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Editorial
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# Evidence-based medicine and needs based medicine

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***Keywords:*** Evidence-based medicine, family medicine, needs

Evidence-based medicine (EBM) collides every day with doctor's needs. Reality is different from aseptic world of clinical research. We know that in daily work we carry out some actions supported by scientific evidence, many based on general consensus, others on common sense and a few on our intuitions. It is evidence in literature as many questions concerning the Family medicine have no response and as most of the scientific evidences are not relevant for daily practice [1].

Family medicine most likely must continue to live with the paradox that the rigor of the inclusion criteria and the type of patient selection required for controlled trials are exactly the opposite of what occurs in daily practice. The different ages of life, polypathology and polytherapy are subject to a very high variability, they constantly affect the activities of prevention and therapy and force regularly choices that deviate from EBM, and they decide the priorities guide actions.

But these are not the only parameters physicians should take into account, there are also those that accompany the legal requirements and that often force to a defensive medicine, a conflict behaviour model with EBM and often with interests of the NHS, sometimes illogical clinically, but considered necessary from a legal point of view. The diagnostic and treatment activities need time, time is listening tool. The time available to the noble activity of diagnosis and care is eroded by anything but medical activities, bureaucratic, economic, administrative and secretarial.

Time is a vital need and if this resource will decrease further the whole system of care delivery will blow.

Patients don't know EBM and in an ideal relationship, where the doctor and patient agree on the diagnostic and therapeutic choices, patient's needs should be placed first [2]. It is natural that the quality of life is considered a priority; it certainly varies according to the needs of individuals and the conception that everyone has the same life, the scale of values, and the priorities that every human being has given to own self.

Exactly for this reason the diagnostic and therapeutic approach must be individualized and cannot be standardized. The needs of a hypertensive or diabetic patient are very different if it is a young active or a senior. People demand a personalized therapy, simplified, understood and shared, otherwise there will be poor adherence and persistence [3].

Also the organisation of care model must take into account the individual needs: the time spent, the waiting time, the type of doctor – patient relationship, the continuity of care and costs are the parameters that

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will determine significant organizational changes. The type of relationship between caregiver and patient must adapt to each individual and can vary from a partnership to a paternalistic model according to the will, the schooling and cognitive skills of each person. Nothing can be encoded while everything is under continuous variability. A future with a return to the past relationship, but changed and conditioned by the requirements of EBM, NHS and probably 'ideological' may appear.

Primary care and family medicine in particular are rapidly changing for a number of factors, including the most significant we have economic, scientific-technological ones and those related to the expectations of the population. he encoded efficiency of NHS collides with a continuous loss of effectiveness, more and more often patients are turning to alternative structures bypassing even those in the NHS should have the gatekeeper function; so the family doctor is becoming your doctor who cannot afford access to private care. And if the future will be the insurance, family medicine will be excluded from the future?

Genetics, bionics (biology – electronics) and biotic (biology – computer science) together with the digital technology will change the diagnostic and therapeutic approach and will give the patients god space for managing their own health. Scenarios can range from a new alliance between doctor and patient to a post-physician era, from the disappearance of the specialties to the recovery of humanities.

Anyway, no change will be independent from innate desire for individual freedom of each individual and of each professional, this desire will be the one that will drive any system for delivering care, this is what we have to keep in mind willing the survival of our Discipline.

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Review

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# Role of lycopene in the prevention of oral precancerous lesions. A review

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## ABSTRACT

***Objectives.*** To systematically review the methodology and results of clinical trials conducted on oral precancer patients using lycopene. ***Methods.*** An internet search using google search engine including key words - "lycopene, oral submucous fibrosis oral leukoplakia and oral cancer"- was done Full text articles in English language of all the clinical trials that were published in journals from the year 2004 to 2016 were obtained and evaluated. ***Results.*** The data available from the clinical trials were analyzed and presented under broad headings of sample size, duration of study, dosage and results and presented in tabular form. ***Conclusions.*** Lycopene is a promising candidate in reducing cancer and oral diseases in human beings. This review discusses the benefits of lycopene in prevention of different oral diseases.

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***Keywords:*** Lycopene, oral cancer, antioxidants, natural sources

## Introduction

Lycopene is one of the most potent antioxidants among all the carotenoids with applications in oral diseases ranging from management of oral pre-cancer to management of periodontal diseases [1]. Studies have indicated that lycopene exhibits anti-artherogenic properties and hence can play a major role in prevention of heart diseases [1]. Besides its role in prevention in of oral cancer recent studies have exhibited that the serum and tissue levels of lycopene are inversely associated with the risk of breast and prostatecancer [2].

