, green tea, quince leaf

## Introduction

Invasive *Candida* infections are one of the major health problems with high mortality and morbidity rates, increasing hospitalization and cost of treatment (1). The incidence of candidemia cases is increased by malignancies, immunosuppressive diseases or treatments, use of broad-spectrum antibiotics and corticosteroids, aggressive chemotherapy and some major surgical procedures all over the world (2,3). According to the Centers for Diseases Control and Prevention (CDC) and the National Healthcare

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Safety Network (NHSN), *Candida* species are the fourth most common sepsis cause in America and Europe (2,4-6).

Azoles are often used in the treatment of fungal infections with the advantage that they have less toxic effects than other antifungal agents. However, this situation recently has caused increased resistance to azole in fungal agents which prompts researchers to search for new antifungal agents, especially for resistant invasive *Candida* infections (7,8).

Green tea *(Camellia sinensis)* and quince *(Cydonia oblonga)* leaf have been used for treatment of various infections in traditional medicine for centuries. And also there are various studies on antimicrobial activity of green tea and quince leaf in the literature most of which showed that green tea and quince leaf both have *in vitro* antimicrobial effects (9-12).

In this study, we investigated *in vitro* antifungal activity of green tea and quince leaf on *Candida* isolates obtained from blood cultures. The minimum inhibitory concentration (MIC) values of fluconazole were also evaluated for each isolates.

## Methods

***Candida* strains:** Fifty *Candida* spp. isolated from blood cultures (BACTEC 9000 System; Becton Dickinson) and sent to Medical Microbiology Laboratory between April 2013-December 2015 were included to the study. Repeated samples of the same patient were not included in the study. Chromogenic agar, tween 80 agar and related typing cards of VITEK 2 automatizated system (BioMerieux, France) were used to identify the isolated *Candida species*. Typed strains were stored at -80˚C with stock preparations as far as the planned schedule of the study. The strains were passaged twice in Saboraud Dextrose Agar (Oxoid, UK) after dissolved at room tempature to obtain appropriate culture. In the study, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 strains were used as control strains.

**Preparation of extracts:** Quince (*Cydonia oblonga*) and green tea (*Camellia sinensis*) leaves were collected from Tortum/Erzurum and Rize in their appropriate season. The dried and powdered leaves (100 g) of green tea and quince were extracted with 1 L of methanol using a Soxhlet extractor (SIGMA-ALDRICH, 322415) for 72 h at a temperature which was not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper No.1 (Dassel, Germany) and then concentrated in vacuo at 40°C using a rotary evaporator (Buchi Labortechnic AG, Flawil, Switzerland). The plant extracts were then lyophilized and kept at 4°C until being tested (13).

**Preparation of stock solutions:** While fluconazole powder (Pfizer) was dissolved and diluted in sterile, pyrogen-free saline to a stock concentration of 256 μg/mL according to CLSI M27-A3 suggestions (14), prepared green tea and quince leaf extracts were both diluted to the concentration of 256 μg/mL as same as fluconazole since there is no standard MIC values for quince leaf and green tea extracts.

**Broth Microdilution (BMD) method:** To obtain MIC values of fluconazole, green tea and quince leaf for *Candida* strains, BMD method was applied by using L-glutamine, bicarbonate free, RPMI 1640 medium (Sigma Chemical Co., St Louis, Mo., USA) according to CLSI M27-A3 suggestions (14). This medium was used in the BMD test after adding 2% dextrose and adjusting pH to 7 with 0.165 M morpholine-propane-sulfonic acid (MOPS; Sigma). In the direction of this method two-fold decreasing serial dilutions of fluconazole, green tea and quince leaf were prepared in sterile, U-based, 96 well microplates by using stock solutions. Then *Candida* strains adjusted to 0.5 McFarland standard turbidity were added in each well of microplates except the last one. The last two wells were separated as sterilization control and reproduction control wells. Thus, the final concentration of fluconazole green tea and quince leaf were between range of 64 μg/mL to 0.125 μg/mL. Plates were evaluated by two different investigators with naked eyes after 24-48 hours of incubation. The MIC value of the well with the smallest amount of drug with 50% or less reproduction relative to the reproduction control was recorded as MIC value. Fluconazole MIC values were evaluated according to the CLSI M27- S4 break points (15). Because of there is no standard MIC break points for quince leaf and green tea extracts, MIC values were evaluated by comparing with fluconazole break points.

### Statistical Analysis

For analysis of the study data Statistical Package for Social Sciences (SPSS; v20.0) statistical program was used. The data were expressed as number, percentage, mean and standard deviation. Suitability to normal distribution of the data was investigated by the Kolmogorov-Simirnov test. The Kruskal Wallis test was used for the comparison of more than two groups of numerical data without normal distribution. The results were considered significant when $p<0.05$.

## Results

Total of 50 *Candida* spp (37 *C. albicans*, 7 *C. parapsilosis,* 3 *C. tropicalis,* 3 *C. glabrata)* were included in the study. *Albicans* species (74%) were found to be more than *non-albicans* species (26%). The most common isolated *non-albicans* species was *C. parapsilosis* (14%).

*Candida* strains isolated from blood cultures of hospitalized patients in 11 different clinics in our hospital were included in the study. The clinics where these strains were most isolated were Anesthesia and Reanimation Intensive Care Unit (22%), Internal Medicine Intensive Care Unit (16%), Internal Medicine Hematology Service (12%), Pediatric Intensive Care Unit (12%) and Medical Oncology Service (10%), respectively. Distribution of isolated *Candida* strains according to the services are shown in the Table 1.

For all tested *Candida* strains $MIC_{50}$ and $MIC_{90}$ values for fluconazole were found to be 0.125 μg/mL-0.25 μg/mL while they were determined as 0.125 μg/mL-64 μg/mL and 0.125 μg/mL-0.125 μg/mL for green tea and quince leaves, respectively.

**Table 1.** Distribution of isolated *Candida* strains according to the services

| Services | *C.albicans* | *C.parapsilosis* | *C.tropicalis* | *C.glabrata* | Total |
|---|---|---|---|---|---|
| Anesthesia/Reanimation ICU* | 7 | 3 | 1 | - | 11 |
| Internal Medicine ICU | 6 | 1 | - | 1 | 8 |
| Internal Medicine Hematology | 4 | 1 | - | 1 | 6 |
| Pediatric ICU | 5 | - | 1 | - | 6 |
| Medical Oncology | 5 | - | - | - | 5 |
| Neurology ICU | 3 | 1 | - | - | 4 |
| Organ Transplant | 2 | - | 1 | 1 | 4 |
| Infectious Diseases | 2 | - | - | - | 2 |
| Pediatric Hematology | 1 | 1 | - | - | 2 |
| Cardiology ICU | 1 | - | - | - | 1 |
| Brain Surgery ICU | 1 | - | - | - | 1 |
| Total | 37 | 7 | 3 | 3 | 50 |

*ICU: Intensive Care Unit

**Table 2.** The results of $MIC_{50}$, $MIK_{90}$ and $MIC_{min-max}$ obtained with BMD method against *Candida* strains for fluconazole, green tea and quince leaf (µg/mL)

| Strains | $MIC_{50}$ | | | $MIC_{90}$ | | | $MIC_{min-max}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Flu | GT | QL | Flu | GT | QL | Flu | GT | QL |
| *C.albicans* (37) | 0.125 | 0.125 | 0.125 | 0.25 | 2 | 0.125 | 0.125-32 | 0.125-64 | 0.125-64 |
| *C.parapsilosis* (7) | 0.125 | 0.125 | 0.125 | 32 | 64 | 64 | 0.125-32 | 0.125-64 | 0.125-64 |
| *C.tropicalis* (3) | - | - | - | - | - | - | 0.125-0.25 | 0.125-0.25 | 0.125-0.25 |
| *C.glabrata* (3) | - | - | - | - | - | - | 0.125-0.25 | 0.125-0.25 | 0.125-0.25 |
| All strains | 0.125 | 0.125 | 0.125 | 0.25 | 64 | 0.125 | 0.125-32 | 0.125-64 | 0.125-64 |

*Flu: Fluconazole, GT: Green tea leaf, QL: Quince leaf, min: Minimum, max: Maximum, BMD: Broth microdilution

There was no significant difference between fluconazole, green tea and quince leaf extract values obtained from *Candida* strains.

When MIC values were evaluated according to CLSI M27-S4 break points, all of the *C. tropicalis* and *C. glabrata* strains were susceptible; 2 *C. parapsilosis* and 1 *C. albicans* were resistant to fluconazole (MIC=32 µg/mL). The MIC values of green tea and quince leaves for fluconazole resistant strains were 64 µg/mL. The results of $MIC_{50}$, $MIK_{90}$ and MIC range obtained with BMD method against *Candida* strains for fluconazole, green tea and quince leaf are shown in the Table 2.

## Discussion

*Candida* spp are one of the important causes of nosocomial bloodstream infections. The incidence of *Candida* bloodstream infections have risen in the past 20 years worldwide. Generally *C. albicans* is the most frequently isolated *Candida* species in blood cultures, however in some studies *non-albicans* species have been isolated more frequently. The distribution of isolated species may vary by region. The most common *non-albicans* species isolated from blood cultures *are C. parapsilosis*, *C. glabrata* and *C. tropicalis.* It has been reported that *C. krusei* is the least frequently isolated species (16-19). In our study *C. albicans* was the most frequently isolated species (74%) while *C. parapsilosis* was the most common among *non-albicans* species.

Today we use various antifungal agents, such as azoles, that raised significant progress in treatment of invasive *Candida* infections. Fluconazole is very often used with the advantage of its broad spectrum and low toxicity than others. Although in our study, fluconazole resistance was 6% (2 *C. parapsilosis* and 1 *C. albicans*), some studies showed that azole resistance have increased recently because of common use (20-23). Due to this current case and inadequate antifungal treatment options, *Candida* bloodstream infections are still common extending the length of stay in hospitals and increasing the cost of care (24,25). That makes researchers look for alternative antifungal agents. There are many plants with medical features. There is a widespread belief that medicinal plants are healthier and more harmless or safer than synthetic ones. These medicinal plants have a rich content that can be used to develop drugs. In recent years, studies on antifungal properties of some plants have been revealed (26-28).

Green tea is an easily accessible plant in Turkey. Previous studies have given positive results on antifungal activity of green tea. According to the results of the studies, in the future the green tea extracts may be considered as a therapeutic adjunct on oral

*Candida* species (10,29,30). In a study, it was demonstrated that green tea was found to be very effective on *C. albicans, Streptococcus mutans* and *Lactobaclli* spp (31). Yang and Jiang (32) have reported that tea polyphenols can inhibit mycelial growth and spore germination of *Rhizopus stolonifer*. Aladag et al. (33) demonstrated that the antifungal activity of green tea leaves may be closely correlated to catechin content which can vary due to different harvest time. In this study fresh, green tea leaves were harvested in May which was the most convenient time and the $MIC_{50}$ and $MIC_{90}$ values were found to be 0.125 μg/mL-64 μg/mL. According to these results, it is interpreted that green tea is *in vitro* effective on *Candida* strains isolated from blood culture.

Quince, which is the other plant we used in this study (*Cydonia oblonga*), belongs to the family *Rosaceae* and Turkey is one of the most important quince producers worldwide (34). Leaf, fruit and seed of quince have very important medicinal effects. In studies with quince, many useful features have been reported such as antibacterial, antioxidant and antiproliferative, antiinflammatory and anti-dysenteric activities. Furthermore, it has been reported that it can also be useful in cancer, bloodstream and skin diseases (27). Fattouch et al. (35) have studied with various parts of quince and reported that quince may be a useful adjuvant agent for the treatment of bacterial infections in addition to antibiotics. Alizadeh et al. (26) reported that the quince leaf's extracts can inhibit the growth of *Aspergillus niger*. However, there is no satisfactory research result on anticandidal properties of quince leaf in the literature. In this study, we have invastegated anticandidal effects of quince leaf's mehtanolic extracts on 50 *Candida* species. The $MIC_{50}$ and $MIC_{90}$ values were 0.125 μg/mL-0.125 μg/mL, respectively and these results indicated that quince leaf extracts have good in vitro anticandidal effects with very low MIC levels.

Although the $MIC_{50}$ and $MIC_{90}$ values of both green tea and quince leaf extracts were very low, the situation was different in fluconazole resistant strains. In our study, 2 *C. parapsilosis* and 1 *C. albicans* strains were resistant to fluconazole and fluconazole MIC values were 32 μg/mL for all of them. When the MIC values of green tea and quince leaf of these strains were examined, it was found that the MIC value was 64 μg/mL for these strains. Because of this result, it was thought that green tea and quince leaf were not effective in fluconazole resistant *Candida* strains.

There are various studies on the use of green tea and quince leaf for therapeutic purposes and their effects on the body. In some of these studies the distribution of green tea components were calculated in blood and urine after oral intake (36). These studies serve as guides to show the distribution of green tea to the part of the body that is infected. One of these studies showed that, a cup of Japanese green tea contained about 150 mg of epigallocatechin and about 3.5 mg of it excreted into the urine per cup of tea (37). There are also many studies showing the phenolic components of quince leaf and the therapeutic effects of these components on the body (27,38,39). Though these studies include only oral use, they are hopeful that green tea and quince leaf contents can be used to develop different form of drugs.

### Study Limitations

In this study, the phenolic profile of the green tea and quince leave extracts could not be analyzed. Since the study was conducted with *Candida* strains isolated from the blood culture samples which were sent to our laboratory in a spesificied time period, no preliminary statistical study was done in terms of the number and types of strains to be used in the study.

## Conclusion

In addition to making contributions to the literature about the antifungal activity of green tea on *Candida* species, this is the first study directly conducted on the evaluation of antifungal activity of quince leaf extracts on *Candida* species isolated from blood culture. According to the low MIC values obtained from the study, it was observed that green tea and quince leaf extracts both have *in vitro* antifungal activity on *Candida* species. However, further extensive *in vitro* and *in vivo* studies should be undertaken whether green tea and quince leaf can be used in the treatment of invasive *Candida* infections.

### Ethics

**Ethics Committee Approval:** This study was approved by the Ethics Committee of Faculty of Medicine of Ataturk University, dated 28.04.2016 and numbered 4/08.

**Informed Consent:** Written informed consent was not received due to the nature of this study.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Concept: H.H., Design: H.H., M.V.C., S.Ş., Data Collection or Processing: H.H., M.V.C., Analysis or Interpretation: H.H., M.V.C., Literature Search: H.H., M.V.C., H.İ., Writing: H.H., M.V.C.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# Factors Leading to Re-revision Surgery Following the Index Total Hip Arthroplasty Revision: Mid-Term Results

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## ABSTRACT

**Objective:** The aim of this study was to evaluate the factors that led to re-revision surgeries in patients who underwent revision total hip arthroplasties (THA).

**Methods:** A total of 352 revision THAs in 274 hips of 252 patients (January 2001-December 2012) were retrospectively analyzed. Patients with a history of a major component revision surgery, replacement of the modular components, debridement and irrigation with liner exchange in the presence of infection and a two-stage revision surgery were included in the study. The mean follow-up period after the revision surgery was 7.5 (range: 2 to 15) years.

**Results:** A re-revision surgery was required in 17.6% of the index THA revision patients (62 THA re-revisions/352 THA revisions). The mean time between the index revision and re-revision surgeries was 60.4 (range: 0.5 to 348) months. The most common reason for the second revision surgery was aseptic loosening (38 THA revisions; 61.2%), followed by instability (8 THA revisions; 12.9%) and infection (6 THA revisions; 9.1%). When the re-revision surgery was taken as end point for assessing the survival rate after the index revision surgery, the cumulative survival rate 10 years after the first revision surgery was found 70.8%. No significant relationship was established between age, gender and the type of fixation and the rate of repeat revisions. However, re-revision rates were significantly higher in acetabular-only component revision cases in comparison to other or both component revisions.

**Conclusion:** In our revision series, the major factor that necessitated a re-revision following index revision surgery was aseptic loosening, followed by instability.

**Keywords:** Revision, total hip arthroplasty, survival

## Introduction

Although the functional outcomes following total hip arthroplasty (THA) have significantly improved, the ever increasing number of revision surgeries and implant failures are still major problems (1-3). The greatest expectation of a revision patient is to recover with good functional outcome and without need of a re-revision surgery (4,5). The rate of re-revision surgeries unfortunately shows that this expectation is met at a very low level (4). Considering that the number of revision surgeries will increase in the future, investigating and evaluating the reasons for re-revision surgeries will be of significant importance in order to decrease the costs and to increase patient satisfaction.

The reasons for revision surgeries following primary THA have been exhibited in several registry system and multicenter studies (1,2,6). On the other hand, the reasons for re-revision surgeries in patients who had already undergone a revision surgery have been rarely reported in the literature. Studies that address the outcomes following revision surgery usually evaluate the surgical techniques, classification of the outcomes based on the acetabular or femoral defect and the success and survival rates of the

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featured implants (7-10). The most common reasons to repeat revision surgery following THA revision surgeries are infection, aseptic loosening, the wear on the interface and osteolysis and dislocation secondary to the wear (1,3,7,11-14). Re-revisions are usually performed within the first two years of revision surgery, and infection and technical errors are the major problems in the first years (6,12,15).

Understanding and recognizing the factors that necessitate re-revision surgeries may play a significant role in the success of re-revision surgery. Therefore, in this study, we aimed to investigate the factors, which led to repeat revision surgery following revision THA surgery and to identify the patient-related and implant-related factors and to evaluate the survival rates following the index revision surgery.

## Method

A total of 352 THA revision surgeries in 274 hips of 252 patients (184 females, 68 males) with varying indications performed between January 2001 and December 2012 were retrospectively analyzed. The mean age of the patients at the time of revision surgery was 59 (range: 22 to 86) years. One hundred fifty one patients were over 65 years of age and 101 were below 65 years of age. Patients with a history of a major (acetabular or femoral) component revision surgery, replacement of the modular components (femoral head-acetabular liner), debridement and irrigation with linear exchange in the presence of infection and a two-stage revision surgery for infection were included in the study. Patients who had undergone closed reduction due to postoperative instability were excluded. The initial diagnosis of the patient that necessitated a revision surgery, the surgical technique employed, the radiological and clinical follow-up after the index revision surgery, and the reasons for the second and third revision surgeries, if any, were retrieved from the FileMaker 10.0 registry system of the senior surgeon (XX) and recorded. The senior surgeon carried out all surgical interventions. The Ethics Committee of Acıbadem University approved this clinical study according to the declaration of Helsinki. Written informed consent was obtained from each participant before commencement of the study.

The THA revisions were classified based on the type of fixation as cemented, cementless, hybrid and reverse hybrid; the component type as acetabular, femoral, acetabular and femoral, and head and linear; and the surface material of the implant as metal-polyethylene, metal-metal, ceramic-polyethylene, zirconium-polyethylene, and ceramic-ceramic (Table 1). The reasons for re-revision surgery after the index revision surgery were re-evaluated based on the type of fixation, the component type and the surface of the implant used.

The MedCalc v.17.9 software (MedCalc Software bvba, Ostend, Belgium) was used in statistical analyses. In evaluation of the study data, descriptive statistical methods (mean, standard deviation, median, frequency, percentage) were used in addition to the Mann-Whitney U test used in intergroup comparisons of the qualitative data that did not show normal distribution due to the number of cases. The Fisher-Freeman-Halton test was employed in evaluation of the quantitative data. The levels of significance were set at $p<0.01$ and $p<0.05$. The revision of any component due to any reason following the index THA revision surgery was considered as the final time point of survival and the survival rates were evaluated with the Kaplan-Meier analysis.

## Results

A re-revision surgery was needed in 17.6% of the index THA revision patients (62 THA re-revisions / 352 THA revisions). The mean time between the index revision and repeat revision surgeries was 60.4 (range: 0.5 to 348) months. The most common reason for the second revision surgery was aseptic loosening (38 THA revisions; 61.2%). Aseptic loosening was observed after a mean period of 67 (range: 1.2 to 368) months after the index revision surgery. The following reasons for the failure of the first revision surgery were instability (8 THA revisions; 12.9%) and infection (6 THA revisions; 9.1%). Instability and infection were mostly observed in the early term, within the first two years of the revision surgery, after a mean period of 26 (range: 1 to 108) months and 28 (range: 2 to 107) months, respectively. Periprosthetic fracture after the index revision surgery was the last variable among the reasons for re-revision surgery. Of those who underwent a re-revision surgery, 13 (15 hips; 5.47%) needed a third revision surgery.

No statistically significant difference was detected between the rates of re-revision surgeries among the patients over 65 years of age and under 65 years of age ($p=0.123$). Similarly, no significant difference was observed between the rates of re-revision surgeries among patients with cemented and cementless fixation types ($p=0.851$). Patients who underwent revision surgeries with isolated acetabular components comprised the group which had the highest rates of repeat revision surgery (28 rTHA/73 rTHA) and the rate of re-revision surgery in this group was significantly higher than those who required the replacement of both components ($p<0.001$). The most commonly used femoral component in patients in which isolated femoral revisions and

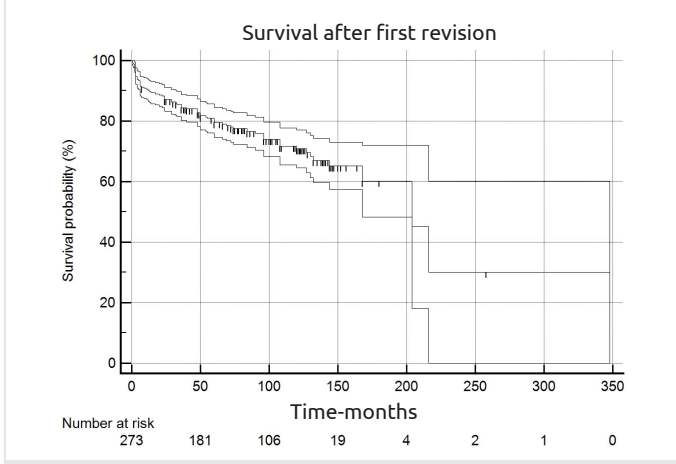


**Figure 1.** Survival rates of the patients following the index revision surgery

**Table 1.** Demographic data of the patients who underwent revision surgery

| | Total | Patients Not Undergone Re-Revision Surgery | Patients Undergone Re-Revision Surgery (n=55) |
|---|---|---|---|
| **Gender** | | | |
| Female | 184 (73.0%) | 148 (80%) | 40 (20%) |
| Male | 68 (27.0%) | 53 (88.3%) | 15 (16.7%) |
| **Age** | | | |
| >65 years | 151 (59.9%) | 113 (75%) | 38 (25%) |
| <65 years | 101 (40.1%) | 84 (83.2%) | 17 (16.8%) |
| **Type of fixation** | | | |
| Cementless | 136 (54.0%) | 103 (75%) | 33 (25%) |
| Cemented | 60 (23.8%) | 47 (78%) | 13 (22%) |
| Hybrid | 32 (12.7%) | 28 (87.5%) | 4 (12.5%) |
| Reverse hybrid | 21 (8.3%) | 17 (80.9%) | 3 (19.1%) |
| Unknown | 2 (0.7%) | 0 | 2 (100%) |
| **Replaced component** | | | |
| Femoral and acetabular | 150 | 128 (85.3%) | 22 (14.6%) |
| Acetabular | 73 | 45 (61.6%) | 28 (38%) |
| Femoral | 21 | 17 (85.8%) | 3 (14.2%) |
| Head and Liner | 5 | 5 | 0 |
| Unknown | 2 | 0 | 2 (100%) |
| **Implant surface** | | | |
| Cobalt-Chromium (CoCr)-Polyethylene | 117 (50%) | 76 (66%) | 39 (33.9%) |
| Zirconium-Polyethylene (ZoP) | 20 (7.9%) | 18 (90%) | 2 (10%) |
| Oxinium-Polyethylene (OxoP) | 50 (19.8%) | 48 (96%) | 2 (4%) |
| Ceramic-Polyethylene (CoP) | 11 (4.9%) | 11 (100%) | 0 |
| Ceramic-Ceramic (CoC) | 54 (21.4%) | 43 (79.6%) | 11 (20%) |
| Metal-Metal | 3 (1.1%) | 2 (67%) | 1 (33%) |

synchronous revision of the femoral and acetabular components were performed was the fully porous-coated cementless cylindrical stems, which provided a distal attachment. In terms of the surface bearings of the implants, the metal-polyethylene group was the one that underwent the highest number of re-revision surgeries. The rates of repeat revision surgeries in other surface bearing groups were comparable.

When the second revision surgery was taken as the final time point for assessing the survival rate after the index revision surgery, the cumulative survival rate 10 years after the first revision surgery was found 70.8% (95% CI: 65.3% to 77.8%) (Figure 1). The cumulative survival rate after 10 years was 76.5% (95% CI: 61.5% to 75.6%) in cases with aseptic loosening, 43% (95% CI: 38.5% to 65.6%) in cases with instability, 68% (95% CI: 57.5% to 72.5%) in cases with infection and 66% (95% CI: 53.5% to 69.6%) in cases with polyethylene wear.

## Discussion

The anticipated increase in the number of THA revision surgeries, expected to reach as high as the number of primary revision surgeries, with the increasing number of primary THAs is a major problem for orthopedic surgeons (1,7,16,17). Investigating and understanding the reasons for re-revision surgeries following the index THA revision surgery will increase the rate of success in re-revision surgeries. In our study designed to establish the abovementioned factors, the most common reasons which led to repeat revision surgeries in order of frequency were aseptic loosening, infection and instability, polyethylene wear, and osteolysis and periprosthetic fractures secondary to the wear.

Infection and instability were seen within two years of the index revision surgery, as it was the case in primary THAs, and aseptic loosening was observed after a mean period of five years after the index revision surgery. In concordance with the findings from

the literature, aseptic loosening and instability were the primary factors that necessitated a re-revision surgery following primary and revision THAs. In our patient series, aseptic loosening was mostly seen in patients who underwent the revision of the acetabular components, a finding in contrast to other studies that reported the highest prevalence of aseptic loosening with femoral components (11,18,19). However, the use of fully porous-coated femoral stems in repeat revision surgeries with femoral components was in concordance with the practices reported in the literature (11,20).

The surgical technique and the type of fixation assessed in our study were not significant factors in necessitating a repeat revision surgery following the revision THA. The need for a repeat revision surgery was significantly higher only in the isolated acetabular component revisions in comparison to other component revisions. There was no statistically significant difference between the rates of repeat revision surgeries among the patients over 65 years of age and under 65 years of age. However, it has been reported that the number of revision THAs increases significantly between 45 and 65 years of age whereas revisions due to dislocations decrease on the contrary (19). Furthermore, obesity has been shown to have a great impact on the success of THA revision surgery in another study (21).

### Study Limitations

The retrospective design of our study, lack of randomization and a control group are some of the limitations of our study. The absence of the demographic data of the patients (BMI, ASA scores) and the classification of the acetabular and femoral defects can be considered as the other limitations. In addition, patients with radiological loosening and/or those scheduled to undergo a second revision were not included in the survival analysis. However, performance of the surgeries by a single senior surgeon with a vast professional experience provides standardization of the surgical technique and clinical follow-up.

## Conclusion

In this study, we evaluated the factors that had an impact on the survival rate following the index rTHA in terms of surgical technique, age and the component type in a mid-term follow-up of the patients in our series. The leading factor which required a re-revision surgery following the index THA revision surgery was aseptic loosening, followed by instability. Cases who underwent isolated acetabular component revision surgery comprised the group with the highest rate of re-revision surgery. The cumulative survival rate 10 years after the first revision surgery was found 70.8% (95% CI: 65.3% to 77.8%).

### Ethics

**Ethics Committee Approval:** ATADEK-2018-4/36

**Informed Consent:** Retrospective study.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Concept: G.D., Design: G.D., V.E.O., Data Collection or Processing: I.R.T., Analysis or Interpretation: G.D., V.E.O., K.K., Literature Search: G.D., K.K., Writing: G.D., K.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# Cytogenetic Studies of Chromosome Abnormalities in Thyroid Cancer Patients Before and After Iodine-131 Treatment

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## ABSTRACT

**Objective:** Thyroid cancer is among the most common endocrine malignancies. Initial management consists surgery and ablation. I-131,which is used to damage surgically unremoved thyroid tissue or treat metastases, is a source for ionizing radiation (IR), also generates potential mutagenic and carcinogenic effects.The purpose of research is to investigate the damage on chromosomes at the I-131 treatment of thyroid cancer patients exposed to the same dose of genotoxic agent.

**Methods:** Peripheral blood of 20 patients and 20 healthy control individuals were used in study. Materials were collected from patients before the I-131 treatment and three times on the 15$^{th}$ day, the first and second month after the I-131 treatment. All chromosome abnormalities, spontaneous chromosomal breakages, and sister chromatid exchange (SCE) were analyzed by cytogenetic methods in the samples.

**Results:** The results obtained from peripheral blood of patiens who received ablation therapy revealed no significant increase for chromosome breakages, chromosome abnormalities, and SCE. Karyotyped metaphase spreads showed none of the structural and numerical chromosome abnormalities.The results of chromosome breakages and SCE have been statistically assessed by Aspin Welch test and did not differ from the control group.The t values of this variability were found to be -1.001,-1.654, respectively, and $p>0.05$.

**Conclusion:** Our results demonstrate that applied therapeutic clinical dose does not show cytogenetic abnormalities risks of the early period in patients. I-131 treatment is a good option for the treatment of thyroid cancer,but also we recommend that physicians should perform long term follow-up examinations for IR exposed patients and prevent possible complications.

**Keywords:** Thyroid neoplasms, cytogenetics, ionizing radiation, chromosome aberrations

## Introduction

Thyroid cancer is the most common type of cancer among malignant tumors of the endocrine system and accounts for 1-2% of all cancers (1). Etiological factor could not identified in most of the patients, while radiation exposure to the neck region in childhood has been clearly shown as the only factor causing thyroid cancer. In the past the relationship between thyroid cancer and radiation was defined and in subsequent studies, it was demonstrated that radiation exposure to the neck region increases the risk of cancer (2-4).

In the treatment of the disease surgery (total or subtotal thyroidectomy) and in addition, in good differentiated thyroid cancers acoording to risk groups, iodine-131 (I-131) ablation/ treatment is applied. I-131 is used to destroy residue thyroid or tumor tissue or metastatic tumor tissue after surgery (5). I-131 is applied successfully in treatment due to its high affinity to the

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thyroid gland. I-131 is a radioisotope with 8-day half-life which emits beta particles and gamma rays and it is used in treatment of differentiated thyroid cancer as well as in thyroid tissue imaging and in treatment of hyperthyroidism. The basis of I-131 treatment is to establish a cytotoxic effect by ensuring I-131 involvement in the target tissue (6-8). I-131 leads to ionizing radiation (IR) and has the potential to produce mutagenic and carcinogenic effects in the genetic structure of cells (8). Ninety percent of the effect of radiation is considered to be due to beta particles (9). Investigation of the biological damage of I-131 exposure, used for therapeutic purposes in patients with thyroid cancer, still continues to be important in terms of clinical and therapeutic approaches.

Cytogenetic analysis of cell deoxyribonucleic acid (DNA) for detection of damage at chromosome level including sister chromatid exchange (SCE), chromosome abnormality and chromosome breakage analysis are considered as *in vitro* indicators of chromosome instability (10). Chromosome instability is the development of abnormalities in chromosomes due to errors in replication, recombination, DNA repair, chromosome separation or cell cycle checkpoints. Small errors are mostly repaired by DNA repair systems, while severe errors stimulate apoptosis, leading the cell to death. The accumulation of errors in the cell is the first step of carcinogenesis, causing mutations (11).

Because the effects of low levels of IR are difficult to be shown by epidemiological studies, it is preferred to be shown using cytogenetic studies (9). In our study, we aimed to perform cytogenetic analysis to determine the damage on chromosomes caused by the I-131 treatment in patients with thyroid cancer. Peripheral blood samples were taken from the patients with thyroid cancer admitting to the nuclear medicine department of our university hospital for ablation treatment, before the treatment and 15 days, 1 month and 2 months after the treatment of 100 milicurie (mCi) I-131. Blood culture was performed and SCE, chromosome abnormalities and chromose breakage were investigated by cytogenetic methods.

## Methods

The peripheral blood samples of 20 patients who were diagnosed as having thyroid cancer, whose ages ranged between 18-72 years and who were decided to undergo I-131 ablation/treatment were used as study materials. None of the patients included in the study were exposed to IR. Blood samples were taken form the patients before the treatment and 15 days, 1 month and 2 months after the treatment. As a control group, peripheral blood of 20 healthy individuals who did not use cigarettes, alcohol and drugs, and who did not have viral disease recently were used. The study was approved by the Ethics Committee of Clinical Research of our university (no: 29619) and the study materials of the cases were obtained after the informed consent form was signed.

All chromosome abnormalities, spontaneous chromosome breakages and SCEs occurring in the samples were investigated with cytogenetic methods. Peripheral blood samples were taken into lithium heparinized tubes (BD Vacutainer® Tubes, BD diagnostics, NJ, USA) and were used for peripheral blood cultures which were administered for 72 hours. For blood culture; 20% fetal bovine serum (FBS), L-glutamine, penicillin, streptomycin and phytohemagglutinin containing RPMI 1640 (Biologic Industries, CT, USA) media was used. For the SCE method; during peripheral blood culture, 100 µL of solution of 5-Bromo-2'-deoxyuridine (5-BrdU) (Thermo Scientific, MA, USA), which is a thymine analogue, was added to the media. The tubes containing 5-BrdU were preserved in such a way that no light was emitted during and after the process. In accordance with the standard technique, spread slides were prepared from the cultured cells and were first left in the Hoechst solution and then shaken with McBouline solution and purified for SCE staining after the hypotonic shock and fixation phase. The slides were then placed in glass petri dishes and completely covered with McBouline solution and kept under UV lamb for 1 hour. The slides were then washed with buffer solutions and stained with 10% Giemsa stain (Figure 1) (12). For the analysis of chromosomal abnormalities; post-culture samples were spread to slides in accordance with conventional cytogenetic technique, aged at 90°C, and then stained (13). In the analysis, karyotyping was performed from at least 20 metaphase sites for

**Figure 1.** Microscopic imaging of sister chromatid exchange observed in metaphase plate of case 20 (SCE observed chromosomes are shown with a red arrow)

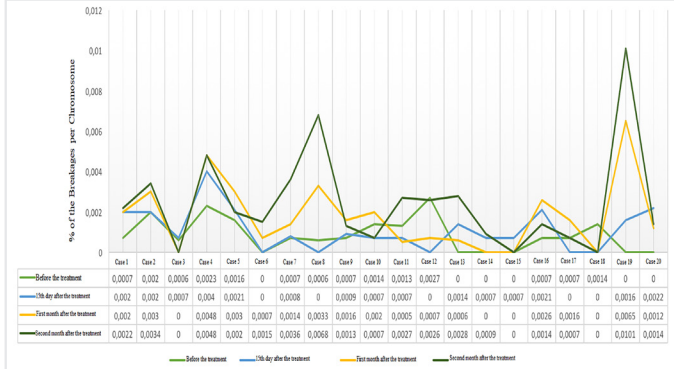


**Figure 2.** Change in chromosome breakage before and after treatment during follow-up in patients

each case with the investigated band level of at least 400 bands. The karyotyping was based on ISCN 2013 (An International System for Human Cytogenetic Nomenclature) (14). For the examination of chromosomal breakage, the preparations spread with solid staining method and dried were stained with 20% Giemsa (Merck, Darmstadt, Germany). All preparations that were ready for imaging after staining were examined with light microscopy (Nikon Eclipse E600, Nikon, Tokyo, Japan), and the fields in the preparations were scanned with the X10 objective lens and were analyzed with the X100 objective lens.

### Statistical Analysis

Statistical analysis was done using the Statistical Package for the Social Sciences Statistics Versiyon 17.0 (SPSS Inc., IL, USA).

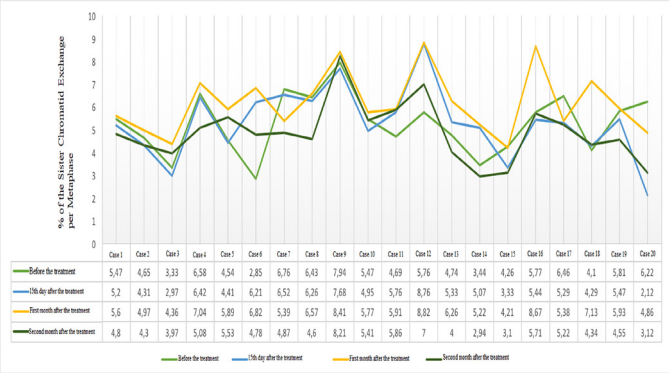


**Figure 3.** Change in sister chromatid exchange before and after treatment during follow-up in patients

**Table 1.** Demograpic data and karyotypes detected during follow-up of the patients

| | Age/ Gender | Karyotype |
|---|---|---|
| Case 1 | 43, F | 46, XX |
| Case 2 | 62, F | 46, XX |
| Case 3 | 18, F | 46, XX |
| Case 4 | 40, F | 46, XX |
| Case 5 | 72, M | 46, XY |
| Case 6 | 42, F | 46, XX |
| Case 7 | 43, F | 46, XX |
| Case 8 | 52, F | 46, XX |
| Case 9 | 44, F | 46, XX |
| Case 10 | 42, F | 46, XX |
| Case 11 | 63, F | 46, XX |
| Case 12 | 38, F | 46, XX |
| Case 13 | 41, F | 46, XX |
| Case 14 | 33, M | 46, XY |
| Case 15 | 62, M | 46, XY |
| Case 16 | 24, F | 46, XX |
| Case 17 | 42, F | 46, XX |
| Case 18 | 44, F | 46, XX |
| Case 19 | 52, F | 46, XX |
| Case 20 | 35, F | 46, XX |

The data analyzed were expressed as mean±standard error. At the end of the investigations, the chromosomal breakage rates and the results of the SCE were evaluated using the Aspin Welch test. The t values of variability of chromosome breakages and SCEs in the study group compared with the control group were -1.001 and -1.654, respectively. The p value was >0.05.

## Results

Demographic data and the karyotypes of the patients were shown in Table 1. Chromosome breakage and SCE findings before I-131 treatment and 15 days, 1 month and 2 months after I-131 treatment and changes in them during process were shown in Figure 2 and 3. Of 20 patients included in the study, the mean age was 44.6 years and 85% of the them (n=17) was female and 15% (n=3) was male. Of 17 females, 35% reported smoking 3-4 cigarettes a day and 33% of 3 males smoked one packet a day. In this study, patients who received 10 mCi I-131 treatment were evaluated and no evidence of abnormality was observed in these patients in terms of cytogenetic analysis during the follow-up. The results of cytogenetic analyses and the clinical and demographic data of the patients were evaluated together. The mean chromosome breakage percentage was 0.0009 in the samples studied before the treatment, and the percentages were 0.0012, 0.0018 and 0.0024, respectively in the samples studies 15 days, 1 month and 2 months after the treatment. Although the percentage of chromosome breakage in patients showed an increase after treatment, it was not statistically significant when compared with control group. The mean percentage before the treatment was 5.26 in the samples studied, and the mean percentages of SCE in the samples 15 days, 1 month and 2 months after the treatment were 5.29, 6.16 and 4.87, respectively. There was no difference between SCE before and after the treatment in the patients. There was no significant difference between the patients treated with I-131 and the control group in terms of SCE frequency. During the follow-up period, no chromosomal abnormality was observed in karyotyped metaphase sites in terms of numeric and structure. There was no difference between the patient and control groups in terms of chromosome breakage and SCE.

## Discussion

As a result of the gradual accumulation of many mutations in a normal cell, instability of the genome occurs. IR; by interacting with intracellular macromolecules and changing cellular metabolism; is a powerful stimulant of instability in cell DNA, indirectly causing oxidation of free radicals and directly causing damages such as base changes and DNA single/double chain breaks in cell (15,16). The examination of chromosomal breakages and SCE, which are indicators of chromosome instability, by various methods are the most effective methods used to determine the damage to cells (10). The loss of genomic stability resulting from gene rearrangement, leading to the formation of different types of cancer and at the same time, increasing the risk of secondary cancers seen after the treatment of cancer is one of the most important pathological stages (11).

I-131 has been used in medical field for diagnosis and treatment since 1942 (17). The benefit of I-131, especially for thyroid cancer patients, is undisputable. Also, it is easy and inexpensive to apply this method which makes it even more convenient. However, during I-131 degradation, radiated gamma rays and beta particles may be harmful to normal cells and tissues due to absorption.

In previous studies, blood samples of patients with thyroid cancer treated with I-131 were reported to show temporary leukopenia, anemia and thrombocytopenia, and persistent cytopenia was observed in patients exposed to high doses of I-131 (18-20). Also, a risk of cancer in patients who exposed to low dose IR for diagnosis and treatment was reported and development of acute and chronic myeloid leukemia was reported in patients who were exposed to IR for a long time. In patients receiving high doses (≥800 mCi) IR therapy, development of leukemia have been reported in various studies (21,22). The important chromosomal abnormalities in acute and chronic myeloid leukemia including t(8;21), t(15;17), inv(16), t(9;11), t(6;9) and inv(3) were not observed in this study.

In this study, we aimed to investigate the effect of 100 mCi I-131 treatment on the treatment of patients with thyroid cancer who were exposed to the same dose of genotoxic agents with cytogenetic tests. Since patients receiving treatment were exposed to harmful effects of gamma rays and beta rays directly affecting tissue, analyses were made to determine chromosome damage in peripheral blood before and after treatment. As in our study, the reasons for choosing peripheral blood as material to examine the effects of the IR are that lymphocytes are highly sensitive to the IR, circulate through the entire body and are numerous, it easy to obtain them and short-term culture techniques are easy to apply (9,11).

When the test results obtained from peripheral blood of patients receiving ablation treatment were evaluated, no significant increase was observed in chromosomal breakage, chromosomal abnormalities and SCE. Our findings suggest that this therapeutic dose did not involve the risk of causing cytogenetic abnormalities that might be observed in the early period. On the other hand, since our investigations were conducted in the metaphase phase of the second cell division in peripheral blood culture, it should be considered that IR-related chromosomal abnormalities in the first division might be overlooked. It should be taken into account that the cells that have been damaged after exposure to IR can not divide in cultures, the rate of division may slow down, or the damaged cells may be completely destroyed. All these may limit the actual damage rates of the analyzed cells. Parida et al. investigated chromosome damage in peripheral blood of 74 hyperthyroid patients before and 3 months after I-131 treatment with micronucleus method and did not find statistical significance similar to that of our study. They suggest that cytogenetic damage caused by low dose therapy may be temporary and reversible (23).

Smoking habits, age and gender factors were not found to affect spontaneous chromosomal damage in the cases we examined. Milosevic-Djordjevic et al. and Vrndic et al. compared the frequency of chromosomal instability between the groups in terms of age, sex, smoking habits and the type of cancer, and found no difference (24,25). When the results are evaluated together, it is thought that the chromosome damage in the treated patients with thyroid cancer is related to the I-131 accumulation and not related to the other factors examined.

Another issue to consider is that the sensitivity of the tissues to radiation varies. Several studies have reported that chromosome instability varies according to the amount and the repeat number of radioactive iodine taken. Also, it was shown in studies that genetic predisposition, occupational hazard, and life style of individuals exposed to IR can also affect the damage to the cell (26,27). Seo et al. (28) showed that incidence of leukemia increases with ≥100 mCi IR. Teng et al. suggested that ≥150 mCi IR increases the risk of secondary cancer development (28,29).

Although the modified effect of IR on chromosome and DNA structure is well known, the results are still in mystery. Various studies on atomic bomb victims have shown that increased chromosome abnormalities in affected individuals continue for at least twenty years (30-32). Although there are long-term studies on chromosomal abnormalities of individuals exposed to high doses of radiation, long-term studies on cytogenetic effects of low doses of IR exposure for diagnosis and treatment purposes are limited. Livingston et al. (11) followed up cytogenetic findings in a patients who underwent total thyroidectomy and ablation treatment, at certain intervals by taking peripheral blood for 20 years. The results showed that cytogenetic effects persisted after treatment due to I-131 exposure. The number of micronucleus was 10 times higher 5 years after treatment than the number of micronucleus before treatment (11). In their study published in 2017, they examined the same patient's peripheral blood sample by multicolor fluorescent in situ hybridization and found a higher proportion of reciprocal and non-reciprocal translocations compared with their previous study (33). These findings show the persistent effects of IR. The cause of the abnormalities in the case examined was explained by the long-lasting T-lymphocytes or hematopoietic stem cells being affected by the IR.

**Study Limitations**

Although our study supports the idea that the effects of IR exposure can be detected, it does not provide sufficient information on long-term results due to the evaluation of a limited number of cases and the short follow-up period.

## Conclusion

In our study, DNA damage was analyzed at chromosome level, and there was no statistically significant cytogenetic finding in peripheral blood examinations in the short-term. We recommend that the use of I-131 is a good option in the treatment of thyroid cancer, but when patients are treated with IR, they should be monitored for a long time and the necessary controls should be performed by the physician to prevent possible complications. Cytogenetic methods and advanced analysis can be used to determine potential DNA damage. Based on the data reported

earlier in the literature, we believe that the effects of IR on the cell should be verified with long-term follow-up in larger number of case-control groups.

**Ethics**

**Ethics Committee Approval:** İstanbul University Cerrahpaşa Faculty of Medicine, Ethics Committee of Clinical Research/29619.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer Review:** Externally peer-reviewed.

**Authorship Contributions**

Concept: S.Ö., S.A., M.D., A.D., Design: S.Ö., S.A., M.D., A.D., Data Collection or Processing: S.Ö., S.A., Analysis or Interpretation: S.Ö., S.A., M.D., A.D., Literature Search: S.Ö., S.A., Writing: S.Ö., S.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# Does Resection of Heterotopic Ossification of the Elbow Result in Satisfactory Functional Outcomes?

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## ABSTRACT

**Objective:** Heterotopic ossification (HO) is a common cause of elbow stiffness following surgical treatment of elbow trauma. In this study, our aim was to evaluate the mid-term functional outcomes of open surgical procedures for HO.

**Methods:** In this retrospective study approved by the institutional review board (IRB), all patients who were diagnosed as having stiff elbow due to HO and underwent surgical resection at a single institution from 2006 to 2013 were included. Intrinsic (inside the joint) pathologies were excluded. Range of motion (ROM) in sagittal and coronal planes, complications, functional scores such as Quick Disabilities of the Arm, Shoulder and Hand Score (Q-DASH), and Mayo Elbow Performance Score (MEPS) were evaluated before and after surgery.

**Results:** There were 19 patients (16 males, 3 females) with a mean age of 38.6 (range 15-69) years. At an average follow-up of 36±8 months, the mean flexion-extension arc was improved from 27.4° to 99.2° ($p<0.001$) and mean supination-pronation arc was improved from 48.9° to 102.3° ($p<0.001$). The mean Q-DASH score was decreased from 68.2 to 17.1 ($p<0.001$) and mean MEPS was improved from 37.5 to 85.6 ($p<0.001$).

**Conclusion:** Excision of heterotopic bone and releasing contracted tissues around elbow can provide a substantial increase in range of motion and improvement in clinical scores.

**Keywords:** Heterotopic ossification, elbow stiffness, elbow trauma, open surgical release

## Introduction

Heterotopic bone formation plays a major role in the extrinsic causes of stiff elbow and has been depicted to arise in several situations including traumatic (intra-articular or extra-articular fractures, dislocations, post-surgery), neurogenic (head or spinal trauma) and congenital (fibrodysplasia ossificans progressiva) conditions andsevere burns (1-5). The pathophysiology of heterotopic ossification (HO) still remains unclear and the condition is likely to be multifactorial (4,6,7). Histologically, HO is the formation of mature lamellar bone in extraosseous soft tissues (8). The energy of trauma seems to be the major determinant of HO formation. Low energy traumas like simple elbow dislocation cause HO formation in 3% of cases and the prevelance increases up to 45% with high energy distal humerus fractures (5,6,9).

HO prophylaxis is controversial and could only be effective by understanding patients' risk factors, utilizing less invasive surgical techniques and implementing perioperative non-steroidal anti-inflammatory drugs (NSAIDs). Low dose irradiation can be preferred in selected cases (6,7,10). Extensive HO can result in clinically significant contractures or complete ankylosis of the elbow (8). If elbow motion does not progress with non-operative

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treatment (physical therapy and splinting), removal of heterotopic ossifications should be taken into account at an appropriate time (4,11). Although surgery is technically challenging, recent studies revealed significant improvements in range of motion after excision of the heterotopic bone with early assistive physical therapy and splinting (11-14).

We, therefore, aimed to evaluate the mid-term functional outcomes of open surgical procedures for HO, regarding the resection of heterotopic bone formation and the release of contracted tissues around the elbow.

## Methods

This is an institutional review board (IRB) approved retrospective study in which all patients who were diagnosed as having stiff elbow due to HO and underwent surgical resection at a single institution from 2006 to 2013 were included. Patients with the diagnoses of intrinsic type stiffness such as osteochondritis dissecans, osteoarthritis, inflammatory arthritis, intra-articular fractures, synovial chondromatosis were excluded. Patients with severe elbow instability or deformity, and with skeletal immaturity were also excluded.

Main indication for surgical excision was clinically symptomatic or disabling HO of the elbow. The patients' demographics, etiology of HO, surgical approach and treatment modality, range of motion (ROM) in sagittal and coronal planes, type of splint used, complications, functional scores such as Quick Disabilities of the Arm, Shoulder and Hand Score (Q-DASH), and Mayo Elbow Performance Score (MEPS) were evaluated. All clinical and functional evaluations were done at two time points; before the operation and at the latest follow-up. All the joint motions were measured utilizing a goniometer. Heterotopic bone formation was classified by the Hastings and Graham classification preoperatively on X-rays (15) (Table 1). Three dimensional computerized tomography (CT) was obtained in selected patients.

### Surgical Procedure

The preoperative evaluation of the initial pathology and the location of the HO were the major determinants of the preferred surgical approach (Figure 1). Each patient was placed in the supine position under general anesthesia. An inter-scalene catheter was placed for pain relief and immediate initiation of physical therapy postoperatively. A tourniquet was applied and the arm was draped free. Firstly, a lateral incision was performed and the lateral extensors (extansor carpi radialis longus and bracioradialis muscles) were first subperiosteally elevated, and the anterior capsule of the elbow joint was exposed (Figure 2). The capsule was then elevated from the lower end of the humerus, exposing the radiocapitellar joint. Debridement of the joint was performed in combination with anterior capsulectomy. If the radial head was hypertrophied or deformed, it was excised to improve the range of motion. A retractor was used during the entire procedure to protect the neurovascular bundle. A subperiosteal dissection provides the exposure of the posterior humerus and release of the capsular adhesions (Figure 3). By the removal of the fibrous tissues from the olecranon fossa, further extension of the elbow can be achieved. Sometimes the tip of the olecranon process was osteotomized and removed. Then,

**Table 1.** The Hastings and Graham classification

| Radiographic and Anatomic Findings | Class |
|---|---|
| Heterotopic ossification without functional limitation | I |
| Partial limitation of (flexion or extension) and/or (pronation or supination) | II |
| Ankylosis with total limitation of (flexion or extension) and/or (pronation or supination) | III |
| Sub-classification of Class II and Class III | Subclass |
| Limitation in flexion/extension plane | A |
| Limitation in supination/pronation plane | B |
| Limitation in both planes | C |

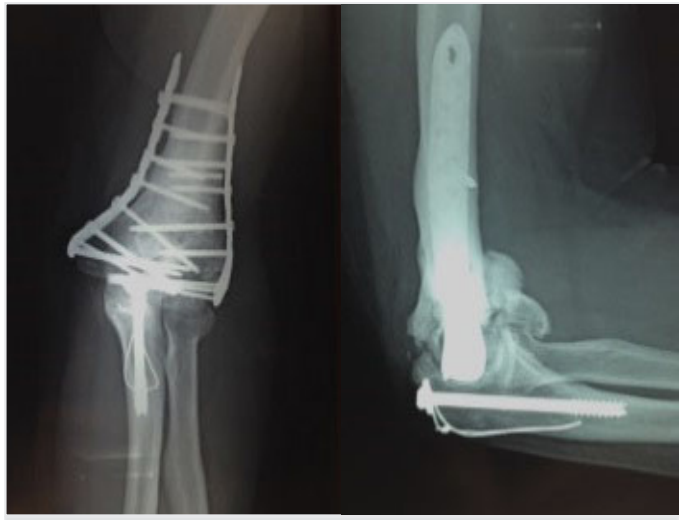

**Figure 1.** Previously operated distal humerus fracture with Hastings and Graham class 2A heterotopic ossification

**Figure 2.** Lateral incision, subperiosteal elevation of lateral extensors (extansor carpi radialis longus and bracioradialis muscles) and dissection of the anterior capsule

medial incision was performed and ulnar nerve was exposed and protected (Figure 4). Next, the flexor and pronator muscle origins were subperiosteally detached and the anterior capsule was stripped with the periosteum from the distal end of the humerus. Further inspection of the humero-ulnar joint was done to find any bony blocks. Any extra-articular ossified masses or osteophytes were excised. The collateral ligaments were released but preserved in selected cases. To avoid bony avulsion, release of the collateral ligament may be necessary. After the soft tissue was completely released, the remaining bony blocks were excised. A V-Y plasty of triceps can be helpful to gain more flexion if flexion more than 90° is not possible due to tightness of the tendon out in this step. Finally, flexion and extension of the elbow were checked passively to evaluate the gain in movement (Figure 5). A meticulous bleeding control should be carried out after the pneumatic tourniquet is released. Subcutaneous ulnar nerve transposition was performed if necessary. After surgery, the wound was closed with suction drainage. The arm was placed in a long arm splint in full extension (Figure 6). A single posterior approach can also be used in particular patients, especially in the presence of a posterior bar between the olecranon and distal humerus. Another case, who underwent lateral and medial release with heterotopic ossification removal is illustrated in Figure 7.