### Biological properties of lycopene

Lycopene is a bright red carotene found mainly in tomatoes and other red- coloured fruits and vegetable like carrots, water-melons, and papayas [3]. Lycopene exhibits the highest physical quenching rate constant with free radicals like singlet oxygen [4]. Lycopene has a high number of conjugated double bonds; therefore it has a higher singlet oxygen quenching capacity in comparison to beta-carotene or alpha-tocopherol [5]. Lycopene has been found to be three times more effective than beta-carotene in arresting

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cell death by neutralizing of reactive nitrogen species [6]. Lycopene has higher antioxidant capacity than that of α-tocopherol [7]. Stahl *et al.* [8] in 1998 ranked the antioxidants as follows: lycopene > α-tocopherol > α-carotene > ß-cryptoxanthin > zeaxanthin = ß-carotene > lutein. Lycopene protects DNA damage caused by 1-methyl-3 nitro-1-nitrosoguanidine and hydrogen peroxide [9]. Lycopene is believed to promote the expression of a gene encoding a gap junction protein. It is also said that this property is independent of its pro-vitamin A or antioxidant properties [10]. Some in vitro experiments have shown that lycopene inhibits the growth process of human neoplastic cells, by interfering in growth factor receptor signaling and reducing progression of cell cycle [11]. Studies have shown that administration of lycopene suppresses DMBA(7,12-Dimethylbenz[a]anthracene)-induced oral carcinogenesis [12]. In vitro studies have revealed thatlycopene in various doses results causes reduced proliferation of oral cancer cells called KB1 human oral cells. These cells originating from a human oral cavity tumor were incubated with different concentrations of lycopene delivered via the cell culture media from stock solutions in tetrahydrofuran [13]. Lycopene is believed to act by stimulation of the immune system or a direct action on the tumour cells [14]. Lycopene is said to increase the resistance of lymphocytes to stress associated with oxidative process [13]. Lycopene has been shown to inhibit hepatic fibro genesis in experimental rats prompting views that it may also exert a similar inhibition on the abnormal fibroblastic activity in oral submucous fibrosis [14]. Lycopene has been reported to increase p53 protein levels which has tumor suppressor properties [15]. Recent animal studies have shown that lycopene may prevent smoke exposure-induced changes in p53, p53 phosphorylation, p53 target genes, cell proliferation, and apoptosis in the gastric mucosa of ferrets [16].

Several recent studies revealed the serum and tissue levels of lycopene are inversely associated with the risk of breast cancer, prostate cancer, coronary heart disease and oral premalignant lesions [17-19]. The physiologic mean plasma range of lycopene extends from 0.22 to 1.06 nmol/ml, and it contributes 21-43% of the total carotenoids [20]. Recent years have also witnessed surged in clinical trials involving lycopene in the treatment of oral cancer and precancer.

**Bioavailability of lycopene**

Researchers have found that in fresh fruits, lycopene is present within the fruit tissue hence only a portion of the lycopene in the fruit is absorbed [5]. Research has revealed that processing fruit into a juice, sauce, paste, or ketchup makes the lycopene more bioavailable by increasing the surface area available for digestion [5].

It is also believed that the temperature changes involved in processing alters the bound chemical form of lycopene to make it more easily absorbed by the body [5]. Since lycopene is fat-soluble absorption into tissues enhanced when oil is added to the diet [5]. Researchers have found isomerization of all trans-isomers to cis-isomers occur under acidic conditions of the gastric juices [21]. Under experimental conditions incubated lycopene derived from capsules with simulated gastric juice for 1 min showed a 40% cis-lycopene content, whereas the levels did not exceed 20% even after 3-hour incubation with water as a control [22]. This experiment was one of convincing evidence regarding the isomerization of all trans- lycopene to cis-isomers, under acidic conditions of the gastric juice. Thus proving the fact that gastric pH and food matrix influence isomerization further subsequent absorption and increased bioavailability of cis-lycopene [22].