**Figure 3.** Elevation of the capsule from the lower end of humerus, radiocapitellar joint debridement in combination with anterior capsulectomy. Neurovascular bundle protection by a retractor. Dissection of the posterior aspect of the humerus to free the capsular attachment. Excision of fibrous tissues and loosing bodies from the olecranon fossa

**Figure 4.** Medial incision, ulnar nerve dissection and protection. Subperiosteal elevation of the flexor and pronator muscle origins and anterior capsule. Further inspection of the humero-ulnar joint to find any bony block, loosing body or fibrous tissue

**Figure 5.** Final inspection of the flexion and extension of the elbow passively

**Figure 6.** Placement of the arm in a long arm splint in full extension


**Figure 7 A-B.** Initial distal humerus T type fracture

**Post-operative Regimen**

Post-operative physiotherapy and medication were chosen for each case upon the pre-operative and peri-operative evaluation of the pathology. Pain relief was obtained by using inter-scalene catheter and intravenous ports. Drains were discontinued and early active assistive motion and continuous passive motion (CPM) exercises for flexion-extension and supination-pronation within tolerable limits of pain were initiated one day after surgery under the supervision of the physiotherapist. Nighttime splinting was continued for the deficient in motion for 3 weeks.


**Figure 7** C-D. Intra-operative X-ray of distal humerus internal fixation as well as olecranon osteotomy

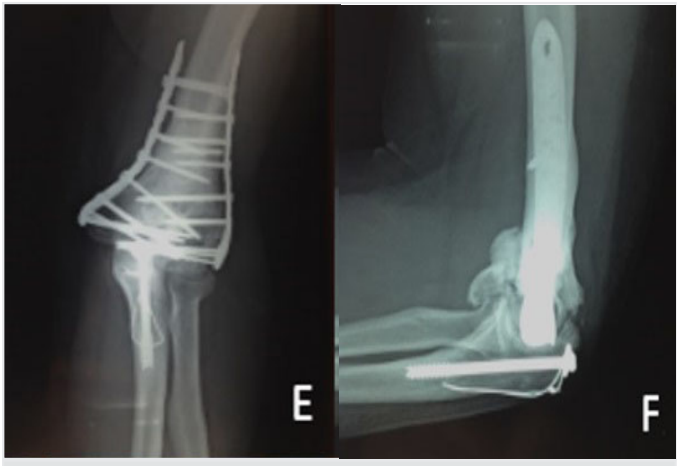


**Figure 7 E-F.** Two year post-operative x-rays showed severe heterotopic ossification formation especially around anterior elbow capsule


**Figure 7 G-H.** Limited elbow range of motion due to heterotopic ossification and joint capsule contracture

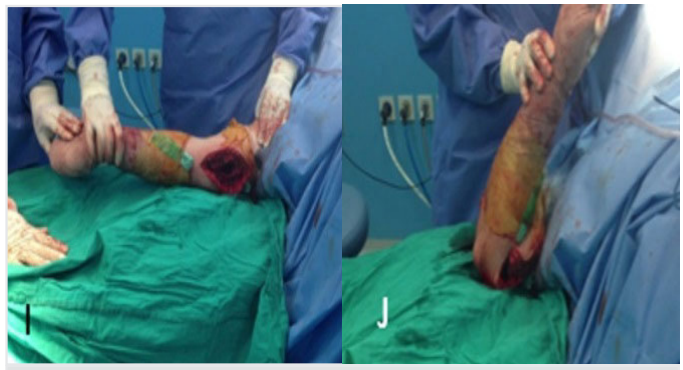


**Figure 7 I-J.** Increased elbow range of motion after medial and lateral release, and removal of heterotopic tissues

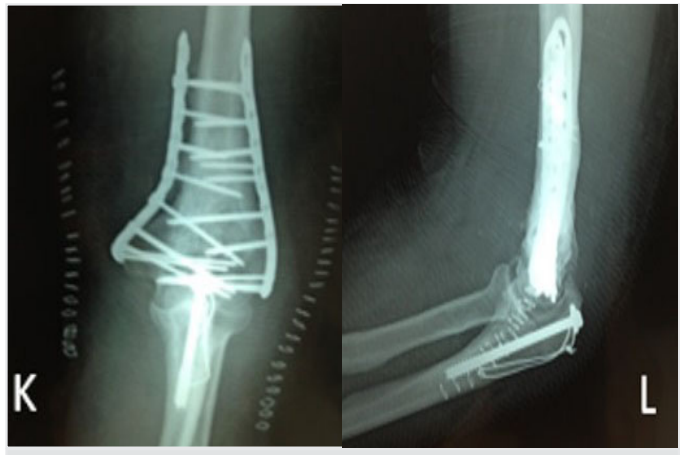


**Figure 7 K-L.** Early post-operative x-ray showed sufficient removal of heterotopic bones

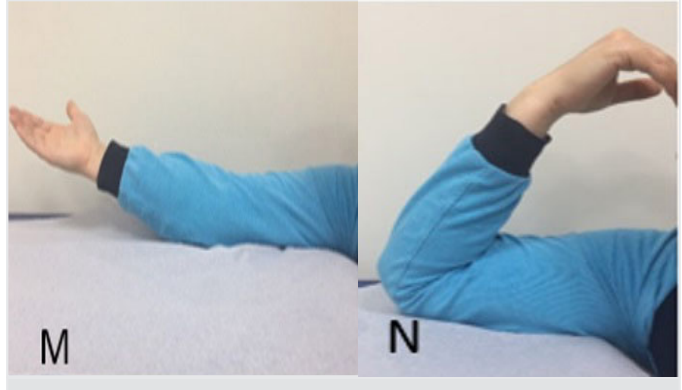


**Figure 7 M-N.** Satisfactory elbow range of motion noticed one year after removal of heterotopic tissues and capsular release

All the patients received oral NSAIDs (indomethacine 75 mg per day) for postoperative pain relief and to prevent the recurrence of HO. The catheters were discontinued 12 hours before discharge. A single low dose (700 cGy) of radiation therapy (RT) was applied in selected cases on the day of surgery. The physical therapy and medication regimen were continued for at least 6 weeks postoperatively. Patients were followed closely for the recurrence of stiffness.

### Statistical Analyses

Statistical analyses were performed using student t-test for parametric data, the Mann Whitney U (Wilcoxon rank test) and Kruskal Wallis test for non-parametric data as appropriate. SPSS Version 20.0 (SPSS Inc, Chicago, IL, USA) was used; a p value less than 0.05 was considered significant.

## Results

There were 19 patients (16 males, 3 females) with a mean age of 38.6 (range 15-69) years at the time of surgery. The etiology of the HO formation was operated extra-articular distal humerus fracture in 8 patients, complex elbow dislocation in 4 patients, isolated head trauma and long term intensive care administration in 4 patients, repetitive low energy trauma in 3 patients. The pre-operative mean flexion-extension arc was 27.4° and supination-pronation arc was 48.9°. According to the Hastings and Graham classification; heterotopic bone formation was 2A in 5 patients, 2C in 6 patients and 3C in 8 patients. Pre-operative mean Q-DASH score was 68.2 and MEPS was 37.5. Radial head resection was performed in 13 patients. Patient demographics and surgical details can be seen in Table 2.

**Table 2.** Demographic and surgical details of the patients

| | Initials | Sex | Age | Etiology | Surgical approach | The hastings and graham classification | Ulnar nerve transposition | Radial head resection |
|---|---|---|---|---|---|---|---|---|
| 1 | UK | M | 16 | Simple elbow dislocation | Combined** | Class IIA | - | - |
| 2 | MO | M | 21 | Operated distal humerus fracture | Posterior | Class IIC | + | + |
| 3 | OS | M | 23 | Head trauma and ICU* administration | Posterior | Class IIIC | + | + |
| 4 | AK | M | 30 | Simple elbow dislocation | Lateral | Class IIC | - | + |
| 5 | MBM | M | 32 | Head trauma and ICU* administration | Posterior | Class IIC | + | + |
| 6 | KS | M | 35 | Complex elbow fracturedislocation | Combined** | Class IIIC | - | + |
| 7 | HM | M | 37 | Complex elbow fracturedislocation | Combined** | Class IIC | + | + |
| 8 | NO | M | 37 | Head trauma and ICU* administration | Posterior | Class IIIC | + | + |
| 9 | TD | M | 37 | Operated distal humerus fracture | Posterior | Class IIA | + | - |
| 10 | CY | M | 41 | Operated distal humerus fracture | Posterior | Class IIA | - | - |
| 11 | HY | F | 41 | Operated distal humerus fracture | Posterior | Class IIA | + | - |
| 12 | HG | M | 43 | Operated distal humerus fracture | Posterior | Class IIIC | - | + |
| 13 | DD | M | 43 | Complex elbow fracturedislocation | Combined** | Class IIC | + | + |
| 14 | TT | M | 48 | Simple elbow dislocation | Combined** | Class IIC | - | + |
| 15 | GM | F | 50 | Complex elbow fracturedislocation | Combined** | Class IIIC | + | + |
| 16 | AK | M | 69 | Operated distal humerus fracture | Posterior | Class IIA | + | - |
| 17 | TO | M | 42 | Head trauma and ICU* administration | Posterior | Class IIIC | + | + |
| 18 | NE | F | 36 | Operated distal humerus fracture | Posterior | Class IIIC | + | + |
| 19 | AC | M | 44 | Operated distal humerus fracture | Posterior | Class IIIC | + | + |

*Intensive Care Unit , **Lateral and Medial approach

**Table 3.** Comparison of pre- and post- operative mean range of motion of elbow lumn5

| | Pre-operative | Post-operative | Improvement | p value |
|---|---|---|---|---|
| Flexion- Extencion Arc | 27.4° | 99.2° | 71.3° | p<0.001 |
| Supination-Pronation Arc | 48.9° | 102.3° | 53.4° | p<0.001 |

**Table 4.** Comparison of pre- and post-operative average functional scores

| | Pre-operative | Post-operative | Improvement | p value |
|---|---|---|---|---|
| Q-DASH * | 68.2 | 17.1 | p<0.001 | Q-DASH * |
| MEPS ** | 37.5 | 85.6 | p<0.001 | MEPS ** |

* Quick Disabilities of the Arm, Shoulder and Hand Score, ** Mayo Elbow Performance Score

At an average follow-up of 36±8 months, significant improvement in flexion-extension arc (from 27.4° to 99; 71.3° improvement) and supination-pronation arc (from 48.9° to 102.3°; 53.4° improvement) were noted (p<0.001) (Table 3). The mean Q-DASH score was decreased from 68.2 to 17.1 and mean MEPS was improved from 37.5 to 85.6 with statistical significance (p<0.001) (Table 4). At their last follow-up, 13 patients described themselves as very satisfied, 3 patients as satisfied and 3 patients as dissatisfied.

We also compared these results with another cohort from the same institution in which all patients underwent the same type of operation in the presence of intrinsic type elbow stiffness (osteochondritis dissecans, osteoarthritis, inflammatory arthritis, intra-articular fractures, synovial chondromatosis). The improvement in ROM in both planes were statistically significantly higher if HO was caused by an extra-articular pathology (30.5° vs. 49.5°, p<0.001).

A peri-operative supracondylar humerus fracture occurred in 2 patients, which was immediately fixed by plate. Two patients were re-operated for recurrence of ectopic bone formation. No neuro-vascular injuries were noted in any patient.

## Discussion

HO is a common extrinsic cause of clinically relevant elbow stiffness, which can affect the performance of daily life activities, especially when the dominant side is affected (1-5). Although the exact cause and mechanism leading to HO formation is not clearly identified, the prevalence in the literature has been reported as 3%-45% after any degree of elbow injury (4-7,9). The main aim of this study was to evaluate a single institution's mid-term functional outcomes of open surgical procedures for HO, including the resection of heterotopic bone formation and the release of contracted tissues around the elbow.

According to Abrams et al. (6) patient-related factors, such as gender and age were not associated with HO. Salazar et al. (8) showed that hypertension, high body mass index, and absence of intraoperative anterior ulnar nerve transposition may negatively affect the total flexion-extension arc. Some recent studies showed that longer time to mobilization after elbow injury may be a significant cause of clinically relevant HO (5,16). Previous studies have also shown that the severity of elbow dislocation correlates with restrictive HO (9,17). The interval from trauma to surgery and the number of surgical procedures within 4 weeks of injury were found to be related with restrictive HO (5). On the other hand, the muscle interval used to approach the elbow during surgery may have an influence to the development of HO (5).

Wiggers et al. (5) reported that although the radiographic evidence of HO could be found in up to 33% of patients who had operative treatment of an elbow fracture after at least 4 months following injury, only 10% of patients had restricted elbow motion. When restriction starts, the timing of surgery is controversial. Charalambous and Morrey (18) recommend surgery for elbow stiffness within a minimum 6 months after injury. Koh et al. (12) showed that surgical results were poor when surgery was performed 19 months after the initial injury. In our study, open release procedure was performed at a mean time of 13 months after first identification of heterotopic ossification formation.

Before the decision of surgical release, both the patient and surgeon should be well aware of the expected benefits and complications after resection of HO. The classic columnar procedure described by Mansat and Morrey (19), is safe and easy procedure to perform and is reliable for an extrinsic elbow stiffness. However, with this lateral approach, it is difficult to release a medial component and ulnar nerve. Capsular release alone cannot resolve the limitation of motion caused by heterotopic bone formation (20). Some authors recommend a posterior approach rather than an anterior, medial, or lateral approach in cases of severe contracture (20). We utilized a posterior approach with using a posterior ulno-humeral bar in cases without severe anterior heterotopic bone formation. We strongly recommend limited dissection in order to prevent hematoma and recurrence of HO regardless of the surgical approach used.

For activities of daily living, an elbow range of motion of 30°-130° flexion/extension and 50° pronation to 50° supination is necessary (21). Although restoration of full elbow motion is

unlikely, improved scores and increased ROM after surgery have been reported (1-3,8,15,22-24). In one of the largest retrospective studies of 39 patients (43 elbows) in whom HO was treated with excision, overall mean flexion-extension arc of motion was improved from 35° to 103° at an average of 13 months follow-up (8). Similarly, in our series, mean flexion-extension arc was improved from 27.4° to 99.2° and mean supination-pronation arc was improved from 48.9° to 102.3°at an average of 36±8 months.

Although patients with an ankylosis gain more increase in motion and in functional scores than those who have limited motion, restoration of normal motion is unlikely (22). The reason for this might be a less extensive surgical approach performed for the patients with partial restriction of motion as compared to patients with ankylosis of the elbow.

Complications are recurrence of HO, fracture during or after operation, infection and wound complications, while nerve palsies are reported as slightly high in the literature (1,8,13). Similarly, we had an overall complication rate of 21% including intra-operative fracture (two cases) and recurrence of heterotopic bone formation (two cases). Recurrence of HO is common after excision. The timing of surgery has always been controversial. Classically, surgery is recommended after maturation of the heterotopic bone (3,4).

### Study Limitations

This study has some limitations. The retrospective nature of the study is an obvious limitation. Large prospective series are unlikely to be published because elbow contractures secondary to heterotopic ossification is not a common situation. In addition, the observers in this study were not blinded and a simple goniometer was used for ROM measurements.

## Conclusion

Open surgical release of contracted tissues around the elbow and resection of heterotopic bone formation is a preferable procedure for treating stiff elbow. However, well programmed surgical technique and rehabilitation are required. Excision of heterotopic bone and releasing the contracted elbow capsule can contribute to a substantial rise in scope of motion and improvement in clinical scores. Surgeons must take into consideration the risk of fracture and neurovascular injury during the operation and patients should be followed up closely for the recurrence of stiffness.

### Ethics

**Ethics Committee Approval:** This IRB approved study includes procedures in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration.

**Informed Consent:** In this study, informed consent was obtained from all participants.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Concept: M.K., A.C.A., Design: M.K., H.D., Data Collection or Processing: M.K., Analysis or Interpretation: M.K., Y.S., Literature Search: A.E., Writing: Y.S.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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Original Article

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# Nutritional Assessment and Physical Activity of the Departments for Nutrition&Dietetics and Nursing Students at a Foundation University

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## ABSTRACT

**Objective:** Nutritional assessment and physical activity habits of nutrition & dietetics students (experimental group) who took a course of The Principles of Nutrition and nursing students (control group) who did not take this course were compared.

**Methods:** Students were completed the questionnaire included sociodemographic data, 24-h recall with food consumption and food frequency tests, and physical activity habits. My plate replicates were also used as visual material. Body mass index was calculated by bioelectrical body analyzer. Data were analyzed using the Nutrition Information System and SPSS software programs.

**Results:** Almost half of students were participated in this study (49.5%). Intake of total energy and nutrients was similar and poor in both groups. It was found that overweight rate and waist circumference measurement of Nursing students are higher and their physical activity are lower than Nutrition and Dietetics students ($p<0.001$).

**Conclusion:** Along with the emergence of inadequate nutrition of the students of both departments, more nutrition education and healthy menu planning are also required for the students of the department of nutrition and dietetics.

**Keywords:** Eating habits, university students, body mass index, nutrients, physical activity

## Introduction

The World Health Organization (WHO) defines health as having a complete physical, mental and social well-being in an individual (1). The two most important determinants of health are adequate and balanced nutrition and regular physical activity (2).

The University period, which is the transition from adolescence to adulthood, is stressful for many students and is a period in which students experience an increase in inadequate and unbalanced eating habits (3,4). In this period, in order to prevent health problems that may arise in older ages, it is necessary to define the nutritional status of individuals, to determine the reasons and to monitor and evaluate the nutritional status of individuals in order to find solutions. Determination of nutritional status is possible with many methods (2). Most frequently used methods are; patient's anamnesis, anthropometric measurements (waist circumference, height-weight account, etc.), 24-hour food consumption scale, food consumption frequency and recall with scale, diet history, biochemical tests, etc. However, using a combination of several of these methods is realistic to determine the nutritional status. For example, they validate the information obtained when evaluated together with the frequency of food consumption and the 24-hour food consumption and gives information about the food recall with pattern (2). Food

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consumption frequency and consumption of food or groups of nutrients can be determined as daily, weekly or monthly and as quantity when requested.

Regular physical activity prevents the formation of obesity and also of many diseases (5). According to a study conducted in the Faculty of Sports Sciences in our country, activity levels of faculty students were higher than the other faculty students (6). According to a Swedish study in 2005, it was found that gender did not make a difference in determining the level of physical activity (7). Another study found that 51% of university students had inadequate physical activity (8).

In the UK, a study showed that university students tended to eat unhealthy foods that cause excessive weight and obesity to become widespread (9). In Germany, it was found that university students ate unhealthy food as a result of lack of time and lack of healthy food alternatives in canteens (10).

It may be useful to investigate the causes of changes in eating habits by qualitative research and to determine the nutritional status of the students (9). Studies in this field in our country were carried out with the students staying in the dormitory or with the students who were training at different faculties of the university such as education and medical faculties (11-18). However, no comparisons between faculties were made in any of these studies, and only comparisons between genders were reported. "Principles of Nutrition" is one of the basic courses given in most of the departments of the faculty of health sciences. The aim of this study was to evaluate the nutritional status and physical activity habits of the second, third and fourth grade students of the department of nutrition and dietetics who took this course and the second and third grade students of nursing department who did not take this course. In addition, the results of these two groups were compared.

## Methods

This study was conducted in accordance with the guidelines in the Helsinki Declaration, and performing procedures involving human subjects/participants was approved by the Ethical Committee on Non-Invasive Research at Bezmialem Vakif University (No: 50222451-050.05.04-9/89). Participants were informed about the purpose of the research and informed consent was taken from participants.

The questionnaire used in the study was modified from the study of Gunes-Bayir et al. in 2015 (19). Filling out the questionnaire was done face-to-face with the participants and using visual materials (my plate food replicas; Nasco, Wisconsin; USA). The questionnaire was applied to students of the (experimental group) who took "Department of Nutrition and Dietetics (DND)" course and to students of the departmet of nursing (DN) (control group) who did not take this course in Bezmialem Vakif University Faculty of Health Sciences (İstanbul, Turkey).

In the first part of the questionnaire, socio-demographic data of the students were taken. In the second part of the study, anthropometric measurements of the students including height which was measured with wall-mount measuring tape (ADE; Tarti medical, İstanbul, Turkey), weight which was measured with bioelectric impedance analyzer (Tanita MC 780; Tarti medical, İstanbul, Turkey) and waist circumference which was measured with tape were recorded. BMI values were calculated by weight (kg)/height ($m^2$) formula and classified according to the WHO's BMI standards (20). In this classification, those less than 18.5 kg/$m^2$ were accepted as weak, those between 18.5-24.9 kg/$m^2$ were normal weight, those between 25-29 kg/$m^2$ were overweight and those 30.0-34.9 kg/$m^2$ were accepted as obese. In the third part of the survey, questions about eating habits such as main dishes and snacks, the frequency of eating outside, daily water consumption, smoking and alcohol habits, etc. Were asked. In the fourth part, there were 24-hour recall with food consumption and food consumption frequency and quantity scales (2). The fifth part included questions that would determine the physical activity status of the students.

### Statistical Analysis

Data obtained from the frequency and quantity of food consumption and the 24-hour recall with food consumption scales were analyzed using the Nutrition Data System (BeBIS; Pacific Electricity, Electronics and Environmental Technology Products Industry and Commerce Ltd., Istanbul, Turkey). Statistical analyses of the data were performed using SPSS 16.0 software program. Qualitative data were calculated with mean and standard deviation. The student-t test was used to compare the groups. Results were considered statistically significant when p value was equal to or lower than 0.05.

## Results

About half of the students (n=171) in the Bezmialem Vakıf University, Faculty of Health Sciences participated in the study (49.5%; n=119). Forty-eight of these students were from and 71 were from. Of the students, 89.9% were under 22 years of age (mean 21.2±1.1 years). The mean height of the students from the DN was 161 cm (148-174 cm) and the mean height of the students from the DND was 164 cm (151-183 cm). Also, the number of overweight (BMI > 25-29.9 kg/$m^2$) students was higher in the DN (14.5%; n=7) than in the DND (5.6%; n=4). Of the students from the DN, 66.6% (n=32) were determined as being normal weight, whereas 77.4% of students from the DND (n=55) were determined as being normal weight ($p<0.001$). Of the students from the DN, 6.25% (n=3) were determined as being obese, whereas 2.81% of students from the DND (n=2) were determined as being obese (Figure 1).

Waist circumference measured with tape was more than 88 cm in 15% (n=7) of the students from the DN and in 13% (n=9) of the students from the DND.

In this study, it was found that 45.8% (n=22) of the students from the DN skipped breakfast and that the 15.4% (n=11) of the students from the DND skipped breakfast. It was found that 72.9% (n=35) of the students from the DN ate meal outside one or more in a week, whereas 63.3% (n=45) of the students from the DND ate meal outside one or more in a week. The

percentage of skipping snacks was 12.5% (n=6) in the students from the DN and 7% (n=5) in the students from the DND. Lack of time was the most important cause of skipping meals in 54% (n=26) of the students from the DN and 43% (n=31) of the students from the DND.

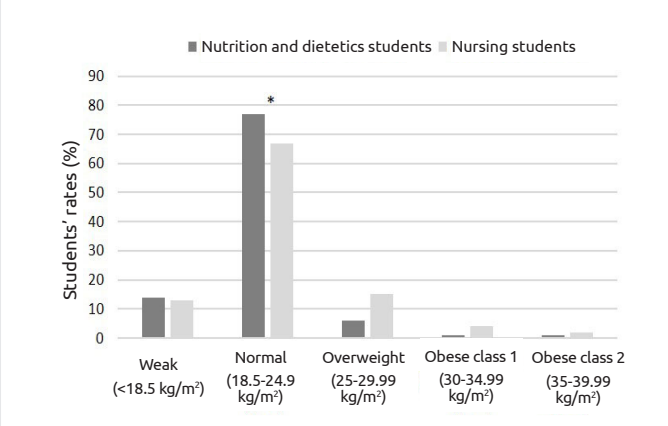


**Figure 1.** *Differences between control group and experimental groups statistically significant (p<0,001).

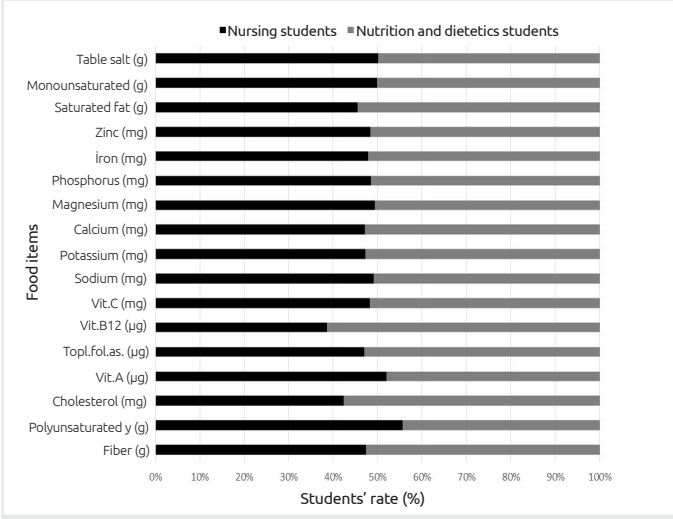


**Figure 2.** Comparison of nutrition consumption of the students (n=119)

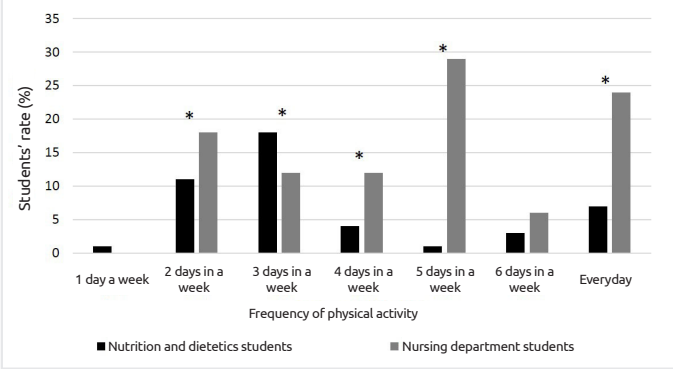


**Figure 3.** *Differences between control group and experimental groups statistically significant (p<0,001).

The mean water consumption was 1400 mL/day in the students from the DN and was 1520 mL/day in the students from the DND and they were very close to each other. In addition, they reported that 10.5% (n=5) of the students from the DN and 18.4% (n=13) of the students from the DND added salt without tasting their meals.

The total energy and nutrient intake of all students were insufficient and there was no statistically significant difference between the two groups (Figure 2). Total energy intake was 1459.2 kcal/day with a distribution of 17.4% protein, 39.1% fat, 42.6% carbohydrate in the students from the DN, while it was 1462.2 kcal/day with a distribution of 17% protein, 41% fat and 42% carbohydrate in the students from the DND. Fiber consumption was 15.9 g/day in the students from the DN, whereas it was 17.7 g/day in the students from the DND. In addition, mean vitamin B12 consumption was 4.10 µg in the students from the DN and on it was 2.59 µg in the students from the DN.

The frequency and quantity of food consumption of the groups in the study differ from each other (Table 1 and 2). Of the students from the DN, 8.3% (n=4) and 18.3% of the students from the DND (n=13) consumed eggs every day. The consumption of solid fat in the students from the DN was statistically significantly higher (60.5%; n=29) than in the students from the DND (p<0.001).

It was found that 55% (n=55) of the students from the DND and 35% (n=17) of the students from the DN were physically active and that this activity was achieved by walking in 34% (n=24) of the students from the DND and 31% (n=15) of the students from the DN (Figure 3; p<0.001).

## Discussion

One hundred and ninety students, aged between 19 and 25 years, participated voluntarily in this study. Of the students participated in this study, 84.8% (n=101) reported that they consumed milk and milk products every day. According to a study conducted at Gaziosmanpaşa University, only 28.67% of the students consumed milk on a regular basis and 33.33% consumed milk products on a regular basis (11). However, this result was not supported by our work.

In a study conducted by Ozdogan et al. (12), 89.5% of the students skipped their main meals and the most skipped main meal was breakfast. According to a study conducted in 2010 at Uludağ University, the most skipped meal was breakfast (24.8%) and 90% of the students paid attention to the consumption of snacks (13). In our study, 88.2% of the students skipped meals and the most skipped meal was found to be breakfast (31.4%). It was observed that 90.7% of the students pay attention to eating snacks. With these findings, it can be said that the students in our research group exhibited the same nutritional behavior as the students in the Uludag University.

**Table 1.** The frequency of nutrition consumption of the students from the Department of Nutrition and Dietetics (n=71)

| Food consumption frequency results table | Everyday | 5-6 per week | 3-4 per week | 1-2 per week | In 15 days 1 | Monthly 1 | Ever |
|---|---|---|---|---|---|---|---|
| Milk and yoghurt | 30 | 16 | 16 | 7 | 2 | 0 | 0 |
| Cheese | 31 | 16 | 12 | 8 | 1 | 0 | 3 |
| Red meat | 0 | 5 | 21 | 39 | 6 | 0 | 0 |
| White meat | 1 | 5 | 19 | 35 | 9 | 2 | 0 |
| Egg | 13 | 7 | 19 | 21 | 4 | 4 | 3 |
| Pulse | 3 | 4 | 30 | 19 | 10 | 4 | 1 |
| Fresh vegetables | 15 | 11 | 26 | 14 | 3 | 0 | 2 |
| Fresh fruit | 24 | 23 | 16 | 6 | 1 | 0 | 1 |
| Bread | 47 | 11 | 5 | 5 | 1 | 2 | 0 |
| Cereals | 11 | 19 | 21 | 14 | 5 | 0 | 1 |
| Liquid oil | 37 | 14 | 13 | 4 | 1 | 0 | 2 |
| Solid oil | 3 | 2 | 5 | 6 | 3 | 7 | 45 |
| Herbal product | 22 | 22 | 7 | 7 | 8 | 1 | 4 |
| Animal products | 10 | 7 | 17 | 18 | 5 | 6 | 8 |

**Table 2.** The frequency of nutrition consumption of the students from the department of nursing (n=48)

| Food consumption frequency results table | Everyday | 5-6 per week | 3-4 per week | 1-2 per week | In 15 days 1 | Monthly 1 | Ever |
|---|---|---|---|---|---|---|---|
| Milk and yoghurt | 20 | 9 | 8 | 8 | 2 | 0 | 1 |
| Cheese | 20 | 10 | 11 | 5 | 0 | 0 | 2 |
| Red meat | 1 | 3 | 5 | 18 | 6 | 11 | 4 |
| White meat | 0 | 4 | 16 | 17 | 8 | 3 | 0 |
| Egg | 4 | 5 | 10 | 21 | 3 | 2 | 3 |
| Pulse | 1 | 2 | 19 | 17 | 8 | 1 | 0 |
| Fresh vegetables | 8 | 9 | 15 | 13 | 1 | 2 | 0 |
| Fresh fruit | 10 | 12 | 13 | 10 | 2 | 0 | 1 |
| Bread | 35 | 3 | 2 | 3 | 0 | 1 | 4 |
| Cereals | 3 | 8 | 16 | 16 | 2 | 0 | 3 |
| Liquid oil | 24 | 2 | 9 | 3 | 0 | 1 | 9 |
| Solid oil | 5 | 2 | 5 | 10 | 1 | 6 | 19 |
| Herbal product | 11 | 4 | 10 | 10 | 2 | 8 | 3 |
| Animal products | 8 | 2 | 3 | 16 | 1 | 2 | 16 |

In a study conducted at Erciyes University, 48.9% of the students ate three meals a day (14,15), while 46.5% of the students ate two meals a day and 44.5% ate three meals a day in a study conducted at Erciyes University (16). In our study, it was found that 47.8% of the students ate three meals, while 16.8% ate four meals.

The reasons for skipping meals of the students were investigated by Memis (17). in a study and it was noted that lack of time was the most common reason for skipping meal in 62.7% of the students. In our study, lack of time was the most common reason for skipping meal with a rate of 47.8%.

Gülec et al. (18) showed that most preferred food was fruits and vegetables in the main meals at a rate of 25.3% in the students. In our study, it was found that milk and milk products were the most preferred food at a rate of 32.7% in the main meals.

Of the students who participated in our study, 89% said "No" to the question of "Have you used cigarettes in the past?". A study at Uludağ University Faculty of Medicine concluded that 82.7% of the students did not smoke (21). According to a study conducted in different faculties at Uludağ University, the rate of non-smoking students was 63.6% (13). Based on these results, we can conclude that the smoking rate of the students in health departments is lower.

In our study, 10% of the students from both departments (n=12) had physical activity for 5 days per week and 15.9% of them (n=19) had activity time for 30 minutes. A study in Florida found that 86.6% of the students had regular physical activity for at least five days a week and 30 minutes per day (22).

## Conclusion

As a result, it was found that the students from the DND had more healthy eating habits compared to the students from the DN, but the students from both departments were malnourished. In addition, higher BMI results were obtained in the DN compared to the DND and therefore the number of students in the risky group was found to be higher. It was found that the physical activity level of the students was higher in the DND. A significant difference between the students from the DND who took the "Nutrition Principles" and the students from the DN who did not take the course in terms of consumption of various food groups which are beneficial/harmful to health, emphasized the importance of this course in education.

### Ethics

**Ethics Committee Approval:** This study was conducted in accordance with the guidelines in the Helsinki Declaration, and performing procedures involving human subjects/participants was approved by the Ethical Committee on Non-Invasive Research at XXX University (No: 50222451-050.05.04-9/89).

**Informed Consent:** Participants were informed about the purpose of the research and informed consent was taken from participants.

**Peer Review:** Externally peer-reviewed.

### Authorship Contributions

Concept: A.G.B., Design: A.G.B., Data Collection or Processing: D.G., A.G.B., Analysis or Interpretation: A.G.B., D.G., Literature Search: D.G., Writing: A.G.B., D.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# The Evaluation of Reasons for Re-admission to Family Physicians and Emergency Services and the Factors Affecting Satisfaction

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## ABSTRACT

**Objective:** Patient satisfaction is an important indicator for measuring the quality of health care services. The quality of health care service is also closely associated with hospital admissions. In this study, patient satisfaction and health care system quality in emergency department (ED) and primary health care (PHC) department were evaluated and its correlation with recurrent admissions was investigated.

**Method:** This is a questionnaire study conducted with 200 patients admitted to a university hospital ED in İstanbul between 1-15 May 2016. The patients were asked 14 question including medical history with demographic data, any admission to ED or PHC department in last month, results of their admissions and satisfactions rate.

**Result:** Of the 200 patients aged between 18 and 92 years, 53% (n=106) were female and 47% (n=94) were males. Within one month, the proportion of those who were admitted to both PHC and ED was 7.5% (n=15). Satisfaction rate was (2.40) significantly lower in this group than in the others (p=0.014). Only admission to PHC was 13.5% (n=27) and to our ED was 18% (n= 6). In patients admitted to PHC or ED, gender, physician's diagnosis and way of resulting of the physician did not have a significant effect on the satisfaction (p>0.05).

**Conclusion:** The satisfaction rates of patients who were admitted to more than one department (ED and PHC) were significantly lower than patients who were admitted to only one department (ED/PHC). On the other hand there was no correlation between decreased rate of satisfaction and being succesfully diagnosed or results of admissions.

**Keywords:** Emergency department, primary care, satisfaction, recurrent admission

## Introduction

While serving patients in the field of health care, it is necessary to develop appropriate solutions to the problems in line with the psychosocial aspects of the services, the different understanding of the patients, and expectations and satisfaction of the patients. Patient satisfaction can be defined as satisfying the wishes and expectations of patients, which is one of the important criteria used to measure the quality of service in the field of health (1). It is also important to determine the evaluation criteria that measure the quality of health care services while planning the steps to be taken to address the deficiencies or problems in the health service presentation. Conducting community-based research in service procurement, determining status, comparing with the past and measuring changes over the years will contribute positively to management and resource allocation (1-3). For this purpose, the patient satisfaction surveys determine whether the expectations of the patients are met and the quality of the health service can be evaluated by this way. Patient satisfaction is closely related to patients' application to health institutions (4). In one study,

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researchers showed that the patient satisfaction in hospital environment was affected by emergency, service and discharge stages. Based on this, they thought that personal relationships were the primary factors affecting patient satisfaction (5). However, there are other important factors. For example, it is very common for old and chronic patients to be admitted to hospital again (6).

In many studies, elderly patients who are admitted in emergency services have been shown to have pulmonary and cardiovascular diseases and hypertension. It was reported that elderly patients had more complex problems during the emergency service admission, and were more likely to need more examinations and intensive care (7-9). Re-admission constitutes approximately half of all hospital admissions and 60% of cost (6,10-12). From this point of view, we aimed to conduct a questionnaire to search the number and satisfaction of patients admitted to the emergency department and primary health care institutions in the last month, including demographic information.

## Methods

The study was carried out between 1-15 May 2016 with the patients who admitted to the emergency service at the University Hospital Bezmialem Vakıf and who agreed to complete the questionnaire. Patients over the age of 18 were included in the questionnaire. The questions were asked to those who were able to speak and they were asked to their relatives in case of the patients were unable to speak or their lives were at risk. The questionnaire was carried out in patients whose examination and treatment were initiated and who were available to answer the questionnaire by a health care personnel after informed consent was taken. Patients under 18 years of age and patients with trauma were excluded from the study. In the questionnaire, patients were asked if they were admitted to primary health care service, an emergency service of a University Hospital, and other emergency services in the last month and if they were satisfied. After presenting the results with descriptive statistics, comparisons were made in terms of demographic characteristics. The choices used by patients for questions were as follows: Very satisfied, satisfied, dissatisfied, very dissatisfied. These answers were used to calculate the satisfaction rate. In the questionnaire, co-existing diseases, whether the patient was diagnosed as having a specific disease and the way of physician in managing were investigated in relation to the frequency of satisfaction or admission frequency. The questionnaire was consisted of 14 questions (Table 1). Descriptive statistics and Kruskal Wallis test were performed with the SPSS 21.0 program and $p<0.05$ was considered statistically significant.

## Results

A total of 200 patients were included in the study; 53% (n=106) were female; 47% (n=94) were male; and the mean age was 45 years. Of the patients only admitted to family physician (n=27) 70.4% were female and 29.6% were male. Of the patients only admitted to emergency room (n=36) 47.2% were female and 52.8% were male. When the participants were questioned about chronic diseases, it was observed that there was 48% (n=96) of those with no disease and 24.5% (n=49) of those with multiple diseases (Table 2).

When the admissions in the last month were examined; 39.5% (n=79) of the patients was not admitted to anywhere, 60.5% (n=121) to at least one institution. Among these, 13.5% (n=27) was admitted to the family physician, 18% (n=36) to our emergency service, 4% (n=8) to another emergency service. The ratios of patients admitted to two institutions were 8.5% (n=17; family physician+our emergency service), 6% (n=12; our emergency service+another emergency service) and 3% (n=6; family physician + another emergency service), respectively. The rate of patients admitted to 3 institutions was 7.5% (n=15) (Table 3).

In all of the participants, 15.5% (n=31) of the patients was admitted to family physician to repeat prescriptions, 7% (n=14) for healthy control, 6.5% (n=13) for newly diagnosed complaints for examination, 3.5% (n=7) for chronic disease control. Of those only admitted to family physicians 47.5% wanted to repeat prescriptions and 20% wanted to be examined due to newly started complaints (Table 4). Of patients admitted to family physician, 66.2% (n=43) did not have a disease to be diagnosed. Of patients, 21.5% (n=14) did have a diagnosis and 41.3% (n=8) did not have a diagnosis. When the management and recommendations of the physicians were assessed, in 16.5% (n=33) of the patients admitted to family physician, physician prepared prescription and 4.5% (n=9) of the patients were recommended outpatient clinic control. The family physician recommended 3% (n=6) of the patients to admit to emergency room and recommended 8% (n=16) of the patients to do nothing. The family physician sent one patient to emergency service with ambulance. Of the patients admitted to family physician, 20.5% (n=41) was very satisfied, 8.5% (n=17) was satisfied, 2% (n=4) was not satisfied, and 1.5% (n=3) was dissatisfied.

Of the patients, 40% (n=80) was admitted to our emergency service in the last month. Of those patients, 45% (n=36) was admitted to emergency service with a newly started disease. Of the admissions, 35% (n=28) was for increase in complaints related to chronic disease, 8.8% (n=7) for wish to get quick result in the emergency, 7.5% (n=7) for control, and 3.8% (n=3) for getting prescription. A diagnosis was made in 26.3% (n=21) of the patients and a diagnosis was not made in 32.5% (n=26) of the patients. Of the patients, 41.3% (n=33) did not have a disease to be diagnosed. Of the patients 35% (n=28) were recommended to get appointment for outpatient clinic and 32.5% (n=26) were given prescription. Of the patients, 16.3% (n=13) were recommended nothing and 11.3% (n=9) was hospitalized. Of the patients, 2.5% (n=2) was recommended to go to family physician and 2.5% (n=2) was sent to another hospital. Of the patients admitted to emergency service, 50% (n=40) was very satisfied, 21.3% (n=17) was satisfied, 16.3% (n=13) was not satisfied, and 12.5% (n=10) was dissatisfied. The rate of admission to another emergency service in the last month was 20.5% (n=41).

**Table 1.** Questionnaires directed to patients or their relatives in the emergency room

| | | |
|---|---|---|
| **1-Age** | | |
| **2- Gender** | • **Female** | • **Male** |
| 3- Existing Diseases, If You Have Any | • None<br>• Hypertension<br>• Renal failure<br>• Cardiac disease<br>• Diabetes mellitus | • Chronic obstructive pulmonary disease<br>• Asthma<br>• Tumor, cancer<br>• Others<br>• More than one disease |
| **4- Admission To Your Family Physician In Last One Month ( If Your Answer Is No, Go To The 5th Question )** | • **Yes** | • **No** |
| A) If yes, why? | • Newly onset disease<br>• My previous disease<br>• For getting my prescription written<br>• For control | |
| B) Did you have a diagnosis? | A diagnosis was made<br>A diagnosis was not made | |
| C) What was your doctor's recommendation at the end of admission? | • Transfer by ambulance<br>• None<br>• Drug (prescription)<br>• Drug+outpatient clinic<br>• Directed to emergency service | |
| D) What was your satisfaction from your doctor? | • I was dissatisfied<br>• I was not satisfied<br>• I was satisfied<br>• I was very satisfied | |
| **5- Admission to emergency service in last one month (If your answer is no, go to the 6th question)** | • **Yes** | • **No** |
| A) If yes, why? | • Newly onset disease<br>• My previous disease<br>• For getting my prescription written<br>• For control<br>• To get a fast result | |
| B) Did you have a diagnosis? | • A diagnosis was made<br>• A diagnosis was not made | |
| C) What was your doctor's recommendation at the end of admission? | • Another hospital was recommended<br>• None<br>• Drug was recommended<br>• Directed to hospital's outpatient clinic<br>• Directed to family physician<br>• Hospitalized | |
| D) What Was Your Satisfaction From Your Doctor? | • I was dissatisfied<br>• I was not satisfied<br>• I was satisfied<br>• I was very satisfied | |
| **6- Admission to Another Emergency Service in Last One Month** | • **Yes** | • **No** |
| Participation in the questionnaire is subject to approval. relatives of patients who are unable to speak may participate in the questionnaire | | |

Family physician satisfaction average was 3.47±81 and the satisfaction average of our emergency service was 3.05±1.13 (Figure 1).

When we looked at the relationship between satisfaction and admission cause to family physician; the highest average satisfaction rate was 3.61 in patients who had their prescription written (n=31) and the lowest average satisfaction rate was 2.71 in patients who were admitted for the control of chronic disease (n=7) (p=0.124) (Table 5). The satisfaction average was 3.3571 in the patients who were diagnosed as having a disease (n=14) and was 3.5 in those who did not have a diagnosis (n=8) (p=0.714) (Table 6). The satisfaction average was 3.66 in the patients who were recommended to go to emergency service (n=6) and was 3.42 who were given prescription (n=33) (p=0.767).

When we looked at the relationship between satisfaction and admission cause to emergency service; we found that the average satisfaction rate was 3.16 in patients who were admitted for control (n=6) and that was 3.14 in patients who were admitted with increase in complaints of a chronic disease (n=28) and these groups had the highest satistaction rate. The lowest rate was 2.71

**Table 2.** Chronic diseases of the patients

| Chronic diseases | N | % |
|---|---|---|
| No chronic disease | 96 | 48.0 |
| Hypertension | 4 | 2.0 |
| Chronic renal failure | 10 | 5.0 |
| Cardiac disease | 14 | 7.0 |
| Diabetes mellitus | 1 | ,5 |
| Chronic obstructive pulmonery disease | 2 | 1.0 |
| Asthma | 6 | 3.0 |
| Tumor, cancer | 5 | 2.5 |
| Others | 13 | 6.5 |
| More than one disease | 49 | 24.5 |
| Total | 200 | 100.0 |

**Table 3.** Causes of admission to the family physician

| Type of Admission | Number of Patients | % |
|---|---|---|
| No Admission | 79 | 39.5 |
| FP | 27 | 13.5 |
| ES | 36 | 18.0 |
| AES | 8 | 4.0 |
| FP+ES | 17 | 8.5 |
| FP+AES | 6 | 3.0 |
| ES+AES | 12 | 6.0 |
| FP+ES+AES | 15 | 7.5 |
| Total | 200 | 100.0 |

FP: Family physician, ES: Our emergency service, AES: Another emergency service

**Table 4.** Distribution of admissions according to institutions in last one month

| Cause of Admission | Number of Patients | Total % | % |
|---|---|---|---|
| Newly onset complaint | 13 | 6.5 | 20.0 |
| Examination for existing chronic disease | 7 | 3.5 | 10.8 |
| For getting prescription written | 31 | 15.5 | 47.7 |
| Routine control | 14 | 7.0 | 21.5 |
| Total admission to family physician | 65 | 32.5 | 100.0 |
| No admission to family physician | 135 | 67.5 | |
| Total | 200 | 100 | |

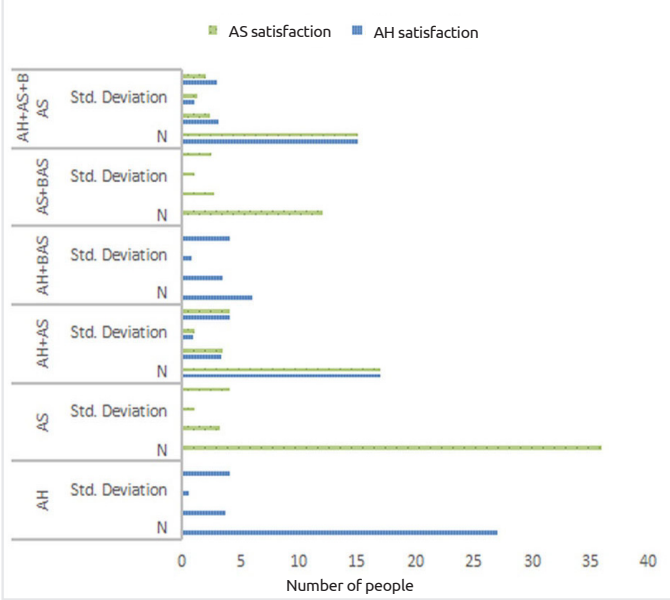


**Figure 1.** Chart showing the satisfaction level of the emergency room of our hospital and emergency department of the other hospitals

FD: Family doctor, OER: Our Emergency Room, AER: Another emergency room

**Table 5.** Satisfaction rates and causes of admission to family physician

| Cause of Admission | Number of Patients | Satisfaction Average | Std. Dev. | Min | Max |
|---|---|---|---|---|---|
| Newly onset complaint | 13 | 3.46 | 0.66 | 2.00 | 4.00 |
| Examination for existing chronic disease | 7 | 2.71 | 1.25 | 1.00 | 4.00 |
| For getting prescription written | 31 | 3.61 | 0.71 | 1.00 | 4.00 |
| Routine control | 14 | 3.57 | 0.75 | 2.00 | 4.00 |
| Total | 65 | 3.47 | 0.81 | 1.00 | 4.00 |

Std.Dev.: Standard deviation, Min: Minimum, Max: Maximum

in patients who wanted to get fast result (n=7) (p=0.751) (Table 7). When we looked at the relationship between satisfaction and making a diagnosis in emergency service, we found the satisfaction average was 3.04 in patients who were diagnosed as having a disease (n=21) and was 3.05 in patients who were not diagnosed (n=26) (p=0.293) (Table 8). When we looked at the relationship between satisfaction and recommendation in emergency service, we found that the highest satisfaction rate was 3.44 in patients who were hospitalized (n=9) and that the lowest satisfaction rates were 1.5 in patients who were sent to family physician (n=2) and 2.84 in patients who were not recommended anything (n=13) (p=0.576).

**Table 6.** Satisfaction rates and diagnosis status in patients admitted to family physician

| Diagnosis Status | Number Of Patients | Satisfaction Average | Std. Dev. | Min | Max |
|---|---|---|---|---|---|
| No diagnosis was expected | 43 | 3.51 | 0.82 | 1.00 | 4.00 |
| A diagnosis was made | 14 | 3.35 | 0.92 | 1.00 | 4.00 |
| No diagnosis was made | 8 | 3.50 | 0.53 | 3.00 | 4.00 |
| Total | 65 | 3.47 | 0.81 | 1.00 | 4.00 |

Std.Dev.: Standard deviation, Min: Minimum, Max: Maximum

**Table 7.** Satisfaction rates and causes of admission to our emergency service

| Cause of Admission | Number of Patients | Satisfaction Average | Std. Dev. | Min | Max |
|---|---|---|---|---|---|
| Newly onset complaint | 36 | 3.02 | 1.02 | 1.00 | 4.00 |
| Examination for existing chronic disease | 28 | 3.14 | 1.17 | 1.00 | 4.00 |
| For getting prescription written | 3 | 3.00 | 1.73 | 1.00 | 4.00 |
| Routine control | 6 | 3.16 | 1.32 | 1.00 | 4.00 |
| Get a fast result | 7 | 2.71 | 1.38 | 1.00 | 4.00 |
| Total | 80 | 3.05 | 1.13 | 1.00 | 4.00 |

Std.Dev.: Standard deviation, Min: Minimum, Max: Maximum

**Table 8.** Satisfaction rates and diagnosis status in patients admitted to our emergency service

| Diagnosis Status | Number of Patients | Satisfaction Average | Std. Dev. | Min | Max |
|---|---|---|---|---|---|
| No diagnosis was expected | 33 | 3.21 | 1.13 | 1.00 | 4.00 |
| A diagnosis was made | 21 | 3.04 | 1.20 | 1.00 | 4.00 |
| No diagnosis was made | 26 | 2.84 | 1.08 | 1.00 | 4.00 |
| Total | 80 | 3.05 | 1.13 | 1.00 | 4.00 |

Std.Dev.: Standard deviation, Min: Minimum, Max: Maximum

When we evaluated satisfaction in groups in all admissions, satisfaction rate was 3.25 in patients who were admitted only to our emergency service (n=36), was 3.47 in those admitted to our emergency service and family physician (n=17), was 2.66 in those admitted to our and another emergency services (n=12) and was 2.4 in those admitted to all three (n=15). The patients who were admitted to family physician, our emergency service and another emergency service had the lowest satisfaction rate (p=0.014) (Figure 1).

## Discussion

This study was based on a questionnaire applied to patients who were admitted to a university hospital with an annual admission number of 258401 to emergency service for 2013. The fact that 60.5% of the participants was admitted to family physicians, our emergency service or any emergency services at least once in the last month pointed to one of the reasons underlying the intensity of the admission to emergency services in our country. The number of admission to emergency services in Istanbul, which has a population of 14.160.467 (4), was 100.081.171 (13). This number constituted 26.97% of all hospital admission and was about 7 times the population of the city.

About half of the admissions to the family physician was consisted of patients who wanted to get their prescription written and only one-fifth of them want to be examined because of the newly started complaints. All these show that family physician is not used as the first admission unit. In a study with 485 people on awareness of family medicine in Kayseri city center, it was concluded that family medicine was not considered as the first place of admissiom. Readmission constitutes approximately half of all hospital admissions and 60% of all costs (6,10-12). It also indicates that patients are receiving inadequate treatment (6,14-16). In our study, more than half of the patients was re-admitted to the emergency room within a month. Calling patients to control by clinicians is expected to be effective in re-admission. Although there is no call for control in the emergency service the re-admission rate was quite high. In the questionnaire, family physicians directed 3% of their patients to emergency services, while the emergency physician recommended 14% of the patients to go to outpatient clinic for control and 1% of the patients to go to family physician. This was expected to be a factor in reducing the rate of emergency admissions.

Early readmission to hospital of high-risk and elderly patients is costly and occurs oftenly. Similar to our results, one fifth of the patients is re-admitted to hospital within 30 days and it costs $26 million per year (17,18). To improve quality of care and prevent unnecessary repeated expenditures; politicians and insurers in the United States set a reduction in 30-day readmission as a priority national target, and searched for underlying causes to ensure this (18,19). One reason is the quality of service offered

to the patients. Health care providers have a desire to reduce the number of hospitalized patients and provide quality care. According to an argument; determining patients with a high likelihood of readmission and providing them with intensive and high quality initial treatment may reduce readmissions (6,20).

There are many benefits for family medicine in increased continuity of service such as better patient-physician communication and improved success of treatment. Repeated visits to the same family health center can also be seen as an indirect indicator of patient satisfaction (4). In our study, almost all of the patients admitted to the family physician and 3/4 of the patients admitted to the emergency department were satisfied. We saw that the readmissions to family physician in whom patient satisfaction was a little higher than the emergency room decreased slightly. Cetinkaya et al. (1) found the rate of very satisfied and satisfied patients as 80.7% in patients admitting to family physician (1).