Factors reducing the absorption of lycopene include certain fibers, fat substitutes, plant sterols and cholesterol-lowering drugs [23]. These agents prevent incorporation of lycopene into micelles, thus reducing absorption. Clinical trials using lycopene have been reported recently. Consumption of dietary fat in the form olive oil or sunflower oil has proven to promote lycopene absorption, primarily by stimulating bile production for the formation of bile acid micelles [24]. One study has demonstrated the positive effect of avocado consumption on lycopene absorption. The effect was attributed to high oleic fatty acid present in avocado, which probably facilitated the formation of chylomicrons and facilitated absorption [25].

**Review methodology**

An internet search using google search engine including key words -"lycopene, oral submucous fibrosis oral leukoplakia and oral cancer"- was done. Full text articles of clinical trials in English language that were published in journals from the year 2004 to 2016 were obtained. Information from case reports and reviews were excluded from the tables.

**Lycopene and oral precancer**

Recently there has been a surge in the literature

**Table 1.** Summary of clinical trials involving lycopene in oral precancer cases

| Researcher and year | Study subjects | Duration | Dose of lycopene | Disease condition | Results |
|---|---|---|---|---|---|
| Singh *et al.* 2004 | 58 | 3 months | 8 mg and 4 mg | Oral leukoplakia | Significant reduction in the clinical and histological results was seen. |
| Kumar *et al.* 2007 | 58 | 2 months | 16 mg | Oral submucous fibrosis | Increase in mouth opening |
| Gowda *et al.* 2011 | 12 | 3 months | 2000 µg | Oral submucous fibrosis | Clinical and histological improvement |
| Karemore *et al.* 2012 | 92 | 3 months | 4 mg and 8 mg | Oral submucous fibrosis | Significantly efficacious in the amelioration of signs and symptoms of OSMF |
| Aung *et al.* 2013 | 72 | 3 months | 10 mg | Oral leukoplakia | Mild improvement in thin leukoplakia cases |
| Selvam *et al.* 2013 | 45 | 3 months | 16 mg | Oral submucous fibrosis | There was significant increase in mouth opening |
| Patel *et al.* 2014 | 41 | 3 months | 3 mg | Oral leukoplakia | Patients receiving lycopene in combination with vitamin E and selenium havestatistically significant improvements both clinically and histologically as compared to those receiving placebo and with no side effects. |
| Patil *et al.* 2015 | 60 | 3 months | 8 mg | Oral submucous fibrosis | Clinical improvements in mouth opening and tongue protrusion were significant |

regarding lycopene in oral precancer. [Table 1]. One of the foremost clinical trials using lycopene in oral precancer was done by Singh *et al.* [12] in 2004. The study involved fifty-eight clinically and histologically diagnosed patients of oral leukoplakia randomly divided into three groups. Two study groups were administered 8 mg and 4 mg lycopene, respectively. The third group was administered placebo. When the outcome was assessed clinically, the patients in the three groups had a mean response of 80%, 66.25% and 12.5%, respectively. Histological evaluation yielded similar results. Based on these results, the researchers suggested that lycopene can be effectively and safely used for the management of oral leukoplakia. Kumar *et al.* [26] in 2007 evaluated the efficacy of oral lycopene therapy in patients with oral submucous fibrosis. Fifty-eight patients with oral submucous fibrosis were recruited for the study and were randomly divided into 3 groups. First group received 16 mg of lycopene whereas the second group received 16 mg of lycopene along with biweekly intralesional steroid injections. The third group was administered placebo. The researchers observed an average increase of 3.4 mm, 4.6 mm, and 0.0 mm in the mouth opening values of the first second and third groups.Based on these results the researchers suggested that lycopene should be used as a first line of therapy in the initial management of oral submucous fibrosis.

Gowda *et al.* [14] in 2011evaluated the clinical and histopathological response of oral submucous fibrosis to Lycopene in 12 adult patients picked from the regular outpatient of dental department. The study subjects were administered 2000 µgof lycopene and the responses were assessed clinically and histopathologically after 3 months. They observed clinical parameters such as mouth opening of the oral submucous patients [14].

Karemore *et al.* [27] in 2012 evaluated the efficacy of lycopene in conjunction with the cessation of causative habit in the treatment of OSMF. Of the 92 study subjects, 46 patients were given lycopene and remaining 46 were on placebo drug. Lycopene group

patients received 8 mg Lycored TM per day in two divided doses of 4 mg each, while placebo group patients received placebo tablet twice a day. Patients were examined for changes in mouth opening and other clinical symptoms of OSMF during three months and were followed up two months. They found that lycopene significantly effective in reduction of signs and symptoms of OSMF. A significantimprovement in the maximal mouth opening was observed in the study subjects administered lycopene [27].