In a randomized multicentric study on this subject, veterans with many disadvantages in benefiting from health care were discussed. By increasing the quality of care they received, they were facilitated to reach a health care institution where a good first care could be made and it was investigated whether readmissions in 6 months were reduced. Although the number of patients hospitalized in the service decreased a little bit, the results appeared contrary to expected. First, the emergence of new diseases that were overlooked in patients receiving a good health care; second, increased readmission request of the patients due to increased communication between patient and health care provider caused increase in readmissions (6). The number of visits to the family health center and satisfaction rate increased as the age increased. Furthermore, increase in the number of visits to the family health centre showed that the level of satisfaction increased (4).

Another reason that affects satisfaction is duration of waiting before examination. Bursch et al. (21) showed that the exact duration of stay in the emergency room did not affect satisfaction of patients much. The actual effect was caused by longer duration of waiting before examination. The maximum waiting time for outpatient treatment in our hospital is approximately one and a half hours in busy hours, while in most of the day, this time is half an hour. Cetinkaya et al. (1) pointed out that although there were many parameters, the most effective issue on patient satisfaction for family physicians was the attitude of the physician towards the patient (18%). Readmissions are often in patients with chronic diseases and with anticipation of hospitalization. This group of patients is small in numbers, but they cause crowding in emergency services with hospitals with limited beds and readmissions. This is usually because the patient does not receive adequate support from family, social environment and primary health care. Readmissions of these patients can be reduced by good care at home or with good protection (22). In our study, 52% of patients had known chronic diseases and 24.5% of the patients had multiple chronic diseases. Although all of these patients were not admitted due to acute attacks of their chronic diseases, it was noteworthy that half of the patients with readmission had chronic diseases. It has become important to reduce the duration of hospitalization, to increase the use of daily procedures, to make home care programs and to develop initiatives aimed at reducing hospitalization. Evidence suggests that even a small decrease in the number of readmissions could provide a financial benefit. Targets for initiatives that can reduce readmission, improved patient and family education and community-based support, primary health care-based contact, early follow-up and ongoing chronic disease management. Initiatives aimed at reducing admission to hospital can reduce the crowd in the emergency room, improve patient outcomes, and contribute positively to existing hospital facilities without requiring an additional cost and additional hospital beds. It was found that a comprehensive good discharge planning and temporary care were effective in reducing readmission to the hospital and facilitated follow-up with primary health care after discharge from the hospital (22).

Most of the patients who are admitted to our emergency service do not have an emergency situation. This is an important and discussed factor that is widespread in our country and it affects the crowding of emergency services.

The patients who were admitted to family physician for getting their prescription written had the highest satisfaction rate and it was 3.61, whereas the patients admitted for control for their chronic diseases had the lowest satisfaction rate and it was 2.71. This could be attributed to the fact that the expectations of the patients who were admitted to get their prescription written were met. The relative decrease in satisfaction of those with chronic disease could be caused by following reasons: Their expectations of hospitalization were not met or not much change in the existing treatments were done.

When we looked at the relationship between satisfaction and admission cause in our emergency service, we found that the patients who wanted to get a fast result had the lowest satisfaction rate which could be due to the fact that they were not fully satisfied with these expectations, because waiting queues were very long in emergency rooms due to admission of patients without emergent situations.

Both in emergency room and family physician admissions, whether a diagnosis was made did not affect satisfaction. This indicates that the actual expectations of the patients are not concentrated on the diagnosis and other reasons should be sought.

Satisfaction rate was significantly lower in patients who were admitted 3 times in last one month than in patients who were admitted one time. Although they were admitted to different institutions, there was no data that could explain their low satisfaction rate. However, we think that the problems of this group should be addressed separately in other studies.

**Study Limitations**

Although we believe that the questions we have prepared to reveal the relationship between satisfaction and admission are very

comprehensive and important questions, the results show that our questions are not closely associated with patient satisfaction.

## Conclusion

Satisfaction rate of patients who were admitted to both emergency room and family physician was lower than patients who were admitted to one institution (emergency room or family physician). Contrary to expectations, it was observed that whether a diagnosis was made or the way of resulting the admission did not have much effect on decreased satisfaction.

Emergency services are very crowded in our country. One of the reasons for excessive admissions to emergency services and family physicians is readmissions. Especially in patients with low level of satisfaction, we see that readmissions are increasing. Reducing such readmissions can reduce both excessive admissions and health expenditures. With patient satisfaction, we believe that many other factors may affect readmissions. However, it seems difficult to tell exactly what affects the satisfaction of the patients. To find out which factors are effective, we believe there is a need for questionnaire studies involving a wide range of questions.

### Ethics

**Ethics Committee Approval:** It was taken by the ethics committee of Bezmialem Vakif University. Approval no: 10/37

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Concept: E.S, B.G., Design: E.S., H.İ.Ç., Data Collection and/or Processing: B.G., H.İ.Ç, Analysis and/or Interpretation: E.S., H.İ.Ç., Literature Review: E.S., B.G., Writing: E.S.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Original Article

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# Frameless Neuronavigation Assisted Brain Biopsy: Safety, Efficiency and Our Experience

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## ABSTRACT

**Objective:** Brain biopsy is a commonly used method for diagnosing deeply located or generalized intraparenchymal brain lesions and determining the further treatment options. A number of comparative studies have been performed with open surgical biopsy, framed or frameless biopsy methods. In this study, we shared the results of the framless neuronavigation assisted biopsy in our clinic and diagnostic sensitivity of this method.

**Methods:** Twenty-four cases of frameless neuronavigation assisted biopsy for cerebral intraparenchymal lesions between 2014 and 2016 were retrospectively reviewed. Seven cases had thalamic and 1 case had hypothalamic deep masses. Four cases had deeply located lesions on eloquent brain areas, 6 patients had diffuse lesions passing to the opposite cerebral hemisphere. Five cases had multiple cerebral masses and 1 case had exophytic mass originating from brainstem.

**Results:** Histopathologic diagnosis in 14 of 24 cases was grade IV glial tumor, diffuse grade III glial tumor in 1 and diffuse grade II glial tumor in 2. Histopathologic diagnosis of 5 cases were reported as B-cell lymphoma, whereas 1 case was reported as having gastrointestinal system metastasis. In one case, the diagnosis could not be made despite the biopsy was performed twice. The mitotic index (ki-67) showing tumor aggressiveness was 28.43% and range was 1-90%. Twenty three of the 24 patients had a definite histopathological diagnosis and 1 patient had no histopathological diagnosis.

**Conclusion:** Frameless neuronavigation assisted brain biopsy is one of the most sensitive, safe and easy to perform stereotactic biopsy methods. The method which will be used with high accuracy and planning of biopsy require surgical experience.

**Keywords:** Biopsy, brain, neuronavigation

## Introduction

In spite of current developments in radiological imaging techniques, accurate histological diagnosis is required to determine the appropriate treatment methods in intracranial lesions. Open surgical methods with mini-craniotomy and framed or frameless brain biopsies were used in diagnosis of intraparenchymal tumors. Neuronavigation devices are used in frameless brain biopsy. The advantages and disadvantages of framed or frameless brain biopsy techniques are still controversial; sampling accuracy, approach to deep brain lesions and sample volume are a few of controversial topics (1-10). In addition to this, there are discussions about the deficiencies of craniotomy with open biopsy and stereotactic biopsy techniques. In the literature, diagnostic accuracy was reported as 66-99% in framed biopsy (3-5,10,11), and as 89-93% in frameless biopsy (10-14)%, in the cases with frameless biopsy. In this respect, today, no method has been shown to have a definite superiority over another. It is obvious that the most

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important criteria determining the accuracy of the diagnosis are patient selection and correct planning.

In this study, we shared our diagnostic accuracy and experience with our results of the patients who underwent biopsy with the help of neuronavigation for intraparenchymal lesions in our clinic.

## Methods

Twenty-four cases of assisted biopsy with frameless neuronavigation (Stealth Station Treon™ Vertek®, Medtronic, Minnesota, USA) for cerebral intraparenchymal lesions between November 2014 and December 2016 in our clinic were retrospectively investigated. The study was approved by the local ethics committee. Informed consent was taken from all patients. Of 24 patients, 15 were male and 9 were female. The mean age was 61 (38-84) years. Of the patients who had intraparenchymal masses, 7 had thalamic masses and 1 had hypothalamic mass. The masses of four cases were deeply located on eloquent brain areas. The masses of the six cases were widespread (diffuse) and spread to the opposite cerebral hemisphere. There were more than one cerebral mass in five cases, whereas there was an exophytic mass originating from the brainstem in one case (Table 1).

### Surgical Procedure

Frameless biopsy using the Medtronic Stealth Treon™ Vertek® (Stealth Station Treon™ Vertek®, Medtronic, Minnesota,USA) neuronavigation system was performed in all patients. Axial T2 constructive interference in steady state (CISS) and contrast enhancing axial T1 magnetic resonance (MR) slices with 1 mm thickness were taken and loaded to the neuronavigation device before surgery to determine the safest route (Figure 1). All patients

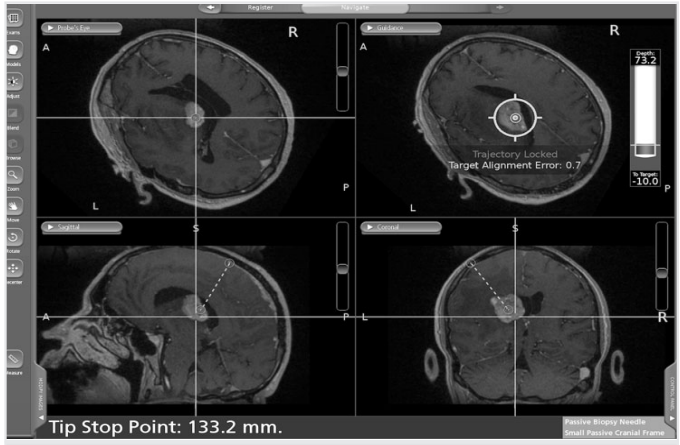


**Figure 1.** Planning of biopsy in neuronavigation device

**Table 1.** Features of the patients

| Gender | Age | Localization | Pathology | KI67 |
|---|---|---|---|---|
| M | 72 | Frontoparietal diffuse | Grade 4 glial tumor | 20% |
| F | 52 | Right temporal deep | Grade 4 glial tumor | 45% |
| M | 51 | Right thalamic mass | Grade 2 oligodendroglioma | 2% |
| M | 63 | Corpus callosum diffuse | Grade 4 glial tumor | 40% |
| F | 72 | Corpus callosum diffuse | Grade 4 glial tumor | 25% |
| F | 40 | Brainstem exophytic | Grade 2 diffuse astrositoma | 2% |
| M | 59 | Thalamic diffuse | Grade 4 glial tumor | 30% |
| F | 71 | Thalamic diffuse | Grade 4 glial tumor | 22.5% |
| M | 51 | Left occipital deep | B-cell lymphoma | 90% |
| F | 38 | Right frontal deep | B-cell lymphoma | 2.5% |
| M | 62 | Right frontal deep | Grade 4 glial tumor | 10% |
| F | 68 | Periventricular bilateral | B-cell lymphoma | 80% |
| F | 84 | Right frontal diffuse | Grade 4 glial tumor | 17.5% |
| M | 63 | Left frontal diffuse | Grade 4 glial tumor | 20% |
| M | 75 | Right hypothalamic | Grade 4 glial tumor | 40% |
| M | 64 | Left thalamic mass | Metastasis (Gastrointestinal) | 30% |
| M | 60 | Right thalamic mass | Grade 4 glial tumor | 22.5% |
| M | 53 | Bifrontal diffuse | Grade 4 glial tumor | 12.5% |
| F | 59 | Corpus callosum diffuse | Grade 4 glial tumor | 40% |
| M | 45 | Parietal | No diagnosis was made | 1% |
| M | 58 | Corpus callosum diffuse | Grade 3 glial tumor | 20% |
| M | 78 | Right parietal deep | B-cell lymphoma | 60% |
| M | 66 | Left temporal deep | B-cell lymphoma | 40% |
| F | 71 | Right parietal deep | Grade 4 glial tumor | 40% |

were fixated with 3-pin Mayfield head holder in an appropriate head position for biopsy plan under general anesthesia. The patients were recorded to the system using the neuronavigation system surface marking technique. With appropriate skin incision and 1 cm burr hole, the Stealth Treon™ Vertek® (Stealth Station Treon™ Vertek®, Medtronic, Minnesota,USA) model neuronavigation instruments were used. Biopsies with 8 mm in length and 1 mm in thickness were taken in different depths and in all of 4 directions and were sent to the histopathology department for frozen biopsy during the operation. Permanent pathological specimens were taken from the patients in whom tumor was detected in frozen biopsy and surgery was terminated (Figure 2).

**Figure 2.** Sampling during surgery

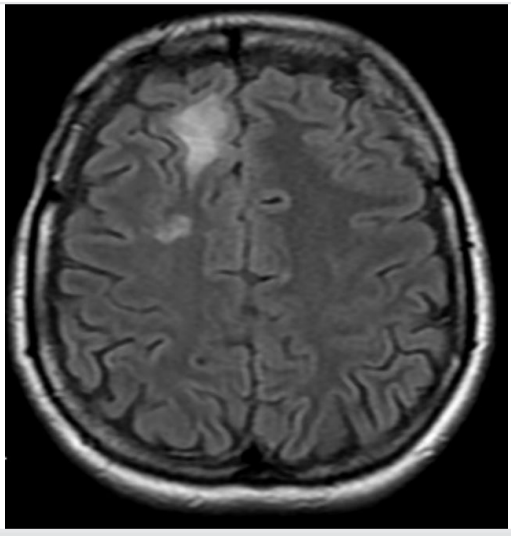

**Figure 3.** Pre-operative axial T1 weighted FLAIR magnetic resonance image

### Complications and Mortality

All patients underwent cranial CT after surgery for the risk of complication in the early period and were monitored for 1 day and followed up in surgical intensive care ünit. There were no early or late surgical complications, new neurological deficits or mortality due to surgery.

## Results

The histopathological diagnosis of the patients who underwent biopsy due to cerebral intraparenchymal mass was reported as stage IV glial tumor in 14 patients, stage III glial tumor in 1 patient and stage II diffuse glial tumor in 2 patients. Histopathologic diagnosis of 5 cases were reported as B-cell lymphoma, whereas 1 case was reported as having gastrointestinal system metastasis. In one case, the diagnosis could not be made despite two biopsies performed with one month interval. The mitotic index (ki-67) showing tumor aggressiveness was 28.43% and range was 1-90%. Twenty three of the 24 patients had a definite histopathological diagnosis and 1 patient had no histopathological diagnosis. Undiagnosed patient was first followed up as cortical dysplasia or low grade glioma. Then, progression in the right frontal subcortical lesion was observed in cranial MR and performing a biopsy was decided (Figures 3 and 4). Despite all histopathological and immunohistochemical studies for diagnosis, pathological diagnosis was not established and progression in the lesion was observed in follow-up (Figure 5). Second biopsy was performed but again definite histopathological diagnosis was not made. Open surgery was planned as a decision of the Council of neuro-oncology, but a decrease in lesion size was observed in cranial MRI performed in the late period (Figure 6). Surgical intervention was abandoned and follow-up was planned.

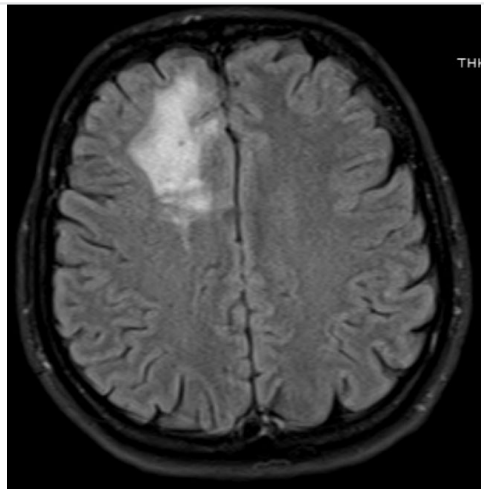

**Figure 4.** Progression in pre-operative axial T1 weighted FLAIR magnetic resonance image

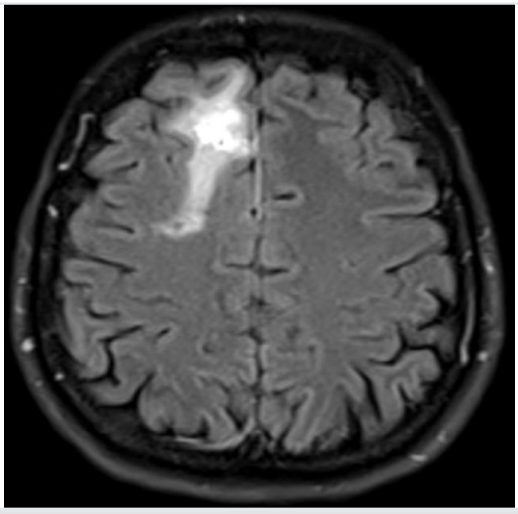

**Figure 5.** Axial T1 weighted FLAIR magnetic resonance image after biopsy

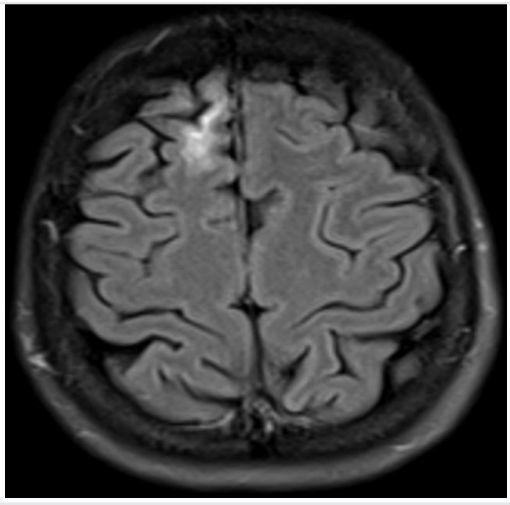

**Figure 6.** Spontaneous regression in the lesion after biopsy in the late period

## Discussion

The main aim of all procedures of biopsy for cerebral intraparenchymal lesions including framed, frameless or open surgery with craniotomy is to be easy to use, to be reliable and to be able to diagnose correctly. Several studies have been conducted in the literature comparing stereotactic biopsy with or without frame and open biopsy based on diagnosis. In all these studies, a success rate of 51-79% was achieved especially in glioma staging and the causes for these low rates were shown as sampling error and low amount of tissue (10-14). With the advancement of technology, especially the number of frameless stereotactic biopsy devices are increasing and they are becoming widely used. In recent publications, there have been studies showing that the use of endoscope in addition to neuronavigation increases success in tissue diagnosis, however, larger series are needed because the number of cases is small (15). The reason of higher diagnostic sensitivity of framed stereotactic biopsy methods, is the excess number of cases in whom these methods are used for biopsy. In our study, the diagnostic sensitivity of frameless stereotactic biopsy which is a new method was 95.8%. In only one of the twenty-four cases, histopathological diagnosis was not made, although biopsy with neuronavigation was performed twice, but it was observed that the subcortical lesion was regressed spontaneously in the long-term follow-up. Frameless stereotactic biopsy was superior than framed stereotactic biopsy in terms of patient comfort and operation duration and had much more lower mortality and morbidity compared with open craniotomy and biopsy (16). In our study, no complication was observed in any of the 24 cases after surgery in the early and late period and no worsening of the neurological condition of the patients due to surgery was observed.

### Study Limitations

Relatively low number of patients and short follow-up periods are the limitations of our study.

## Conclusion

Frameless neuronavigation assisted brain biopsy is one of safe and easy-to-apply stereotactic biopsy methods with high diagnostic sensitivity. The method which will be used with high accuracy and planning of biopsy require surgical experience.

### Ethics

**Ethics Committee Approval:** 08/146

**Informed Concent:** Informed consent was taken from the patients.

**Peer Review:** Externally peer-reviewed.

### Author Contributions

Concept: E.Ö., Design E.Ö., Data Collection or Processing: E.Ö., Analysis or Interpreting: E.Ö., Literature Search: S.K., Writing: E.Ö.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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Review

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# Current Approaches in Premenstrual Syndrome Management

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## ABSTRACT

Premenstrual syndrome (PMS) is a health problem that occurs with physical and psychological symptoms presenting about five days before menstruation, end within a few days after the onset of menstruation. The most common symptoms are appetite changes, edema, breast tenderness, abdominal pain, back pain, headache, fatigue, insomnia, depressive affect, irritability, angry outbursts andanxiety. Most women's half of lifes go through with premenstrual problems and quality of life is affected negatively. PMS prevalence is notably high in the world and in our country. In this respect, it is important to increase awareness of health professionals and women about PMS. The aim of this review is to explain current approaches of PMS management in the context of international guidelines. Getting detailed anamnesis for the diagnosis of PMS, if necessary, consultations and laboratory investigations are carried out. In addition, the diagnosis of PMS is confirmed by assessing women's symptom records for at least two months showing the type of symptoms and the time of appearance of these symptoms in the menstrual cycle. PMS management is carried out gradually by multidisciplinary team that adopted an integrative holistic approach in the direction of individualized plan. The first step includes creating awareness about PMS, teaching to women self-screening, lifestyle changes, regulation of diet, methods of coping with stress. In the second step, cognitive behavioral therapy and complementary alternative therapies are implemented. If the problem continues, there is pharmacological treatment in the third step. Finally, surgical treatment is applied in the fourth step.

**Keywords:** Premenstrual syndrome, disease management, women's health

## Introduction

Repeated menstruation every month in women's life is considered to be a sign of being healthy. However, the symptoms that occur in the second half of the menstrual cycle negatively affect the life of most women (1). The American College of Obstetricians and Gynecologists defines the period which occurs approximately five days before menstruation and ends in a few days after menstruation starts and is accompanied by physical and psychological symptoms as premenstrual syndrome (PMS) (2). The late twenties and mid-fifties are the periods when PMS is seen commonly (3). In the premenstrual period, changes in appetite such as excessive eating and craving, weight gain, edema, breast tenderness, and swelling and pain in the joints, abdominal pain, stomach problems, back pain, headaches, vertigo and dizziness, sweating in hands and feet, fatigue, skin problems such as acne, insomnia and short-term drowsiness, decreased libido, depressive mood, anger outbursts, irritability, crying spells, anxiety, restlessness, and confusion have been observed (2,3). Because of the problems associated with PMS, women experience change in body perception, decrease in self-confidence, social isolation and interpersonal relationships are disrupted (4,5). It is also stated that PMS leads to drug addiction, increased tendency to have an accident and to commit crime, economic losses, and decline in academic achievement (4,6).

The prevalence of PMS was examined, Royal College of Obstetricians and Gynaecologists reported that 4 out of 10 women have premenstrual symptoms and 5-8% of them is severely

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affected by PMS (4). In addition, PMS is seen in France at the lowest rate (12%) and Iran at the highest rate (98%) (7). PMS is also very common in our country and especially women in young age group are reported to have a high rate (66%-91.8%) of PMS in studies (8-10). Although the prevalence of PMS is influenced by cultural characteristics, PMS is a common female health problem. Given the fact that half of women's lives have passed through premenstrual period problems, it is important to raise awareness of health workers and to teach women the necessary self-care practices for PMS management. This review aims to present current approaches to PMS management accompanied by international guidelines.

**Etiology of PMS**

The causes of PMS are multifactorial and are still unclear (4,11). Nowadays, theories suggest that there is relationship between PMS and overian hormone levels, serotonin levels, and gamma-Aminobucyric acid (GABA) levels. The first theories were based on the fact that PMS was associated with ovarian hormone levels. The absence of PMS before puberty, during pregnancy, after menopause, during treatment with gonanotropin relasing hormone (GnRH) analogues suggests this situation (4,5). However, there is no significant difference between symptomatic and asymptomatic women in terms of progesterone levels (5). This is explained by the theory that some women are more sensitive to progesterone (4).

One of the theories is that serotogenic activity in the brain is influenced by overial hormones. Serotonin receptors are sensitive to estrogen and progesterone. Progesteron makes an individual prone to depression by increasing monoamine oxidase which is associated with the transport of serotonin, while estrogen creates an antidepressant effect. Therefore, low estrogen levels in luteal phase and high progesterone levels in luteal phase trigger depressive mood (4).

One of the theories in the etiology of PMS is based on the relationship between progesterone and GABA. Allopregnanolone, which appears as the metabolite of progesterone, regulates the level of GABA in the blood. GABA receptors are less susceptible to allopregnanolone, since they are exposed to high concentrations of allopregnanolone prior to the luteal phase. Therefore, low concentration of allopregnanolone in luteal phase causes anxiety, depressive mood and aggression. Indeed, allopregnanolon levels were shown to be lower in women suffering from PMS (4,5,12).

PMS is more likely to develop in women whose mothers have had severe PMS, and also PMS is more frequent in monozygotic twins than in dizygotic twins, suggesting that PMS has a genetic component (11). In addition to psychosocial theory, cognitive and social learning theories, and socio-cultural theory; BMI, exercise and diet are also thought to have a role in the etiology of PMS (3,13).

**Diagnosing PMS**

Symptoms of depression, anxiety, perimenopause, chronic fatigue syndrome, irritable bowel syndrome, and thyroid disease are similar to the symptoms of PMS (2,13). At the same time, half of women seeking treatment for PMS have at least one of these conditions. Depression and anxiety most often mimic the psychological symptoms of PMS. In this context, it should be determined whether the woman's mood disorder is related to the menstrual cycle (2). Therefore, women who are thought to be experiencing PMS should be consulted appropriately to rule out these diseases (5). Although there are no laboratory tests for PMS diagnosis, blood and urine analysis can be used to exclude other diseases (3,13). In addition, the relationship between PMS and sexual abuse and posttraumatic stress disorder is known (14). Therefore, it is important to take detailed anamnesis of medical, reproductive and menstrual conditions in order to evaluate these aspects (4,5).

The diagnosis of PMS is based on the type of symptoms and the time of emergence in the menstrual cycle (3). Premenstrual symptoms occur during the period covering the luteal phase of the endometrial cycle and ovulation phase of the ovarian cycle. The severity of symptoms increases just before the start of menstruation, and towards the end of menstruation, all symptoms are resolved. There is an asymptomatic period between the end of menstruation and the beginning of ovulation (5). In order to associate the symptoms with PMS, at least one physical and psychological symptom should occur five days before menstruation, these symptoms should end four days after menstruation, they should continue at least three menstrual cycles and should adversely affect daily activities and interpersonal relationships. It is also recommended that the structure of premenstrual symptoms be confirmed by the health worker (2). In this context, health workers should use PMS scales and prospective daily records held for at least two months (2,4,5). Thus, the diagnosis made with the scale can be confirmed by diary (5,15). Since women have difficulty in keeping a diary, practical mobile applications for this purpose have been developed. "PreMentricS" is one of these applications. It graphically documents premenstrual symptoms, helps healthcare workers diagnose and monitor treatment (5). These applications may be recommended for women in the young age group who are particularly concerned with technology.