Although most of the clinical trials were carried out in India, one study was carried out in Burma by Aung *et al.* [28] in 2013 also suggested improvement in oral leukoplakia cases when treated with lycopene. Selvam *et al.* [29] in 2013 evaluated the efficacy of oral lycopene therapy when used in combination with conventional intralesional steroid therapy in the management of oral submucous fibrosis. Forty five patients with oral submucous fibrosis were included under the study and were randomly divided into 3 groups. First group received 16 mg/day oral lycopene with biweekly intralesional steroids and hyaluronidase. The second group received oral antioxidant capsules with biweekly intralesional steroids and hyaluronidase whereas the third group received biweekly intralesional steroids and hyaluronidase alone. After 6 weeks there was significant increase in mouth opening among all the 3 groups but the group receiving lycopene in combination with intralesional steroids and Hyaluronidase showed the maximum improvement in mouth opening when compared to other groups.

Patel *et al.* [30] in 2014 studied the efficacy of lycopene in combination with vitamin E and selenium in the treatment of oral leukoplakia. Forty-one patients of leukoplakia were randomly categorized in two groups. First group was administered a combination of lycopene (3 mg), vitamin E (200 I.U.) and selenium (100 mcg) twice daily whereas the second group was given placebo capsules once daily for a period of 3 months. Post-treatment clinical and histopathological evaluation showed that the patients receiving lycopene in combination with vitamin E and selenium have statistically significant improvements compared to those receiving placebo and with no evidence of any side effects.

Patil *et al.* [31] in 2015 compared the efficacy of two antioxidants, lycopene and aloe vera in the management of OSMF. One hundred and twenty clinic-pathologically diagnosed OSMF cases were divided equally into two groups. First group was administered 8 mg lycopene in two divided doses of 4 mg daily whereas second group was given 5 mg aloe vera gel to be applied topically thrice daily for 3 months over the lesion. The researchers observed clinical improvements in mouth opening and tongue protrusion were significant in lycopene group. Although subjective symptoms of burning sensation pain associated with the lesion, and difficulty in swallowing and speech improved in both the groups, but the improvement was not statisticallysignificant. The researchers concluded that lycopene can bring about significant clinical improvements in the symptoms like mouth opening and tongue protrusion when compared to aloe vera [31].

In almost all the studies subjects were counseled and evaluated for cessation of tobacco and alcohol [12, 27, 28, 30]. Study subjects with oral submucous fibrosis were counseled to stop the habit of using areca nut and a complete oral prophylaxis was done to improve the oral hygiene and simultaneously to motivate the patient to cease the habit [28].

Besides it has a positive effect on controlling periodontal diseases through its action in response to periodontopathic bacterial colonization. A strong relationship exists between periodontitis and risk of congestive heart failure, and high monthly total consumption of lycopene appears to affect this relationship in a positive direction in periodontitis subjects has been recorded.The results of a recent clinical study suggested that modulation of the free radical production is important for the inhibition of tissue destruction, and hence treatment with most potent antioxidant like lycopene, will block the production of free radicals and therapeutically effective [32].

**Adverse effects of lycopene**

According to the available literature a dose of 3 g/kg/d of dietary or formulated lycopene did not show any adverse effects [1]. There are reports yellow-orange discoloration of skin accompanied by elevated lycopene levels in serum due to prolonged, excessive, consumption of tomato juice. However after 3 weeks of lycopene free diet the skin discoloration reduced completely [33].

## Conclusion

In conclusion it can be stated that carotenoids, have a powerful antioxidant agent with a various

activities inside the human body as they act as precursors for vitamin A. Lycopene is a fat-soluble carotenoid natural constituent of red fruits, vegetables, fungi and of certain algae. Lycopene has been hypothesized to prevent carcinogenesis and atherogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins, and DNA. The anticancer activity of lycopene has been demonstrated both in vitro and in vivo tumor models [34]. The mechanisms of actions could involve reactive oxygen species scavenging, up-regulation of detoxification systems, interference with cell proliferation, induction of gap-junctional communication, inhibition of cell cycle progression. Based on the data available from the studies it can be concluded that lycopene has biological properties with promising role in oral cancer chemo-prevention. It has also to be taken into consideration that lycopene is a naturally occurring substance in comparison to other chemically synthesized chemo-preventive agents with substantive antioxidant properties.