**Management of PMS**

It is recommended that PMS Management be carried out gradually by a multidisciplinary team that has adopted an integrated holistic approach. At the same time, an individualized management plan should be applied because the number, type and severity of premenstrual symptoms vary from person to person (4,12). If PSM is mild to moderate, lifestyle changes and diet causes cure and if the symptoms start to have an adverse impact on daily life pharmacological treatment is recommended (2). First step in PMS management is to create awareness with education and consultancy, to teach women self-screening and self-care practices. These are followed by non-pharmacological and pharmacological strategies and surgical methods are applied as the last option (5,16) (Figure 1).

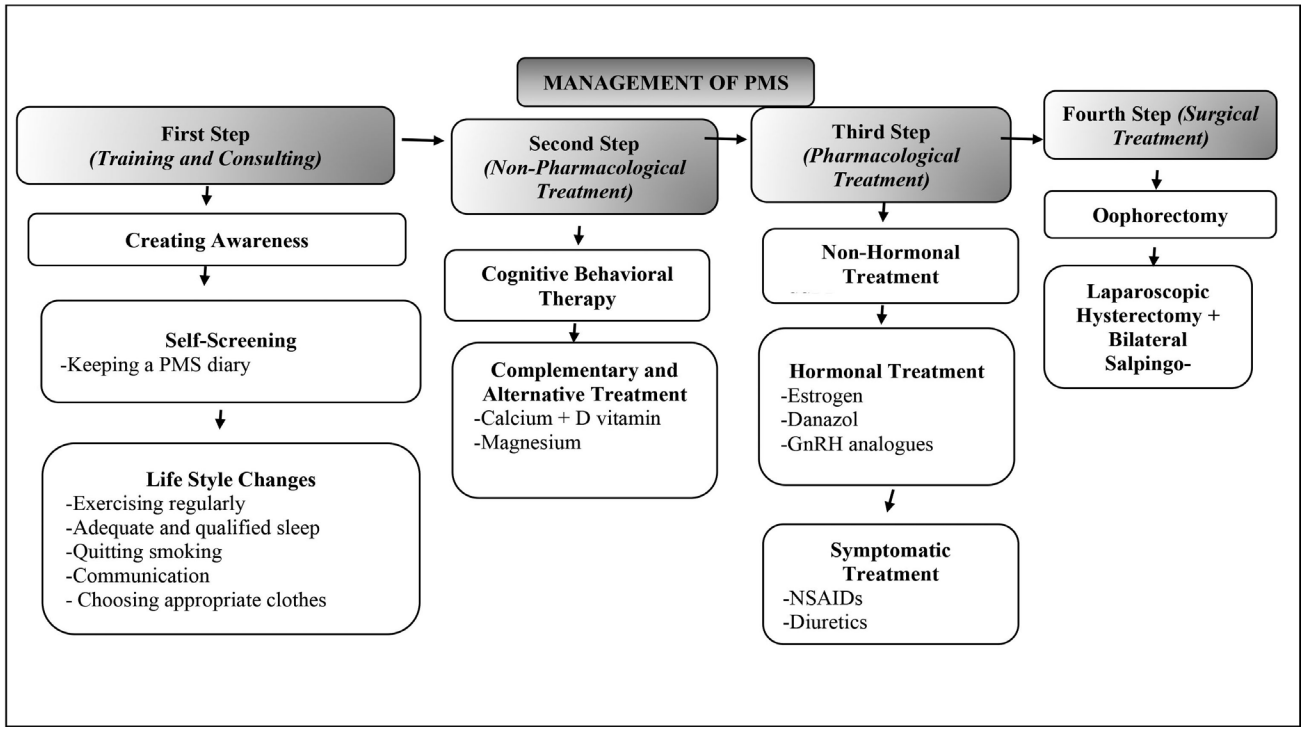


**Figure 1.** Premenstrual syndrome management

**1. First Step (Education and Consulting)**

**1.1. Raising Awareness**

Because most women feel premenstrual symptoms as required during the reproductive period, PMS is socially justified. For this reason, the vast majority of women do not seek treatment for PMS. Although this situation varies according to cultures, women in our country do not think that PMS can be treated and do not refer to the health care institution because they hesitate to talk to others about it (17). Therefore, health workers are advised to evaluate all women admitted to health institution during the reproductive period in terms of PMS, to organize trainings to make them aware of PMS management and to make them consulting (12).

**1.2. Self-Screening**

Women diagnosed with PMS should be informed about the events occurring on ovaries and uterus every month in order to create awareness of menstrual cycle. It should be explained that most women have PMS and can manage PMS. So the woman who has the awareness of PMS and the belief that PMS can be managed will ask the question "How can I help myself?". In this case, firstly, it is suggested that the woman should keep a PMS diary so that she can recognize the symptoms she has experienced and determine the type and severity of symptoms, when and how they occur, and how much they affect her life. For example, women who hold a diary can rearrange their meetings that correspond to the premenstrual period and that have the potential to pass through difficult, and begin to work early on their exams (17).

**1.3. Lifestyle Changes**

Most women can only cope with PMS by making lifestyle changes (18). In this context, it is important that health workers guide women. As lifestyle changes in the management of PMS;

-Women are advised to exercise at least 30 minutes a day. Aerobic exercises, including walking, running, cycling, and swimming increases heart rate, lung function, sleep quality and endorphin levels, while reducing fatigue and depressive mood (2,3,19,20).

-Adequate (at least 8 hours a day) and qualified sleep is recommended to reduce fatigue and depressive mood (3).

-Smoking is recommended to be stopped, because it is known that nicotine worsens premenstrual symptoms (3).

-It is recommended that women communicate with their spouses, family members and friends and share their feelings in order to be understood about how they feel during this period and to receive help from social support systems (3,13,21).

-If there is a general edema in the body, clothing which is not tight should be preferred; if edema is in feet, comfortable and supportive shoes should be worn; and if edema is in breasts, supportive bras and elastic waist band are recommended (3,22).

**1.4. Diet Regulation**

It is important to note that the prevalence of PMS in women with high BMI is higher and diet regulation is important in management of PMS (23). In this context, nutrition should be modified as follows;

-Six small meals, rather than three main meals per day should be preferred ( 2,3),

-Less fat, sugar, alcohol consumption and more fiber, vegetables and fruit consumption are recommended (2,3),

-To reduce irritability and insomnia caffeine (tea, coffee, cola) intake and to reduce edema, salt consumption should be reduced and natural diuretics such as grapefruit to should be added to diet (2,3,20),

-The consumption of whole grain bread, barley, brown rice, beans and lentils should be encouraged, as well as feeding with nutrients which contain high level of protein, pulp and complex carbohydrates can reduce psychological symptoms and craving (2),

-Iron-rich foods should be consumed with sources of vitamin C to replace iron lost in menstruation,

-Walnuts, chia seeds and flax seeds (1 spoonful/day) which are rich of omega-3 and fatty fishes should be added to the diet (24),

-Consuming calcium-rich foods such as yoghurt and green leafy vegetables is recommended (2),

-Healthy snacks (whole grain cereals, dried fruits, nuts, oily seeds) is recommended to be preferred (12,22).

**1.5. Coping With Stress**

Because stress increases the severity of PMS, coping with stress significantly simplifies PMS management (3). In this context, the recommended methods of coping with stress are;

-Breathing exercises,

-Relaxation exercises such as meditation and yoga,

-Having a bath, sleeping enough, dealing with a hobby, massage, biofeedback, autohypnoses and acupressure (22,25).

**Second Step (Non-Pharmacological Treatment)**

If daily life, work life or interpersonal relationships are negatively affected, then a woman is in search of PMS's treatment. In this case, non-pharmacological treatment in the second step is recommended to women. Non-pharmacological treatment methods include cognitive behavioural therapy (CBT) and complementary and alternative treatment (CAT) (3).

**2.1. Cognitive Behavioral Therapy**

In the management of PMS, with CBT which is a routine treatment option, the woman's cultural perception of PMS and negative menstrual attitudes are changed (3,4,15,22,26,27). At the same time, relaxation, stress management and assertiveness are provided with CBT. With a successful CBT, drug treatment can be avoided (3,4). Although the studies show that CBT do not provide rapid improvement in the management of PMS as well as antidepressant treatment, it is at least as effective as antidepressant treatment and has a longer lasting effect (5,12).

**2.2. Complementary and Alternative Treatment**

Although some CAT practices are known to be useful in the management of PMS, there is no advanced evidence that the majority of them are effective. In this regard, women should be advised to consult their physician before applying CAT. The physician should consider two points in CAT practices: The first is that the data obtained from clinical studies on CAT practices are limited and not strong. Second, if the individual has drugs to use, it is possible that drugs may interact with the CAT practices (2-5).

The most convincing of the recommended CAT practices in the management of PMS is calcium + vitamin D (2,4). Studies show that blood calcium and vitamin D levels are lower in women who suffer from PMS (4,12). Daily average 1000 mg calcium supplements are recommended to improve nearly all symptoms (15,16).

Another recommended CAT practice in the management of PMS is magnesium supplementation (2,4). Daily 400 g magnesium intake improves premenstrual symptoms associated with depressive mood and fluid retention. The combination of magnesium + B6 is also recommended in the management of PMS (16,28). In addition, there are studies showing that vitamin E, vitex agnus castus, saffron, ginkgo biloba, evening primrose oil, lemon grass (lemon balm), curcumin, wheat seed, isoflavones, St Jhon's Wort oil, multivitamins, refloxology and acupuncture are effective in the management of PMS, but there is insufficient evidence (2-5,12,13,16,20,22,29,30).

**Third Stage (Pharmacological Treatment)**

In PMS, there are treatment options suitable for etiological theories. The first option is non-hormonal therapy to increase the central seratogenic transmission, and the second option is hormonal therapy to increase the ovarian hormone level. At the same time, symptomatic treatment may be applied to improve some premenstrual symptoms (11).

**3.1. Non-Hormonal Treatment**

SSRIs are the first choice in non-hormonal therapies and their use increases serotonin activity in the brain to improve PMS (4,12,15,31). Indeed, studies show that SSRIs are effective in PMS management compared to placebo (5,16,31,32). However, some of the drugs in this group may have quite a lot of side effects (4). In this regard, women should be informed about the possible side effects of SSRIs such as nausea, insomnia, drowsiness, fatigue and decreased libido in continuous use (4,5). In order to avoid withdrawal symptoms, women should be told to stop taking the medication gradually. In addition, intermittent use of SSRIs may be recommended for the reduction of side

effects (4). While intermittent use of SSRIs is as effective as continuous use in reducing psychological symptoms, there is no similar situation in reducing physical symptoms (5). If SSRIs are to be used intermittently, they should be started before PMS occurs and continued throughout the luteal phase (4.5). In addition, if anxiety is a major problem among premenstrual symptoms and SSRIs can not solve this problem, an anti-anxiety drug is recommended (4). In addition, women receiving SSRIs should be advised that SSRIs are not appropriate for use during pregnancy (4).

**3.2. Hormonal Treatment**

Hormonal therapy can improve physical symptoms by suppressing ovulation and reducing hormonal fluctuations, while psychological symptoms can not be treated (3,4). Estrogen, danazol and GnRH are used in hormonal therapy.

**-Estrogen:** Oral contraceptives containing drospirenone are the first choice among hormonal interventions (4,33). It is reported that the use of hormone pills for 24 days and inactive pills for 4 days has improved significantly in PMS compared to placebo (5,15,33). Estrogen can be used as a transdermal patch and subcutaneous implant. However, oral contraceptives are not suitable for every woman as they pose a risk of venous thromboembolism. At the same time, if the estrogen is not met with progesterone, it causes hyperplasia in the breast and endometrium tissue, and the data on the safe dose of estrogen is insufficient. Since synthetic progestestin causes PMS-like symptoms, it should be given in minimal doses (the dose should not exceed the maximum amount produced from corpus luteum). Alternatively, progesterone can be applied directly to the endometrial layer with an intrauterine device containing levonorgestrel (4,5). If pregnancy is not desired, the woman should be informed about the use of a barrier method and not rely on estrogen to suppress ovulation (4). In addition, studies have shown that the use of older generation combined oral contraceptives and progesterone alone are not useful in the treatment of PMS (4,5,12,34)**.**

**-Danazol:** Low-dose danazol therapy at luteal phase is effective in reducing the sensitivity of the breasts. However, the potential for irreversible virilism should be taken into consideration. Because danazol may cause virilism also in the fetus, the woman should be told about the importance of using a reliable contraceptive method in this process (4).

**-GnRH:** GnRH analogues are highly effective in the treatment of severe PMS and are not recommended for use in routine. If there is a severe PMS that requires effective and long-term treatment, then GnRH should be preferred. Hot flashes, night sweats, insomnia and depressive mood are the short-term effects and vaginal atrophy, increased cardiovascular disease and osteoporosis risk are the long-term effects of hypoestrogenic condition. Therefore, GnRH analogues should not be used for longer than six months and if re-use is planned, bone mineral density should be evaluated prior to use it again (4,5,16).

**3.3. Symptomatic Treatment**

**-Nonsteroidal Anti-inflammatory Drugs (NSAIDs):** Analgesic and anti-inflammatory effects of NSAIDs that inhibit prostaglandin synthesis are used to improve PMS. NSAIDs can help reduce cramps, headaches, back pain, sensitivity in the breasts. However, women should be informed about the long-term use of these drugs, which may lead to stomach bleeding or ulcers (2,3).

**-Diuretics:** Diuretics used to treat severe premenstrual symptoms associated with fluid retention may cause undesirable effects when taken together with some drugs. For example, concomitant use of diuretics with NSAIDs may cause kidney problems. In this respect, the woman should be questioned whether she is taking another drug before the diuretic is recommended (2,4).

**Fourth Stage (Surgical Treatment)**

**4.1. Oophorectomy**

Oophorectomy alone removes PMS, but estrogen replacement is needed after surgery. In this case, preventive progesterone therapy is needed to prevent endometrial hyperplasia (5).

**4.2. Laparoscopic Hysterectomy + Bilateral Salpingo-oophorectomy**

Prevention of ovulation by performing hysterectomy with bilateral salpingo-oophorectomy is highly successful in the treatment of severe PMS (3,4). However, since hysterectomy + bilateral salpingo-oophorectomy is a serious treatment method, it should only be preferred if pharmacological treatment fails, long-term use of GnRH analogues is required or there is a different gynecological condition requiring surgery (4). This treatment method should be decided considering the anesthetic risks, surgical complications, infertility and the development of surgical menopause. If a hysterectomy + bilateral salpingo-oophorectomy is to be performed, it is recommended to be performed laparoscopically, because it is less invasive. Hormone replacement therapy is recommended for women under 45 years of age after the surgery (4,5). In addition, studies have shown that hysterectomy alone or endometrial ablation is not sufficient in the treatment of PMS (5,16).Prevention of ovulation by performing hysterectomy with bilateral salpingo-oophorectomy is highly successful in the treatment of severe PMS (3,4). However, since hysterectomy + bilateral salpingo-oophorectomy is a serious treatment method, it should only be preferred if pharmacological treatment fails, long-term use of GnRH analogues is required or there is a different gynecological condition requiring surgery (4).

## Conclusion

PMS is a common health problem, and women spend half their life with premenstrual problems and experience a reduction in self-confidence, social isolation, decline in academic achievement, increase in accident tendency and loss of Labor. With the management of PMS, the quality of life can be improved. The first step in PMS management is to create awareness, to make women self-screen, adopt lifestyle changes, apply nutrition

suggestions and use stress coping methods. If these methods are insufficient, CBT and CAT in the second step are applied. If PMS continues to affect daily life negatively; hormonal, non-hormonal and symptomatic treatment in the third step; oophorectomy or laparoscopic hysterectomy + bilateral salpingo-oopherectomy in the fourth step are applied. In this context, it is important for women suffering from PMS to be monitored by health workers and it is recommended to establish our national PMS management guide and to develop models in PMS management and to increase evidence-based research. At the same time, it is recommended that women who are admitted to primary health care institutions should be evaluated routinely in terms of PMS. Also it is recommended to give awareness training for young people in high schools and universities, to set up counseling units, and to organize social awareness campaigns using the press organs.

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Review

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# The Importance of Polyphenols as Functional Food in Health

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## ABSTRACT

Polyphenols which are produced by plants are very important functional foods in our nutrition. Because of their diverse chemical structures, they are subject to main classification among them. The main groups of polyphenols are; flavanoids, lignans, stilbenes and phenolic acids. At the present time, the health effects of them have been investigated in several *in vivo* and *in vitro* studies. In addition to their antioxidant effects, they are known to have a pro-oxidant character. Polyphenols have important effects in protecting the body against external factors and the cleansing of reactive oxygen species which occur as a consequence of some diseases. Furthermore, polyphenols have effects on protection from specific diseases and stop their progression with certain mechanisms. In this review, various effects of polyphenols including antioxidant, pro-oxidant, cytotoxic, anti-inflammatory, antihypertensive, anti-diabetic are discussed which obtained from *in vitro*, *in vivo* experimental animal and clinical investigations.

**Keywords:** Polyphenols, functional food, antioxidant, pro-oxidant, biological activities

## Introduction

Plant-based foods in our diet consist of primary and secondary ingredients produced by plants. Plants synthesize secondary metabolites such as terpenes, saponins, glycosides and polyphenolic compounds that are not present in all living things as well as primary metabolites such as carbohydrates, proteins and fats in order to maintain their growth and liveliness (1). Most of the secondary metabolites are consisted of polyphenols. Polyphenols make up most of the components found in all vegetative organs, fruits and flowers. Plants produce polyphenols as secondary metabolites to protect themselves and interact with other plants. Also, polyphenols have an effect on the formation of bitter taste in plants.

### Chemical Structure And Classification of Polyphenols

Polyphenols contain at least one aromatic ring and one or more hydroxyl groups, in addition to other components of them (2). According to their chemical structure, they are classified as follows: Flavanoid, Lignan, Stilben and phenolic acids. The main classification of polyphenols is shown in Figure 1.

### Flavonoids

All flavonoid species are formed as a result of the biosynthesis of shikimic acid and acetate-malonate pathways together (3). Flavonoid subspecies: a) Flavonol, b) Flavanol, c) Isoflavon, d) Flavon, e) Flavanon, f) Anthocyanin (4).

**a)** The richest sources of flavonols are onion (1.2 g/kg of wet weight), broccoli, leeks and blueberries. Tea and red wine contain about 45 mg/L flavonols. In general, these components are glycosylated with glucose and ramnose. Five to ten kinds of flavonol glycosides are in the content of fruits. For biosynthesis of flavonols in plants, exposure to sunlight is required. Greenish leaves of leafy vegetables such as lettuce and cabbage have 10 times more flavonol glycosides than the light-colored leaves inside (4).

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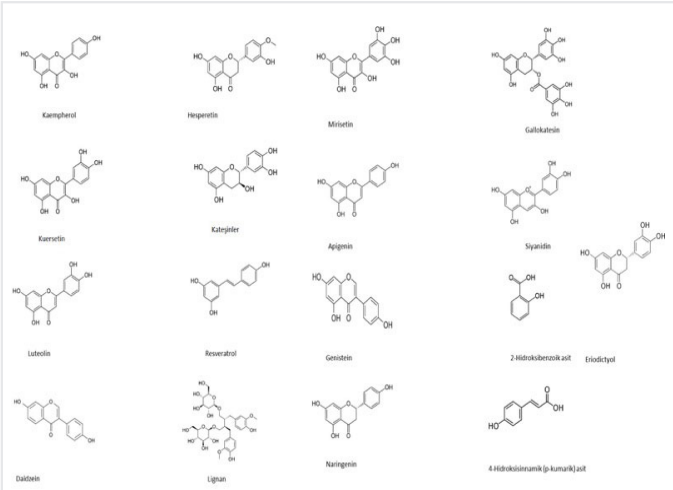


**Figure 1.** General classification of polyphenols

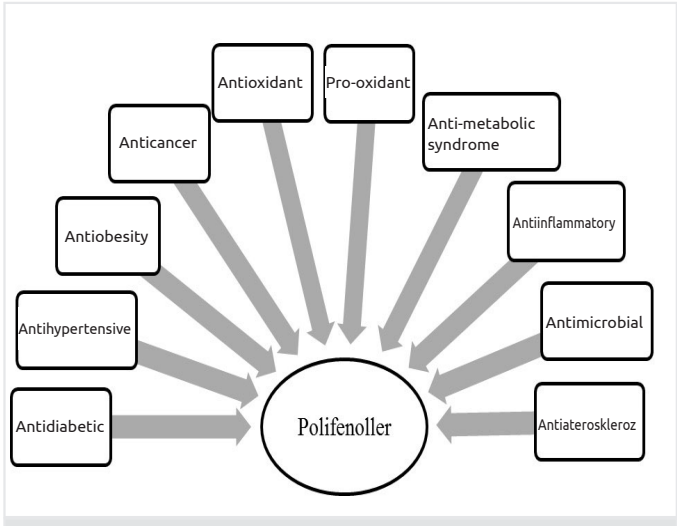


**Figure 2.** Biological activity of polyphenols

**b)** Flavanols have two forms: Catechin in monomer structure and proanthocyanidin in polymer structure. Most catechins are found in fruit species. The richest sources are green tea, cocoa and red wine. However, black tea contains less flavanol in monomer form because green tea is oxidized during fermentation but is rich in theaflavin and thearubigine which are polyphenols in polymer form (4).

**c)** Isoflavones are similar to estrogen as chemical structure and are most commonly found in soybean products. Because it binds to estrogen receptors in the body, it is classified as phytoestrogen. It usually contains three main molecules: Genistein, daidzein and glisitein (4).

**d)** Flavones are most commonly found in dry tea leaves (especially chamomile), parsley, celery, chicory (5).

**e)** Flavanones are found in citrus fruits with the highest concentrations. The most important flavanones are hesperidin, naringen and eriodictyol. The major flavanone glycosides in orange (*Citrus sinensis*) are narirutin, a derivative of hesperidin and naringenin (6).

**f)** Anthocyanins are found in eggplant, red fruits, cherry, strawberry and black grape (4). Anthocyanins are dominant pigments that give plants the color of red, purple and purple pink. The pigmentation of flowers is determined by the type and amount of anthocyanin in the contents of the flower (7). When lingonberry *(Vaccinium vitis-idaea)* which is grown in Poland and blueberries (*Vaccinium myrtillus L.*) are compared in terms of phenolic ingredients they contain, blueberries have shown more antioxidant activity. It was found that increased anthocyanin content in blueberry increased the effect of radical cleansing and antioxidant activity (8).

**Lignans**

Flax seed is the richest source of lignan called secoisolariciresinol diglycoside (SDG). The lignan complex isolated from flax seed contains 34-38% SDG, 15-21% sinnamic acid glucoside and 9.6-11% hydroxymethyl glutaric acid. It is metabolized by anaerobic intestinal microorganisms and converted to lignans called enterolactone and enterodiol (9).

**Stilbens**

The most common of stilben subtypes is resveratrol. It is found in grapes, red wine and some forest fruits. It is very limited to use in the body because it is taken in a very little amount in daily diet. However, recent studies were carried out to increase bioavailability of resveratrol in the body and to understand the various functions of resveratrol. It is known that it has anticancer effect and has remarkable properties in the treatment of neurodegenerative diseases (10).

**Phenolic Acids**

Phenolic acids are divided into 2 types: 1-) Benzoic acid and derivates, 2-) Cinnamic acid and derivates

**a)Benzoic acid and derivates:** The concentration of hydroxybenzoic acid in plants is usually very small, a certain amount of it is found in red fruits, onion and black radish (4).

**b)Cinnamic acid and analogues:** They are caffeic acid, ferulic acid and p-coumaric acid. Hydroxycinnamat, the major phenolic substance in the cherry, is found at highest concentration in the outer membrane of the cherry (11).

**Bioactivities of Polyphenols**

Polyphenolic compounds are known to have many biological activities. The most common features of polyphenols with a wide range of biological activities are their antioxidant and pro-oxidant characters (12). Figure 2 shows the biological activities of polyphenols. The potential role of polyphenols in preventing and/or treating chronic diseases such as cardiovascular and neurodegenerative diseases and cancer is extremely important which (13).

**Anti-oxidant Activity**

Reactive oxygen species (ROS) are characterized by a non-stable high-energy oxygen atom containing a double-formed electron in the outer orbitals (14). ROS are mainly composed of superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^-$). ROS production occurs in most aerobic organisms. Ultraviolet rays around us, radiation, cigarette smoke, aging, pathogenic invasion are factors that increase the formation of ROS (15,16). Low doses of ROS in the body play a role in cell signal transmission. However, excessive ROS cause some harmful effects. For example, oxidative stress occurs as a result of the imbalance between ROS production and metabolism. However, the development of oxidative stress and its effect on the cell depends mainly on the ability of the organism to maintain its dynamic balance of redox state (16). As ROS accumulate, the function of cells and tissues deteriorates and this accumulation causes various conditions such as cancer, atherosclerosis, and neurodegenerative diseases (17,18). ROS production occurs in plants as well as animals. Plants produce ROS to protect themselves against pathogens and external stress. Plants and animals use similar systems to respond to ROS (19). The human body needs endogenous antioxidants as well as exogenous antioxidants to minimize effects of ROS. In this respect, polyphenols that show antioxidant activity are extremely important in terms of human health (15). Polyphenols protect cells and tissues against oxidative damage by acting as antioxidants. They do this by cleaning radicals and by protecting DNA from oxidative damage and LDL from peroxidation (12).

Low doses of phenolic terpens called carvacrol in essential oils of plants from the *Lamiaceae* family were shown to play an important role in the prevention of diseases such as cancer due to their antioxidant activity (20).

The drug, cisplatin (CP), used in the treatment of cancer, has toxic effects on liver, kidney and genes. In a study conducted on rats, it was shown that when rats were first treated with naringenin-oxime (NOX) which is produced from naringenin and then CP was injected to rats, NOX showed an antioxidant effect and reduced the oxidative stress and DNA damage (21).

**Pro-oxidant Activity**

Polyphenols are often known for their antioxidant structure. However, high doses of some polyphenolic compounds were shown to cause DNA damage, apoptosis, and cell death. (12,20). Free radicals damage DNA, lipids and proteins in the cell. Phenoxyl radicals, which are produced during cleaning of free radicals by some plant polyphenols, oxidize proteins and lipids and thus show pro-oxidant activity. Flavonoids and tannic acid, for example, cause DNA degradation due to the formation of hydroxyl radicals as a result of Fenton Reaction in the presence of copper (II) (12,22). In addition, some flavonoids may show pro-oxidant activity in the presence of a free radical, NO (nitric oxide), and cause DNA damage (12,23). Although tea polyphenols are known as antioxidants, there is evidence that these components have pro-oxidant activity. High dose tea polyphenols show pro-oxidant activity in hepatocytes and cause toxicity (24). High doses of polyphenols may inhibit carcinogenesis as a result of their effects that initiate apoptosis. Carvacrol and tea polyphenols increase ROS in cancer cells with a dose-dependent manner which is a sign of their pro-oxidant activity (16,20). Therefore, the dose of antioxidants in the treatment is extremely important.

**Anti-cancer Effect**

Since the normal level of antioxidant defense system in the cell is often insufficient to neutralize free radicals of endogenous or exogenous origin, there is a need for antioxidants of exogenous origin (25).

Polyphenols have a protective effect against certain types of cancer. They inhibit the growth of tumors, and on the other hand they have cytotoxic effects on cancer cells, induce apoptosis in cancer cells and stimulate the activity of various enzymes (25). Cytotoxic effects of polyphenolic compounds on cancer cells were found to be associated with their pro-oxidant activities. Pro-oxidant activity is related to the dose and molecular structure of polyphenolic compounds, oxidative state of the environment and the presence of free iron and copper.

An *in vitro* study on human breast cancer cells revealed that, between 5 flavonoids, myricetin showed the highest cytotoxic effect in the presence of copper ions. This suggests that the chemical structure of the polyphenols is different and that their cytotoxic effects on cancer cells are different (22).

In an *in vitro* study, the effect of polyphenols which are abundant in grape seed and shell contents on human epidermoid carcinoma cells was investigated. According to the results, grape seed and shell stimulated apoptosis in skin cancer cells and caused various morphological changes. Therefore, alkaloids, flavonoids, saponins and tannins in grape content are of therapeutic importance (25). Curcumin, the main active ingredient in turmeric, which has been used as a spice for many years, is a pigment and phenol that stands out with its anti-cancer and anti-inflammatory properties. Curcumin inhibits the division of tumor cells and increases the rate of death of cancer cells in some types of cancer. For example, in clinical trials performed on curcumin, it was determined that it was effective on breast, pancreas, and stomach cancers (26). Tea and tea polyphenols have a protective and therapeutic effect on many organs. The potential antioxidant effect of tea polyphenols was observed in different animal carcinogenesis models. In these models polyphenols inhibit carcinogenic activity by activating various enzymes such as detoxifying glutathione S-transferase and superoxide dismutase, and they prevent the onset of cancer. According to results obtained from *in vivo* experiments, tea and tea polyphenols are antioxidants, as well as they inhibit colonization of tumor-initiating cells. Thus, it restricts the progression of carcinogenesis to early dysplastic stage in the treatment process (27).

**Anti-atherosclerotic Effect**

Polyphenols from various plant foods have a protective effect on cardiovascular system by various mechanisms (preventing oxidation of LDL, increasing HDL, reducing LDL and triglycerides). The most studied herbal foods with anti-

atherosclerotic proporties are; extra virgin olive oil, bitter chocolate, black and green tea, forest fruits, citrus fruits and grapes (28). LDL, which is made up of lipophilic structures, is one of the causes of atherosclerosis when oxidized. Several studies have shown that some polyphenols protect LDL from oxidation (4). Olive oil polyphenols protect LDL from oxidation and also contribute to down regulation of gene expression that causes it (29). It is reported that resveratrol (30) and tannic acid (31) have anti-atherosclerotic effects by improving endothelial dysfunction in cell culture studies and animal and human experiments.

**Anti-metabolic Syndrome**

Metabolic syndrome is a condition caused by the clustering of cardiometabolic factors such as dyslipidemia, high blood pressure, blood sugar regulation disorder, abdominal obesity (32). The possible mechanism of influence of polyphenols on metabolic syndrome is to improve these factors.

In a study of Polish adults, the association between daily polyphenol intake and prevalence of metabolic syndrome was examined. When daily polyphenol intake was grouped according to age, taken total energy and gender, significant results were achieved (33). The body mass index (BMI) , waist circumference (WC), blood pressure and triglyceride levels were significantly lower in individuals taking more polyphenols, while a linear correlation was found only with WC and BMI. High polyphenol intake in women was negatively correlated with blood pressure, high LDL cholesterol and triglyceride levels, whereas high polyphenol intake in both genders was negatively correlated with fasting plasma glucose. Polyphenols including phenolic acid and stilben are significantly associated with metabolic syndrome. It was found that lignans and stilbens were associated with WC, phenolic acids with blood pressure and triglycerides and flavonoids with fasting plasma glucose (33).

Basu et al. randomized 35 individuals with obesity and metabolic syndrome into groups consisted of 3 individuals based on age and gender in a randomized controlled clinical trial. Water (4 cups/day), green tea (4 cups/day) and green tea extract (2 capsules/day) were randomly given to individuals in the groups for 8 weeks. In binary comparisons, weight loss and reduce in body fat mass in individuals who consumed green tea or green tea extract compared to control group (34).

Curcumin has a significant protective effect on fatty liver disease, which can develop due to the fat dysfunction seen in the metabolic syndrome. In a prospective cohort study of 100 patients with metabolic syndrome, patients were given 400 mg of curcumin per day for one year. At the end of the study, morphological changes in liver were visualized by ultrasound and improvement was observed (35).

**Anti-obesity Effect**

Polyphenols affect obesity by inhibiting pre-adipocyte differentiation, reducing adipocyte proliferation, stimulating adipocyte apoptosis, suppressing lipogenesis and contributing to lipolysis and beta oxidation of fatty acids in *in vitro* conditions.

The anti-obesity effect of epigallocatechin gallate (EGCG) and green tea extract has been shown in cell culture, animal and human models (34,36). On the other hand, EGCG reduces the release of resistin, an inflammatory adipokine, which is a derivative of adipocyte. Resistin is also associated with insulin resistance and cardiovascular disease risk. Because visceral fat stores contribute to chronic inflammation in obesity, it is important to reduce oxidative stress and ROS molecules (37). In this regard, since green tea acts as an antioxidant, it can be effective in reducing the destructive effect of ROS molecules and in suppressing the inflammation in the development of obesity (10).

**Anti-hypertensive Effect**

Cocoa is a plant that is extensively searched in scientific interventional research on human beings. Cocoa beans contain polyphenolic component up to 6-8% of their dry weights. Cocoa accommodates plenty of flavonols, especially epicatechin, catechin and procyanidin within. It is known that flavanol-rich cocoa products improve endothelial function, reduce the sensitivity of LDL to oxidation, and increase endothelium-dependent vasorelaxation (38,39). The effect of cocoa flavanols on endothelium-dependent vasodilation is accepted by the European Food Safety Authority ( EFSA) (38).

In a study by Grassi et al. (39), 19 hypertensive patients with glucose tolerance disorder were randomized and a group of patients were given 100 grams of bitter chocolate rich in flavanol for 15 days and the other group was given the same amount of flavanol-free white chocolate. Fifteen days later, treatments were exchanged. Insulin resistance and systolic and diastolic blood pressures decreased and insulin sensitivity increased in bitter chocolate eaters. In a meta-analysis, it was found that the quercetin was significantly effective in reducing blood pressure (40).

**Anti-microbial Effect**

Anti-microbial activity of polyphenols on a wide range of microorganisms is investigated. However, especially flavonoids have more anti-microbial effects than other polyphenols. In addition, most flavonoids are highly important because they suppress microbial virulence and show synergism with antibiotics (41).

In a study on thyme plant rich in polyphenols and flavonoids, the antibacterial effect of thyme extract on gram-positive bacteria (*Staphylococcus* and *B.subtilis*) and gram negative bacteria (*E.coli* and *P. Aeruginosa*) was investigated. According to this; thyme extract inhibited the growth of tested bacteria from medium to high level. Maximum effect was observed against *P.aeruginosa* (42). EGCG, the tea polyphenol, has anti-microbial, antifungal and antiviral effects. It inhibits the binding of the influenza virus to the target receptor on the cell (41). In a study by Seo and Choi (43), mouse raw 264.7 macrophage cells were treated with five flavonoids including EGCG, quercetin, fisetin, daidzein and ECG. According to this, the release of antiviral agents was regulated up by all flavonoids and neuroviruses were significantly inhibited (43). Polyphenols in olive oil provide bacterial

inhibition. Various extra virgin olive oils obtained from different geographical regions of Turkey have provided microbial inhibition of some important nutrient pathogens (*E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella enteritidis*). However, this effect was not observed in refined olive oil, hazelnut and canola oils (44).

**Anti-diabetic Effect**

A diet rich in polyphenols reduces the risk of development of diabetes. Various polyphenols positively regulate insulin and (glucagon-like peptide-GLP-1) pathways. In addition, polyphenols increase insulin sensitivity in peripheral tissues (45). In a randomized and controlled study of overweight/obese adult individuals; the effect of diets rich in polyunsaturated fatty acids, in long chain omega-3 fatty acids and in both on blood glucose for 8 weeks. Foods rich in polyphenols are bitter chocolate, extra virgin olive oil, decaffeinated coffee green tea, cranberry jam and polyphenol-rich vegetables. In a study, polyphenols taken with diet reduced blood glucose levels and increased early insulin secretion in 3-hour OGTT (oral glucose challenge test) at the end of eight weeks compared with 3-hour OGTT performed before the study (46). It is known that cinnamon has many beneficial effects on hyperlipidemia and use of glucose. In a study conducted on rats, cinnamon extract reduced visceral fat mass, liver weight, serum glucose level and insulin concentration (47). Palm has gastric-protecting, anti-cancer, anti-inflammatory, and anti-hyperglycemic effects and is a very common fruit in some communities. Palm contains phenolic acids from polyphenols, lignans, isoflavones, flavonoids and tannin. In addition, it strongly inhibits the α-glucosidase and α-amylase enzymes and thus regulates the absorption of glucose from the intestines and contributes to the normal glucose balance. Therefore, it is a potentially effective fruit in preventing and controlling diabetes mellitus (48).

**Anti-inflammatory Effect**

Some plant polyphenols have effects on immune system and inflammatory cells. Some phenolic agents influence cytokine and cytokine receptors, while others affect the secretory process (12).

Chlorogenic acid is a phenolic compound formed by esterification of caffeic acid and quinic acid. This compound has been shown to have an anti inflammatory effect in various animal experiments. Ohkawara et al. (49) injected 20mg/kg or 40mg/kg chlorogenic acid to rats, and pancreatitis was subsequently initiated. The histological effects of pancreatitis decreased in rats given 40 mg/kg chlorogenic acid. The macrophage migration inhibitory factor (MIF) level, which is a pro-inflammatory cytokine, decreased in serum and pancreas (49).

The grape peel extract, rich in Anthocyanidin, when combined with a high-fat diet on mice caused various metabolic changes (weight gain, dyslipidemia, insulin resistance, change in some hormones released from adipose tissue), but inflammatory markers such as TNF-a, IL-6 decreased significantly (50).

## Conclusion

Polyphenolic compounds, which are secondary metabolites produced by plants, are a large group of molecules of different chemical structure. In addition to the effects of polyphenols on many diseases such as anti-hypertensive, anti-microbial, anti-obesity, anti-diabetic effects in humans and animals; the most remarkable and well-they antioxidant effect prevents the formation of ROS when used in low doses and prevents many diseases such as cancer. However, high doses of certain polyphenols may have a pro-oxidant effect and cytotoxic and apoptotic effects especially on cancer cells. This situation also offers the option of polyphenols for the treatment of such disease.

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**Authorship Contributions**

Concept: A.G.B., Design: A.G.B., Data Collection or Processing: A.G.B., A.A., Analysis or Interpretation: A.G.B., A.A., A.K., Literature Search: A.A., A.G.B., Writing: A.G.B., A.A., A.K.

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Case Report

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# Posterior Reversible Encephalopathy Syndrome in a Thalassemia Minor Woman after *in vitro* Fertilization

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## ABSTRACT

Posterior reverzibl ensefalopati (PRES) sendromu, nöbet, başağrısı, vizüel defisitler, fokal nörolojik bulgular ile karakterize, bilinç düzeyinin değişik derecelerde etkilenebildiği bir klinik antitedir. Literatüre göre, preaklampsi ile ilişkili PRES olguları daha iyi seyirli ve kendisini sınırlayan bir tablo gibi görünse de, *in vitro* fertilizasyon sonrası gelişen gebelikler sonrası gelişen PRES seyri ile ilgili veriler oldukça sınırlıdır. Bu olgu sunumunda, *in vitro* fertilizasyon sonrası ikiz gebelikle sonuçlanan gebelik dönemi sonrası, doğum eyleminin hemen sonrasında PRES gelişen 40 yaşında talasemi minör tanılı bir primigravida tartışılmıştır.

**Anahtar Sözcükler:** Posterior reveverzibl ensefalopati, *in vitro* fertilizasyon, talasemi

## Introduction

Posterior reversible encephalopathy syndrome (PRES) as a complication of severe preeclampsia occurs most frequently in the third trimester of pregnancy and may evolve unfavourable outcomes increasing fetal and maternal mortality (1). Herein, we report a thalassemia minor patient with twin pregnancy who developed PRES. Most studies indicate that IVF-pregnancies and oocyte donation are associated with more obstetric complications increasing risk of maternal mortality when compared to naturally conceived pregnancies (2-5).

## Case Report

We present a case of 40-year-old thalassemia minor primigravida who underwent *in vitro* fertilization (IVF) ensuing twin pregnancy. At the gestation age of 36 weeks and 3 days, cesarean section (c/s) was performed under spinal anesthesia. The new borns were two girls weighing 2130 and 2060 g. Two hours after the c/s, patient experienced generalized tonic clonic seizures and lost her consciousness.

She was reported to have high arterial pressure up to 180/110mmHg during c/s. She did not have any history of hypertension and documented blood pressures during her pregnancy were normal.

Systemic examination was unremarkable except the high blood pressure (around 170/100mmHg). Neurologic examination after postictal period revealed loss of vision in both eyes with regard to perception of hand movements. Pupillary reactions and fundoscopy were normal and plantar reflexes were flexor. Biochemical blood tests including liver and renal functions, coagulation and blood count were all normal except urine examination which showed 1+ proteinuria.

Cranial magnetic resonance imaging (Philips Medical Systems 1.5 Tesla, The Netherlands) showed diffusion restriction (Figure 1) with focal ADC map hipointensity involving bilateral occipital lobes and FLAIR hyperintensities were spotted which were suggestive of typical PRES findings (Figure 2,3) .

Treatment with magnesium sulphate (Onfarma, Samsun, Turkey) infusion was started immediately. She required addition

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of calcium channel blocker and beta blockers to restore blood pressure. Levetiracetam (UCB Pharma, İstanbul, Turkey) was also added and the dose increased to optimal level till magnesium infusion was stopped. The patient gained full consciousness after seizure and aggressive blood pressure control. She was discharged from intensive care unit to neurology service next day.

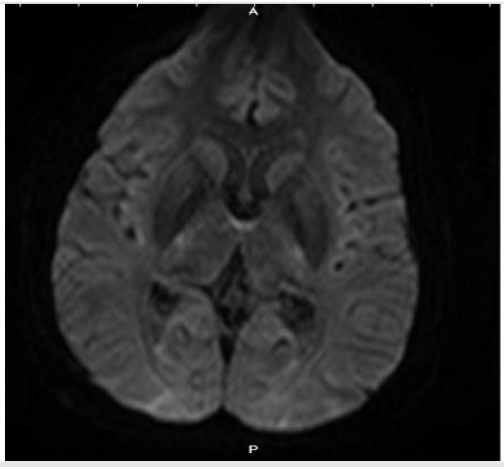

**Figure 1.** Diffusion restriction at bilateral occipital lobes at DW scan

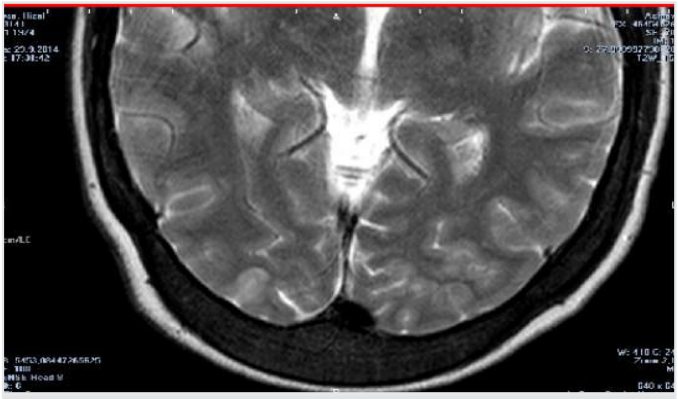

**Figure 2.** FLAIR hyperintensities at bilateral occipital lobes with cortical thickening on right

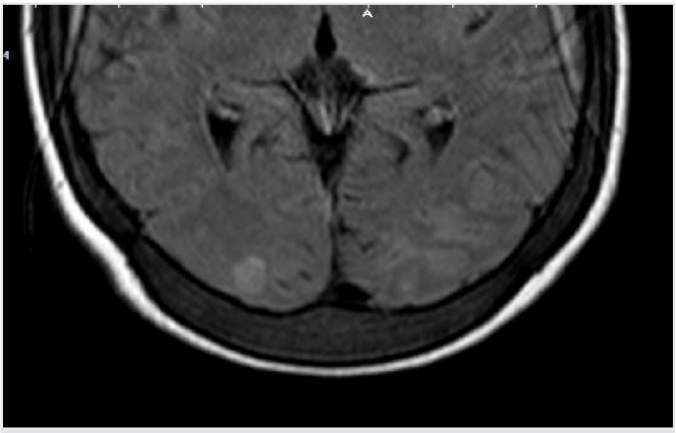

**Figure 3.** T2 hyperintensities at bilateral occipital lobes with cortical thickening on right Diploe thickening at cranial bones in Thalasemia minor patient

Patient's clinical condition was stabilized but the next day she had difficulty in breathing and oxygen saturation from the pulse oximetry decreased till 75. She was diagnosed to have pulmonary embolism. Low molecular weighted heparine was initiated after diagnosis.

All the symptoms and improved and disappeared completely on day 6 of hospitalization and she was discharged. The MRI was reviewed 2 weeks later which showed complete disappearance of the previous imaging findings. Levetiracetam was stopped gradually in the third month as both MRI and EEG were normal. She currently has no discomfort.

The informed consent was taken from the patient.

## Discussion

PRES is a clinical entity characterized by acute or subacute clinical presentation of altered mental status, encephalopathy, headache, new onset seizures, and visual disturbances.

Neuroradiologic findings are essential in diagnose of PRES. The typical MRI lesions are symmetrical white matter vasogenic edema, usually located parieto-occipital region sparing calcarine and paramedian regions, which may show progression to cytotoxic edema, infarctions, hemorrhages, laminar necroses and glioses (2).

Although PRES is generally assumed to be related to hypertensive encephalopathy, there is a long list of predisposing factors and diseases including inflammatory or auto-immune diseases, electrolyte imbalances, spinal injuries, vasoactive drugs and chemotherapy agents, preeclampsia and eclampsia. Preeclampsia is a significant, multifactorial, multiorgan disease which is one of the five major causes of maternal death worldwide (2). It is classically characterized by increase in blood pressure and proteinuria in the third trimester. It is known that IVF, twin pregnancy and women being pregnant at advanced age are all risk factors for pre-eclampsia. IVF-pregnancies are associated with more obstetric complications and increased risk of maternal mortality than naturally conceived pregnancies. An increased risk of preeclampsia, gestational hypertension and pregnancy induced hypertension especially in oocyte-donated pregnancies, when compared *in vitro* fertilization with own oocytes and natural conception. For nulliparous women this risk is even more significant (3). In another study, the risk factors independently associated with preeclampsia in twin pregnancies were reported as egg donation and pre pregnancy obesity (4).

It is shown that PRES relating to preeclampsia tends to present better evolution and reversibility. There is very limited data in the literature about the prognosis in PRES cases related to preeclampsia after IVF treatment. There are reports of maternal death after oocyte donation due to neurologic complications and pregnancy terminations at early gestational age due to preeclampsia (5).

PRES is a life-threatening neuro-radiological entity which tends to be reversible once the underlying cause is identified and treated

early. It should be kept in mind that in vitro fertilization, twin pregnancy, nulliparity and advanced maternal age may increase the risk of developing this entity as stated in our case.

**Ethics**

**Informed Consent:** A consent form was completed by all participants.

**Peer Review:** Externally and internally peer-reviewed.

**Authorship Contributions**

Analysis or Interpretation: S.A., Literature Search: P.G., Writing: P.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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Case Report

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# Enostosis of Clavicle Causing Severe Dyspnea by Compressing the Trachea Externally: Case Report

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## ABSTRACT

Clavicle is the bone that forms anterior border of shoulder arch. It lies on anterosuperior of thorax with first rib. Clavicle is very near to major vascular structures, brachial plexus, esophagus and trachea at thoracic inlet. Because of this, clavicular lesions fractures and sternoclavicular dislocations -especially posterior dislocations- may cause symptoms due to compressing symptoms due to these structures. In this article we present a case with enostosis of clavicle causing respiratory failure by compressing on trachea.

**Keywords:** Clavicle, enostosis, repiratory failure

## Introduction

Clavicle is the bone that forms anterior border of shoulder arch. It lies on anterosuperior of thorax with first rib (1). At the upper thoracic inlet, around sternoclavicular joints area, both of clavicle are anotamically near with subclavian arteries and veins, both brachial plexuses esophagus and trachea, both carotid arteries and jugulary veins. Because of nearness to major vascular structures, brachial plexus, esophagus and trachea at thoracic inlet clavicle lessions, fractures and sternoclavicular dislocations -especially posterior dislocations- causes symptoms due to these structures being affected

## Case Report

A six-year-old girl was referred to pediatric pulmonology outpatient clinic with complaint of irritative cough and dyspnea that continues for a long time in spite of medical treatment. Three months before, she was hospitalized in intensive care unit because of occurrence of respiratory failure after a caughing crisis. Flexible bronchoscopy revealed a pulsatile externally compressing lesion on anterior wall of trachea (Figure 1).

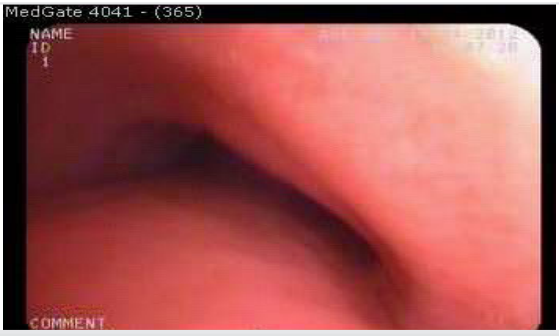


**Figure 1.** Pulsatile externally compression narrowing the lumen of trachea on right anterolateral

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Thorax CT angiography revealed a lession protruding into mediastinum which was thought to behyperthrophy of the bone or osteophyte. It was below the right subclavian artery and was compressing on trachea at the junction of right common carotid artery and right subclavian artery (Figure 2).

Mediastinal vascular structures were normal. On physical examination, thoracic inlet was close-fitting because of wide interclavicular ligament. It was thought that pulsatile, external compression seen at flexible bronchoscopy was due to the lession of clavicle, and operation was decided. Clavicle was reached by an incision started at upper point of sternum through laterally to the medial head of clavicle. Approximatelly, 1 cm long and 4-5 mm wide, hard and tight interclavicular ligament was seen and excised. Head of right clavicle was explored by opening the periost (Figures 4-5).

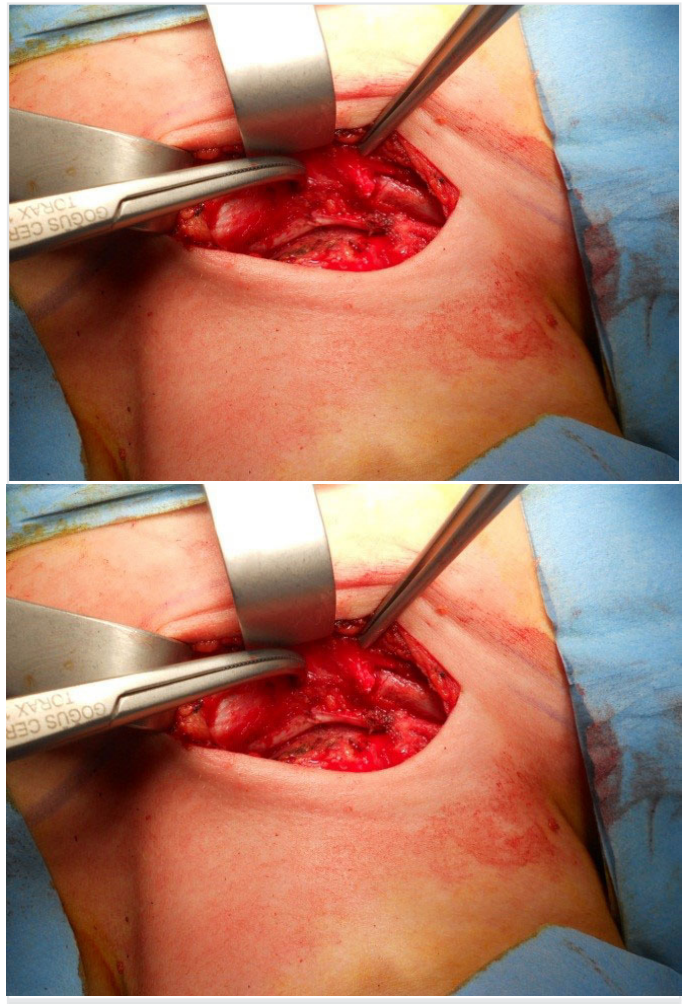

**Figure 2.** Preoperative thorax CT angiography: Hypertrophic osseosis lession on the head of right clavicle compressing on vascular structures and the trachea


**Figure 3.** Postoperative thorax CT: Lession excised, Shape of the trachea and vascular structures are circle

Hypertrophic tissue protruding posteriorly was excised. Mediastinum was controlled by finger disection as it was relaxed. Procedure was finished after placing a minivac drain into the disection area. At postoperative CT, compression on mediastinal structures was disappeared (Figure 3). The patient was discharged on the first postoperative day. Pathology was reported as enostosis (bone island). The patient was seen on the postoperative sixth month and she was having neither cough nor dyspnea.

## Discussion

Posterior dislocations may cause some symptoms due to compression of head of clavicle on contiguous structures and injuries or lacerations may occur due to direct effect or trauma. In the literature, there have been cases of injury or laceration of innominate vein, innominate artery, esophagus, trachea, brachial plexus, and even vena cava or compression on vena cava because of posterior dislocation and cases having symptoms due to compression of tumours of head of clavicle (2-9). We did not



**Figures 4-5.** Thick interclavicular ligament and view after interclavicular ligament excised and clavicle was turned subperiostally

encounter such a case withenositosis in the literature. Pathologies on this area may have crucial risks. Until now, mortality because of posterior dislocation of clavicle due to injury was reported in five cases (10). In this article, we aimed to emphasize the importance of pathologies in the head of clavicle in cases with external compression to trachea causing respiratory failure.

Bone Iisland (enositosis) is a focus of compact bone located in cancellous bone. This is a benign entity that is rarely symptomatic and that is usually found incidentally in radiological studies. Symptoms are pain and protuberance due to enlargement of the lesion. In our case, in physical examination there was no protuberance but it was determined that thoracic inlet was close-fitting because of wide interclavicular ligament. In radiological examination, there was a lesion on the head of right clavicle protruding to mediastinum which was considered as hyperthrophy or osteophyte. Under this lesion, right subclavian artery was bending to posterior and the lesion was compressing the trachea at the junction of right common carotid artery and right subclavian artery.