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# Study designs in biomarker research

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## ABSTRACT

In order to advances in technology, nowadays science is facing to a large variety of biomarkers. Issues of selecting appropriate study design for biomarkers, facing with a large number of biomarkers, multiple biomarkers, and usefulness of a new biomarker the today is more complicated. Current study is an overview of the issues discussed in studies of biomarkers.

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***Keywords:*** Biomarker, study design, trial, ideal biomarker

## Introduction

Biomarkers as biological disease signs play important role not only in diagnosis of disease but also in understanding disease process and even in cure process. In recent years along with advances in technology, recognizing new biomarkers has been subject of many studies. Moreover, because of possible classification errors, authors have considered to select powerful biomarkers. Difficulty in selection process and variety of biomarkers power in diagnosis, suggest authors to consider study designs for biomarkers more carefully. Since each design can use for any biomarker, the subject of study deign depends only on purpose of study. In many studies, different research methods have addressed also for the classification of biomarkers, biomarker power, biomarker misclassification, and the discovery of new biomarkers for disease diagnosis.

### Biomarkers

What is biomarker? A biomarker is a sign or an indicator of disease, and it can be answer to question that physician asks. NCI dictionary in the definition of cancer terms define biomarker as: "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. biomarker may be used to see how well the body responds to a treatment for a disease or condition, Also called molecular marker and signature molecule [1]."

Moreover, biomarker is defined by the Biomarkers Definitions Working Group as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [2]."

Biomarkers should have some features, they

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should easily measure and biomarker activity should remain almost constant in patients. In addition, an ideal biomarker should show the intensity and severity of the disease in the entire region of biomarker expression. Diagnosis biomarkers should be associated with the specific disease and not be related to any other situation.

Furthermore, an ideal biomarker should have suitable sensitivity and specificity, and must have high predictive power. Unfortunately, such ideal biomarkeris difficult to find,therefore, some researchers suggest the combination of different biomarkers for enhance their power.

**Biomarker Classification**

In many different studies biomarkers have classified in base of different reasons. The subjects that biomarkers have been intended can summarize as: Diagnosis, Screening, Risk prediction, Treatment selection and Monitoring. One can clarify these captions as follows: In a disease process first of all, absence or presence of disease should investigate (Diagnosis), how disease process is progressing (Screening), what is the risk of disease (Risk prediction), which design should be selected for treatment (Treatment selection) and during therapy how is disease status (Monitoring). According to these intended uses of biomarkers, researchers have classified biomarkers by these usages or by merging some of them.

Therefore, for example in Oncology Clinical Trials 2010 three types of biomarkers have introduced:

-Prognostic biomarkers are a biological measure of whether the patient will respond to a particular drug endpoint.

-Predictive biomarkers: Biological markers that predict the speed of the progression of the underlying disease.

-Surrogate and Pharmacodynamic biomarkers, when the change in biomarker is the controlled parameter , in other words, it is an endpoint and if it is use for drug activity or optimize dose, the biomarker is Pharmacodynamic Biomarker. In evaluating effectiveness of a specific treatment, surrogate biomarkers take place of a clinical endpoint in clinical trials [3, 4].

A researcher measures biomarkers once before treatment or several times before, during and after treatment, measurement once before treatment results prognostic and predictive biomarkers, whereas measurement in other way can result every three type of biomarkers.

In another classification way, two type of biomarkers is defined: exposure biomarkers, and disease biomarkers. Exposure biomarkers are used in risk prediction and disease biomarkers are used in screening and diagnosis and monitoring of disease progression [5].

**Biomarker Based Trials and Designs**

Researchers in choice of design should consider to what they know about trial,what are the treatment and biomarker effects? They should know about type of trial (discovery or confirmation)? If trial will be done again, and what is the type I and type II errors in terms of biomarkers?

Gosho *et al.* [6] had a comprehensive study on biomarker study designs as follows:

- Standard Randomized Clinical Trial (RCT) Design
- Biomarker by Treatment Interaction Design
- Biomarker - Strategy Design
- Enrichment Design and Hybrid Design
- Adaptive Signature Design
- Biomarker - Adaptive Threshold Design
- Adaptive Accrual Design
- Bayesian Adaptive Design

Factually, selected design is so variable in order to researcher, available facilities and sources. Interested readers can have access comprehensive information about above mentioned designs by Gosho *et al.* [6].

*Standard Randomized Clinical Trial (RCT) Design*

In clinical studies, Randomized Controlled Trials (RCTs) can use to identify new biomarkers. In this design patients divide in order to levels of biomarkers (especially in two level: biomarker positive, biomarker negative), and after randomization take test or standard treatment. This is similar to conducting two independent RCTs to compare different treatments.

*Biomarker by Treatment Interaction Design*

In this design we use the biomarker status for approve a treatment effect, than we use the biomarker as a stratification factor. Biomarker positive and biomarker negative groups after randomization take standard and test treatments. This process is similar to implementing RCT in both biomarker positive and biomarker negative arms [7].

*Biomarker - Strategy Design*

Biomarker - Strategy design allocate patients in classification; biomarker based strategy or non-biomarker strategy. In biomarker based strategy arm, patient takes test or standard treatment due to the biomarker result (positive or negative). In other arm, non-biomarker based strategy patient can take only standard treatment or maybe either due to randomization [8].

*Enrichment Design and Hybrid Design*

Studies that are limited to patients who are most likely to be affected by the use of the experimental drug are "target design" or "enrichment design". In these designs, the researcher uses a validated diagnostic test limitation of eligibility in comparing test group and control group [9]. In this design before screening we have a step for patients that are selected for the study based biomarker status. First, all patients divide to groups based on biomarker status and only the wanted status is will considered, in a binary biomarker, patients with biomarker positive status will considered and others take out of study (Enrichment design) or maybe they take standard treatment (Hybrid design). In both two designs patients with biomarker positive status after randomization take test treatment or standard treatment [10].

*Adaptive Signature Design*

In adaptive signature design, the researcher has not got a test or signature that identifies patients. Firstly, patients randomly allocate for test and standard treatments, the researcher perform a statistical test for comparing the difference at α1 significance level. If there was a significant difference and the test treatment is better, then the analysis is finished. If there is not, the second stage starts. In next stage comparison for test and standard treatment perform in only biomarker positive patients at α2 significance level. If there was a significance difference and the test treatment is better the analysis is done again, if there is not, researcher should accept fail in efficacy show of test treatment [6, 11].

*Biomarker - Adaptive Threshold Design*

Biomarker - Adaptive threshold design is developed to identify the sensitive patients to test treatment. Mainly with this design researcher can identify a cut-off point that makes comparison between test treatment and standard treatment easier. General procedure for adaptive signature and biomarker-adaptive threshold is: first, researcher does a basic RCT design, and for all patients comprise between test and standard treatment at α1 level of significance, if there were a significant evidence, therefore the RCT succeeded in showing efficacy of test treatment. If there were not a significance evidence, for biomarker positive subgroup, researcher makes comparison between test and standard treatment at α2 level of significance. In this stage if there were significance evidence, then we succeeded in showing efficacy, if not, we can result test treatment in showing efficacy is failed [12].

*Adaptive Accrual Design*

In this design after conducting a basic RCT between test and standard treatment, in the biomarker negative patients, researcher considers an interim analysis for comparison test and standard treatment. If interim analysis fails to show any significance difference in biomarker negative group, the comparison will restricted to biomarker positive patients. If there was unclarity, comparison for test and standard treatment will conduct between all patients and biomarker positive patients [13]. The word accrual indicates stop in occurring biomarker negative in interim analysis or continue in take them for next stage.

*Bayesian Adaptive Design*

The Bayesian Adaptive Design is an outcome based randomization design and it use a Bayesian hierarchical framework for assigning patients to test or standard treatment. In this design we have more than two biomarkers. Patient based on status of biomarkers are assigned to groups, for example patient with positive biomarker status for all biomarkers assigned to group 1 and etc. Then these groups based on researcher's prior knowledge or based on randomization take test or standard treatment [14].

**Sources of Bias in Biomarker Performance Studies**

Many of biomarker studies because of small sample size or wrong definition of resources and maybe endpoints suffer of lack of precision, they may have considerable bias, and need to use statistical methodology for minimize bias. Same of bias resources in biomarker studies are as follows:

- Selection bias: convenience sampling of available specimens
- Spectrum bias: advanced stage of disease vs.

healthy patients, enrichment with cases outside of IU population

• Verification bias: disease status not verified in all subjects by the reference standard

• Imperfect Reference Standard Bias

Ordering bias: order in which results are taken by test, comparator, and reference standard is not randomized; order in which disease and non-disease subjects are tested is not randomized. For predictive tests, test result is taken after onset of target condition.

• Missing biomarker results

• Test interpretation, integrity, and context bias: Device users / operators not masked to true disease status.