Tracheal stenosis caused by external compression may cause recurrent pulmonary infections, symptoms like wheezing, chronic cough and even respiratory failure. Diseases with vascular abnormalities, aneurysms, mediastinal masses, lymphadenopathies may cause tracheal stenosis. Lesions on the head of clavicle are extremely rare. In differential diagnosis of this clinical situations, lesions compressing to trachea externally, and foreign body aspirations must be considered. For diagnosis, flexible bronchoscopy and thorax CT with intravenous contrast must be used. So, that localization and severity of stenosis, and lesion causing stenosis may be determined and treatment may be planned.

In cases with external compression to trachea causing respiratory failure, pathologies of the head of clavicle must be kept in mind. Bone island is one of them. In treatment, removing the compression on vascular structures or trachea by excising the head of clavicle partially may be performed and results are satisfactory in terms of symptoms.

**Ethics**

**Informed Consent:**

**Peer Review:** Externally peer-reviewed.

**Authorship Contributions**

Concept: S.Z., O.C.A., Ö.S., Design: N.M.E., E.Ç., N.B., Data Collection or Processing: Ö.S., M.B., A.K., Analysis or Interpretation: M.B., N.B., N.M.E., Literature Search: O.C.A., Writing: S.Z., O.C.A., Ö.S.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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Case Report

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# Pseudohypoparathyroidism Type Ia with Normocalcemia

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## ABSTRACT

Pseudohypoparathyroidism (PHP) is a heterogeneous group of disorder with parathormone target organ resistance, characterized by hypocalcemia, hyperphosphatemia and high blood parathormone (PTH). Typical phenotypic symptoms and additional hormonal resistance can be observed in type Ia, which is also known as Albright hereditary osteodystrophy. Our patient was an eight-year and nine-month old girl with typical Albright's hereditary osteodystrophy phenotype including short stature, obesity, round face, low nasal bridge, shortened metacarpals, and mild mental retardation. In her biochemical examination, high PTH level and hypothyroidism is detected in spite of normal calcium and phosphor levels. As a result of clinic and laboratory tests, the findings were consistent with PHP type Ia with normocalcemia. In her guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1 (*GNAS 1*) gene serial analysis, C-308T>C (p1103T) transformation was detected, which was previously reported in a PHP type Ia patient. In this report, we've aimed to emphasize the fact that calcium and phosphor level in the blood of the patient with PHP type Ia can be measured normal.

**Keywords:** Pseudohypoparathyroidism, albright hereditary osteodystrophy, normocalcemia, short stature

## Introduction

Pseudohypoparathyroidism (PHP) is an autosomal dominant disorder, which is related with parathormone target organ resistance resulting from mutations in guanine nucleotide binding protein (G protein) and alpha stimulating activity polypeptide 1 (*GNAS1*) genes. It is characterized by hypocalcemia, hyperphosphatemia and elevated PTH levels (1). The disorder occurs as a result of maternal transmission of the mutation. As Gs alpha protein activity is necessary for other hormones such as thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), resistance against these hormones may also exist (2-6). Characteristic phenotypic features such as short stature, obesity, round face, low nasal bridge, shortened metacarpals, shortness and thickness in distal phalanges, subcutaneous calcifications, polyostotic fibrous dysplasia, developmental delay, and besides, mental retardation can be observed in these patients (1,7). This phenotype is named as "Albright hereditary osteodystrophy" (AHO).

Some cases with PHP type Ia are present with heterogeneity in terms of their phenotypical and biochemical characteristics (8-11). In this paper, an eight-year and nine-month-old female with normocalsemic PHP type Ia is reported.

## Case Report

Eight-year and nine-month-old female patient was admitted to the hospital with the complaint of having short stature with a height of 117.2 cm (<3p, -2.5 SDS) and weight of 28.9 kg (50-75p, 0.124 SDS). She was term born, birth weight was 3100 gr (25-50 p) and birth height was 50 cm (50-75 p), respectively. Her parents were

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third-degree relatives. Her father's and mother's heights were 165 cm (-1.65 SDS), and 135.9 cm (-4.2 SDS), respectively. She had healthy four siblings. Her physical examination at presentation showed a short stature, obesity, round face, low nasal bridge, and shortened metacarpals (picture 1 and 2). Her pubertal stage was in tanner phase 1. She was evaluated as mentally retarded in a mild degree.

**Figure 1.** Brachydactyly

**Figure 2.** Short stature, round face, obesity

Skeletal maturation assessment by direct hand wrist radiography revealed shortened metacarpals and metatarsals (picture 3) and her bone age was consistent with her age. Her thyroid ultrasonography and cranial magnetic resonance imaging were both reported as normal. Hypothyroidism and elevated PTH levels with normocalcemia and normal 25-OH vitamin D levels were detected in her laboratory tests. Laboratory findings including complete blood counts, biochemical parameters, TSH, free T4, calcium, phosphorus, alkaline phosphatase, PTH level, 25-hydroxyvitamin D, insulin like growth factor 1 (IGF-1) were given in Table 1.

As a result of clinic and laboratory tests, the findings were consistent with PHP type Ia with hypotyroidism and normocalcemia, her GNAS gene sequencing analysis was performed; C-308T>C (p1103T) transformation was detected, which was previously reported in PHP type Ia patient. Oral L-thyroxine treatment was initiated to the patient. Her growth velocity and calcium and phosphorus levels are still on follow-up.

Written informed consent was obtained from the patient's parents for publication of this case report.

**Figure 3.** Hand and wrist radiograph with diagnosis of brachydactyly and bone dysplasia

**Table 1.**

| | | | |
|---|---|---|---|
| Hemoglobin (g/dL)* | 12.9 (11-13)** | ALT (U/L) | 15 (8.7-39) |
| MCV (fL) | 85.6 (75-90) | AST (U/L) | 192 (100-500) |
| Platelet (10*3 u/L) | 227 (142-424) | fT4 (pmol/L) | 8.71 (11.2-18.6) |
| Leucocyte (10*3 u/L) | 7.2 (6-17) | TSH (mIU/L) | 9.2 (0.51-4.82) |
| Sodium (mmol/L) | 140 (139-146) | Calcium (mg/dL) | 9.7 (8.8-10.8) |
| Potassium (mmol/L) | 4.4 (4.1-5.3) | Phosphorus (mg/dL) | 5.8 (3.78-6.19) |
| Chlorine (mmol/L) | 105 (98-106) | ALP (U/L) | 205 (135-537) |
| BUN (mg/dL) | 12.5 (5.1-16.8) | PTH (pg/mL) | 436.9 (15-68.3) |
| Creatine (mg/dL) | 0.44 (0.35-0.59) | 25 OH vit D (ng/mL) | 28 (20-70) |
| Fasting Glucose (mg/dL) | 80 (50-80) | IGF-1 (ng/mL) | 197 (51-303) |

MCV: mean cell volume, BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, fT4: free tetraiodothyronine, TSH: thyroid stimulating hormone, ALP: alkaline phosphatase, IGF-1: insuline like growth factor-1, *Laboratory measurement units; **Reference Values

## Discussion

Pseudohypoparatyroidism Ia is the most prevalent PHP type. Along with typical phenotypic findings, cases showing heterogeneity have also been reported (12-14). Our patient had typical AHO phenotypes.

Transport system which is sensitive to parathormone consists of three sections namely receptor, adenyl cyclase and protein G. The *GNAS* gene encodes the alpha-stimulatory subunit (Gs) of the intracellular G protein, which stimulates the production of cAMP under certain physiologic conditions (7). Gs alpha protein activity in PHP-Ia is low as a result of *GNAS 1* gene mutation therefore, sufficient c-AMP response to PTH cannot occur in receptor level (15-18). Resistance to other hormones which uses protein G as a second messenger, may also be observed. Moreover, the most common resistance is observed against TSH, which affects more than 90% of PHP patients type Ia (19,20). Previous reports have shown hypothyroidism may be the first manifestation of PHP type Ia in absence of hypocalcemia and elevated PTH levels (8-21). In our case, hypothyroidism with elevated TSH and decreased sT4 levels was detected.

The characteristic findings of the disorder are hypocalcemia, hyperphosphatemia and high level of PTH. However, previously normocalcemic cases have also been reported (10,22-24). Tamada et al. (24) reported a case with R358H mutation with normocalcemia. We have detected C-308T >C (p1103T) change in our case. The mechanism behind the fact of normocalcemia cannot be explained completely. It is proposed that normal serum calcium concentrations in these patients may be explained by the presence of normal bone responsiveness to PTH (10,25,26). Further studies are needed on this issue.

In conclusion, we have presented a PHP type Ia patient with normocalcemia and hypothyroidism caused by C-308T >C (p1103T) change in the *GNAS* gene. In children with hypothyroidism without apparent etiology, *GNAS1* gene mutations should be also considered even calcium levels are normal.

### Ethics

**Informed Consent:** Written informed consent was obtained from the patient's parents for publication of this case report.

**Peer Review:** Internally peer-reviewed.

### Authorship Contributions

Concept: E.K., İ.T.Ö., G.Y., Design: E.K., İ.T.Ö., Y.C., Data Collection or Processing: E.K., İ.T.Ö., G.Y., Analysis or Interpretation: İ.T.Ö., Y.C., G.Y., Literature Search: E.K., G.Y., İ.T.Ö., Writing: E.K., İ.T.Ö.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Case Report

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# *Actinomyces neuii* subsp. *neuii* Isolated from Perineal Abscess; Case Report

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## ABSTRACT

*Actinomyces* are gram positive bacilli which generally colonize in mouth, colon and vagina. The members of genus *Actinomyces* are facultative anaerobic or microaerophilic organisms and have a branching flamentous structure. They cause classical actinomycosis. Among the *Actinomyces* species; *A. israelii, A. viscosus, A. naeslundii, A. odontolyticus, A. bovis* and *A. neuii* are the mostly isolated organisms from clinical cases. A rarely encountered member of this group, *Actinomyces neuii* does not show branching and is catalase and CAMP positive and is a coryneform shaped bacillus. Although *Actinomyces* is mostly found as contaminating organism, in some cases it is reported as a pathogen. *Actinomyces neuii* has been reported in chorioamnionitis, neonatal sepsis, vertebral osteomyelitis, cervical lymphadenitis, breast abscess, fatal bacteremia and postoperative endophthalmitis. In our case, A. neuii was isolated from a perineal abcess and it was not previously reported. In our case, *Actinomyces neuii* was identified by commercial identification systems. For this purpose; VITEK MS and VITEK®2 Compact (both by bioMérieux, France) were used in the clinical microbiology laboratory and then this identification was confirmed as the *Actinomycess neuii* subsp. neuii by the 16S rRNA sequencing. Also, the positivity of CAMP was demonstrated in the laboratory. As in the cases of other actinomycosis, the treatment of the abcess caused by the *Actinomycess neuii* is through the surgical debridement. The antimicrobial susceptibility testing is not performed since the organism is reported to be susceptibile to common antibiotics. Beta lactam antibiotics are acknowledged as the proper selection for antibiotic treatment.

**Keywords:** *Actinomyces neuii subsp*. neuii, perineal abscess, *Actinomyces*

## Introduction

As member of the oral flora, actinomyces are organically anaerobic or microaerophilic and sporless microorganisms. These bacteria with difteroid basil morphology that do not react to acid-fast stain and do not show motility are gram positive bacteria and show branching (1). *A. israelii, A. viscosus, A. naeslundii, A. odontolyticus, A. bovis* and *A. neuii* are species isolated from many clinical cases among *Actinomyces* species.

*Actinomyces neuii* is aerobic and catalase positive member of this family. *A. neuii* was involved in the CDC coryneform group 1 bacteria until 1994. In a study by Funke et al. (2) on the metabolic and cellular fatty acid pattern of bacterium and 16S ribosomal RNA (rRNA) sequence; bacteria were defined as *Actinomyces neuii* subsp. *neuii* and *Actinomyces neuii* subsp. *Anitratus*.

*A. neuii* was previously isolated as an infection agent in soft tissue in a study conducted by Gomez-Garces et al. (3). In our study, *A.neuii* was first isolated from a perineal abscess sample in our country.

## Case Report

Fifty-two-year-old male patient was admitted to urology outpatient clinic with tenderness and swelling in the groin. He had scrotal lesions for 20 years and he said that these lesions merged and pain increased for one week. In addition, it was learned that he

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had type 2 diabetes mellitus, which was not regulated for 5 years and he was followed up with hypertension. Physical examination revealed a fever of 37.2°C and a swelling in 4x4 cm dimensions, stretching from perineum to scrotum, giving perineal induction and suggesting abscess. The number of leukocytes in the complete blood count was 18.5x10$^3$/uL, CRP was 4.36 mg/dL(<0.5) and glucose was 4+ in complete urine examination. The surface tissue ultrasonography revealed a heterogeneous hypoechoic collection with dense content and thickening of subcutaneous tissue which was 35x16 mm in diameter, partially extended from the posterior of the scrotum to the perineum, and had debris and septations within the thick wall. The contents of the 30 cc abscess were completely drained with 1 cm incision from the apse surface and sent to the microbiology laboratory. The patient was hospitalized and started empirically intravenous ceftriaxone 2x1 g/day and gentamicin 3x3 mg/kg/day. In addition, antidiabetic treatment for blood sugar regulation was started. In the first follow-up day, mild serous drainage was observed and no collection was observed in the abscess area of the patient with flow in the incisions during the day. Control leukocyte count was 11.8x10$^3$/uL and CRP was 0.92 mg/dL. Due to improvement of clinical symptoms, he was discharged with 2$^{nd}$ generation cephalosporin on the 3$^{rd}$ day. On the 1$^{st}$ week control, the number of leukocytes and CRP levels were normal and no perineal collection was detected and antibiotic therapy was terminated. Also perineal region examination was evaluated as normal on 1$^{st}$ and 2$^{nd}$ month controls. In the Clinical Microbiology Laboratory, gram positive coryneform bacilli and leukocyte were found in direct microscopic examination of perineal abscess sample (Figure 1). Simultaneously, cultures were prepared at 5% sheep blood agar (Becton Dickinson, USA), MacConkey Agar (Becton Dickinson, USA) and chocolate agar (Becton Dickinson, USA) and they were incubated in incubator with $CO_2$ at 35°C for 72 hours. In the culture examination, round colonies of 0.5-1.5 mm diameter in white color which were opaque, smooth-shaped and non-hemolytic were produced (Figure 2). Microscopic examination of these colonies showed gram-positive, non-branching coryneform-shaped, catalase-positive, inactive and sporeless bacilli. Breeding colonies were identified with conventional methods and Vitek MS (bioMérieux, France) system as *Actinomyces neuii.* At the same time, with 16s rRNA analysis (Sentegen Biotechnology, Ankara), *Actinomyces neuii* subsp. *neuii* was confirmed. In addition, Staphylococcus aureus ATCC 29213 strain and CAMP formation were investigated and a relationship was found between them (Figure 3).

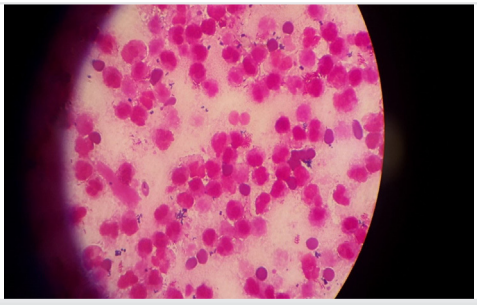

**Figure 1.** Direct microscopic examination from the abscess sample sent to the laboratory

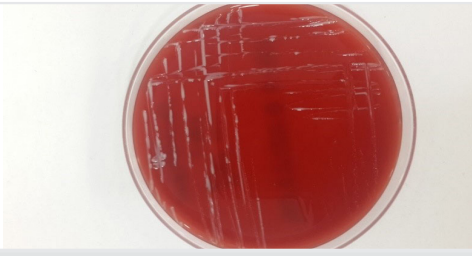

**Figure 2.** Colony image of abscess sample sent to laboratory and incubated in 5% sheep blood agar at 35oC for 48 hours

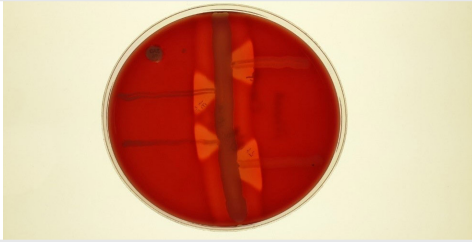

**Figure 3.** CAMP test performed with *A. neuii* and *Staphylococcus aureus*

Since *A. neuii* is sensitive to many antibiotics, antimicrobial susceptibility tests are not performed and β lactam antibiotics are considered appropriate. In this case, a similar treatment regimen was given and an uncomplicated recovery was observed.

## Discussion

*Actinomyces* species have been isolated from clinical specimens such as blood, wound, bone, abscess, bronchial wash, gallbladder fluid, pleural fluid and urine (1). Although they generally show better reproduction in anaerobic conditions, some species such as *A.neuii* can be isolated under aerobic conditions and 5-10% CO2 conditions.

*A. neuii* is catalase and CAMP positive and coryneform-shaped basil. These features separate it from other Actinomyces species. Also, *A. neuii* has a short basil appearance that does not show branching, unlike other species (2-4). It fermentates glucose, maltose, sucrose, mannitol, lactose, mannose, trehalose and xylose. Negative catalase reaction of *A. neuii* has also been reported. Brunner et al. (5) defined catalase negative *A. neuii* in a patient with breast prosthesis infection.

*Actinomyces neuii*, isolated from scrotal abscess sample, is a very rare microorganism seen in the laboratory as an infection agent and was not reported as agent in our country before. This case was presented because *A. neuii* is a clinically important and rare microorganism. In the literature, it was reported as pathogenic microorganism in different clinical specimens. For example, Mann et al. (6) isolated *A. neuii* as agent in a patient with neonatal sepsis and chorioamnionitis. In various studies, *A. neuii* was reported to cause endoftalmit, bacteremia, endocarditis,

pericarditis, osteomyelitis and prostatitis (7,8). Lacoste et al. (9) isolated *A.neuii* in breast abscess.

Surgical debridement is recommended in the treatment of soft tissue infections caused by *Actinomyces species*. It is sensitive to many antibiotics and β-lactam antibiotics is primarily preferred (10).

*A.neuii*, isolated from perineal abscess as a pathogen agent, which was a rarely seen agent, was reported here. It is important for the prognosis to determine the appropriate treatment approach with the correct identification of species with new microbiological methods. Isolation of A.neuii for the first time in our country adds specificity to the study.

**Ethics**

**Informed Consent:** A consent form was completed by all participants.

**Peer-review:** Externally peer rewieved.

**Authorship Contributions**

Concept: B.S.G., E.A., A.C., C.E., M.Z.D., Design: B.S.G., E.A., A.C., C.E., M.Z.D., Data Collection or Processing: B.S.G.,E.A., A.C., Analysis or Interpretation: B.S.G., E.A., A.C., Literature Search: B.S.G., E.A., C.E., Writing: B.S.G., E.A., C.E.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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Case Report

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# A CADASIL Case Presenting with Progressive Bulbar Palsy Caused by Acute Simultaneous Multiple Subcortical Infarcts

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## ABSTRACT

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset inherited small vessel disease of the brain caused by NOTCH3 mutations. Clinical characteristics of CADASIL include recurrent infarctions, migraine with aura, mood disturbances and cognitive impairment. We report a 35 year-old migraine-free patient with unusual presentation with acute simultaneous multiple subcortical infarctions causing progressive bulbar palsy as the initial manifestation. Hepresented with slurred speech progressing to anarthria and bilateral palsy of lower motor cranial nerves within 24 hour. Initial acute subcortical infarcts enlarged and new infarcts developed in bilateral hemispheres. There was diffuse leukoencephalopathy involving temporal horns and the patient had positive familiy history of migraine and young stroke. CADASIL was diagnosed genetically when NOTCH3 mutationwas shown. The patient improved after treatment with liquid hydration and antiplatelet agent. We experienced with the patient that CADASIL may present with unusual progressive manifestations and bilateral simultaneous infarctions. Irrespective of clinical presentation, patients with leukoencephalopathy and positive familiy history should be tested for NOTCH3 mutation.

**Keywords:** CADASIL, simultaneous infarcts, progression, bulbar palsy

## Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is caused by the mutation in *NOTCH3* gene, is the most common hereditary small vessel disease of the brain in adults. The main clinical features of this autosomal dominant inherited disease are recurrent transient ischemic attacks and stroke, migraine, mood disturbances and cognitive impairment (1). Transient ischemic attacks and ischemic strokes are the most frequent manifestations in CADASIL, occurring in up to 85% of patients (2). As being a small vessel disease, most of the ischemic events appears in the form of subcortical infarctions causing lacunar syndrome clinically. The uncommon clinical and radiological manifestations of patients with CADASIL have been rarely reported in the literature. Here, we report a patient with a progressive bulber palsy and acute simultanously multiple subcortical infarctions as the first manifestation of CADASIL.

## Case Report

A 35-year-old male was admitted to the Bezmialem University Emergency Department with a complaint of worsening slurred speech for 6 hours. His past history was unremarkable including drug or substance use. There was no history of consanguineous marriage. His father had had ischemic stroke a few times and he had become bedridden 2 years before his death at 56 year-old age. Also, his two brothers and one sister had history of migraine headache. On admission, his blood pressure was 120/70 mmHg and heart rate was 82 bpm (regular), and body temperature was 36.8ºC. There was no abnormality in his electrocardiography. The neck was supple and Kernig sign was

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absent. Neurologically, he was alert and oriented. His speech was severely dyshartric . There was no facial paralysis or no tongue deviation. The soft palate elevation was good bilaterally. There was no sensory or motor deficit in his extremities and cerebellar examination was normal. His complete blood count, liver and kidney function tests, blood glucose, electrolytes and thyroid function tests, and vasculitic panel was normal. Brain Computed Tomography scanning disclosed periventricular chronic ischemic changes, predominantly in frontal regions. Diffusion weighted magnetic resonance imaging (DWI- MRI) revealed multiple hyperintensities in bilateral subcortical white matter with low ADC values (Figure 1 A, B). Fluid-attenuated inversion recovery (FLAIR) images showed extensive ischemic gliotic changes in centrum semiovale, periventricular and subcortical white matter, basal ganglia, temporal lobes and also in pontin area bilaterally (Figure 1 C, D). He was hospitalized and started dual antiaggregant treatment (asetylsalysililc acid 100 mg and clopidogrel 75 mg daily). 24 hour after his admission, he had a progression with anarthria and bilateral palsy of lower motor cranial nerves. Repeated DWI-MRI demonstrated enlargement of ischemic lesion as well as appearance of new ischemic infarctions in bilateral border-zone areas (Figure 2). There was no hypo-hypertensive episode during follow up period. Extra-intracranial CT Angiograhy, transthoracic and transesophageal echocardiography, 24-h Holter ECG analysis were all normal. He improved in 3 weeks with supportive treatment and antiaggregant treatment. Because the patient had diffuse encephalopathy involving temporal lobes and also family history of migraine and stroke at young age, CADASIL was suspected and *NOTCH3* gene mutation was found positive.

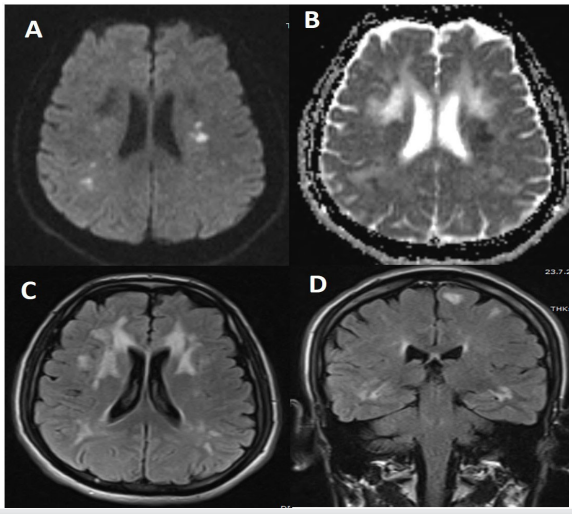


**Figure 1.** Diffusion Weighted magnetic resonance imaging (DWI- MRI) and FLAIR imagings; multiple hyperintensities in bilateral subcortical white matter with low ADC values (A,B); diffuse leucoencephalopathy involving temporal lobes (C,D)

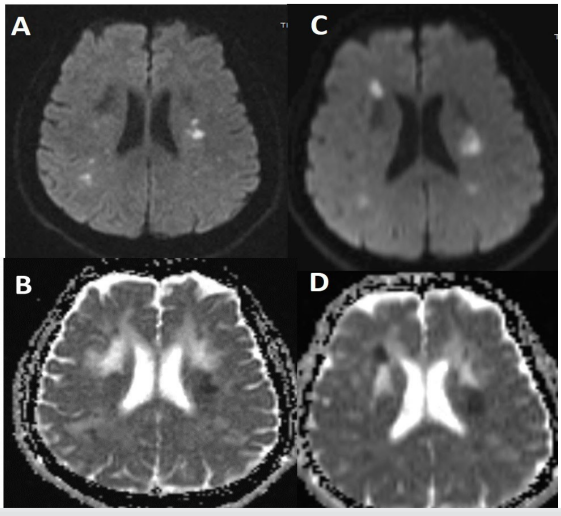


**Figure 2.** DWI-MRG at onset (A,B) and after clinical progression (C,D) the enlargement of ischemic lesion as well as appearance of new ischemic infarctions in bilateral border-zone areas

Informed consent of the patient was taken for publication.

## Discussion

As being a small vessel disease of the brain, ischemic events are almost invariably subcortical and present in 67% of patients as lacunar syndromes such as pure motor, pure sensory or sensorimotor deficit, ataxic hemiparesis and dysarthria-clumsy hand syndrome in CADASIL (1). Generally, if there is acute simultaneous ischemic infarctions in a patient with stroke, the etiological cause is usually attributed to cardiac or carotid arterial emboli (3). However, etiological work-up did not demonstrate any evidence of embolic etiology in our patient. In the literature, there were 7 cases with CADASIL with multifocal cerebral infarctions and these infarctions were supposed to be related with hemodynamic fluctuations (4-9). One of these reports, global white matter hypoperfusion was demonstrated in MRI perfusion study (6). To the best of our knowledge, there is no case with CADASIL presenting with progressive bulbar palsy probably caused by acute bilateral subcortical lesions in the literature. There was no evidence of hemodynamic failure, as in cases in the published literature, in our patient. The precise pathophysiology of these infarcts remains unclear. In a recent study, small vessel disease of the brain have been proved to be an important cause of acute simultaneous small subcortical infarcts (10).

With this case, we emphasized that CADASIL patients may present with acute simultaneous infarctions and may show a progressive course in the early days. Family history should be taken in detail and brain MRI should be evaluated for diffuse leucoencepholopathy with temporal lobe involvement.

### Ethics

**Informed Consent:** A consent form was completed by all participants.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: E.G., N.M., Design: E.G., N.M., Data Collection or Processing: E.G., N.M., Analysis or Interpretation: E.G., N.M., Literature Search: E.G., N.M., Writing: E.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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