**New Biomarkers**

*Ideal Biomarkers*

A big family of biomarkers is using today. Nowadays new biomarkers in whole of biological system have introduced with developed tools. Many of these biomarkers are simple biomarkers like binary biomarkers that show presence or absence of a disease or a status in body. With introducing new biomarkers for a specific disease, researchers face with selection and evaluating of them. They also look for biomarkers that are valuable to measure. Since discovery of biomarkers in many contexts have developed in order to advance in technology over the past two decades, one can measure many biomarkers for a special disease. But generally an ideal biomarker for determining disease condition should have below features:

- Safe and easy to measure
- Cost efficient
- Modifiable with treatment
- Consistent across gender and ethnic groups

*Evaluation of New Biomarkers*

When there are multiple biomarkers for a disease, biomarker selection process is difficult to decision. Some researchers prefer to select biomarkers due to biomarker performance metrics, others make a combination of appropriate biomarkers. There are many biomarker performances metric due to type of biomarkers. ROC curves is one of suitable graphical metrics for evaluating a biomarker performance. It is also used for evaluating the accuracy of medical diagnostic systems.

In studies with multiple biomarkers, usefulness of diagnostic test increases by adding a new biomarker. Quantification of this usefulness can be done with standard methods including statistical significance and area under the ROC curve. Nevertheless, recent studies have introduced some new and useful indexes for the quantification [15].

The net reclassification improvement (NRI) and integrated discrimination improvement (IDI) is defined as two way for evaluate performance of diagnosing improvement by adding new biomarkers: NRI offers improvement offered by new markers by calculating NRI as probability of moving correctly to categories minus moving incorrectly based on new biomarkers or new algorithms.

In multiple biomarkers consider adding a new biomarker, one can consider changing in new probabilities of an event or new classification in disease based on the new biomarker, then we have new probabilities or new classification versus the old one.

Define upward movement (up) as a change in move to a higher category based on the new marker and downward movement (down) as a change in to lower category. The net reclassification improvement is defined as:

$$NRI=P(up \mid event)-P(down \mid event)+P(down \mid nonevent)-P(up \mid nonevent) \quad (1)$$

Second measure of assessing improvement diagnosing performance is integrated discrimination improvement (IDI). Since NRI consider to reclassification tables for patients and others(if there is an event or there is not event) then NRI quantifies movement in categories: if there is an upwards movement then the subject is an event and vice versa. The IDI does not consider at categories, IDI calculate sensitivity and specificity for full model and for model with removing new biomarker. Let IS denote integral sensitivity over all possible cut-off, and IP represent the corresponding integral of 'one minus specificity' [16]. The IDI is as follows:

$$IDI=(ISnew-ISold)-(IPnew-IPold) \quad (2)$$

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# Colorectal surgery in octogenarian patients: is it safe?

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## ABSTRACT

***Objectives.*** The aim of this study was to evaluate the efficiency and safety of resection for colorectal malignancy in elderly patients, by comparing the data from octogenarian (80+ years) patients with other patients of different ages undergoing this procedure. ***Methods.*** Data from 80 patients who underwent elective surgery for colorectal malignancy,by a single surgeon in the same center between the dates of May 2013 and May 2015, were evaluated retrospectively. For comparison purposes, patients were classified into three age groups: 65 years and under; between the ages of 66 and 79; and 80+ years old. Demographic features; the presence of comorbidity; colon tumor location; permeation features during colonoscopy; resection type and method; morbidity and mortality; lymph node metastasis and stage of the disease; follow-up period; recurrence during follow-up; and survival data of the patients were recorded and evaluated. ***Results.*** Statistically, no significant difference was detected between the three age groups in terms of demographic features; presence of comorbidity; colon tumor location; permeation features in colonoscopy; resection type and method; morbidity and mortality; lymph node metastasis and stage of the disease; follow-up period; recurrence during follow-up;or survival data. ***Conclusion.*** Curative resections for colorectal cancer can be safely carried out in octogenarian patients.

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***Keywords:*** Octogenarian, colorectal malignancy, surgery

## Introduction

With the improvement in standards of living, the number of elderly patients in our communities is rising. The incidence of cancer increases with age [1], and the prevalence of colorectal malignancy,in particular, at advanced ages is becoming an important health problem.

The aim for elderly patients with colorectal malignancy should be a positive outcome for both the oncological condition and their quality of life. Surgical intervention emerges as the only serious treatment option, especially in elderly patients with colorectal malignancy in its early stages. Surgical interventions in cancer cases may be either curative or palliative resections, depending on the patient's cancer stage.

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There may be a decrease or loss of organ function due to aging, accompanied by comorbidities such as degeneration in nutritional, cognitive or general condition, dementia or limitation of movement, which render the elderly less able to cope with surgical stress and thus increase the risk of mortality [2-4]. Nowadays, with increased experience of minimally invasive laparoscopic surgery; an improvement in preoperative and postoperative patient management; and multidisciplinary approaches; surgical resections can be performed with a lower risk of morbidity and mortality [5]. Currently, however, there is no formal process for deciding on the optimal surgical treatment method for patients in the 80+ age group [6, 7].

In this retrospective study, we have examined whether surgical attempts are effective and safe for patients of an advanced age, by comparing the data of octogenarian patients with patients from other age groups operated on during the same period for colorectal malignancy.

## Methods

Demographic characteristics, the presence of comorbidity, colonic cancer localization, permeation features in colonoscopy and preoperative metastasis presence in the three groups are shown in Table 1.

The accompanying comorbidity rate in the three groups was equal, with the most prevalent comorbidities being hypertension, diabetes mellitus and chronic obstructive pulmonary disease (COPD). In the preoperative period, liver metastases were detected in 2 (4.7%) patients in Group 1, 4 (15.4%) patients in Group 2 and 2 (18.2%) patients in Group 3, with no statistically significant difference found between the groups ($p$=0.221).

We considered normal lesions to be those that allowed easy passage during colonoscopy, and constricting lesions those that required an enforced passage. Although 81.8% of constricting lesions were detected in Group 3, which was composed of patients aged 80 and over, statistically there was no significant difference in ease of passage between the groups ($p$=0.177).

Evaluation of ASA (American Society of Anesthesiologists) scores, revealed an ASA 1 score in 7 (16.3%) patients in Group 1 and 2 (7.7%) patients in Group 2; an ASA 2 score in 36 (83.7%) patients in Group 1, 22 (84.6%) patients in Group 2 and 9 (81.8%) patients in Group 3; and an ASA 3 score in 2 (7.7%) patients in Group 2 and 2 (18.2%) patients in Group 3. The most common ASA score among the groups was ASA 2, but no statistically significant difference was found between the groups ($p$=0.065).

Resection types and methods used in the three groups, along with development of postoperative morbidity and mortality data are given in Table 2. The most frequent resection type used with both groups 1 and 2 was LAR (62.8% and 42.3%, respectively); whereas for group 3 it was right hemicolectomy (45.5%). Laparoscopy was the most frequent surgical approach in all three groups and statistically, there was no significant difference between the three groups regarding resection type or method ($p$=0.072 and $p$=0.327, respectively). Loop ostomies were formed in 9 (20.9%) patients in Group 1 and in 3 (11.5%) patients in Group 2. End colostomies were created in 3 (7%) patients in Group 1, 2 (7.6%) patients in Group 2 and 2 (18.2%) patients in Group 3.

**Table 1.** Demographic characteristics, comorbidity presence, colonic localization, permeation features in colonoscopy and preoperative metastases presence in the groups

| | Group 1 | Group 2 | Group 3 | *p* |
|---|---|---|---|---|
| Age | 55.7 (35-65) | 72.4 (67-79) | 81.4 (80-84) | |
| Gender (F/M) | 16/27 | 10/16 | 4/7 | 0.091 |
| Comorbidity | 22 (51.2%) | 20 (76.9%) | 7 (63.6%) | 0.102 |
| Colonic Localization | | | | 0.036 |
| Right colon | 4 (9.3%) | 9 (34.6%) | 5 (45.5%) | |
| Left colon | 9 (20.9%) | 3 (11.5%) | 2 (18.2%) | |
| Rectum | 30 (69.8%) | 14 (53.8%) | 4 (36.4%) | |
| Passage during colonoscopy | | | | 0.177 |
| Normal | | | | |
| Narrows | 20 (46.5%) | 13 (50%) | 2 (18.2%) | |
| | 23 (53.5%) | 13 (50%) | 9 (81.8%) | |
| Preoperative Metastasis | 2 (4.7%) | 4 (15.4%) | 2 (18.2%) | 0.221 |

F=female, M=male

**Table 2.** Resection type and method, postoperative morbidity and mortality development in the three groups.

| | Group 1 | Group 2 | Group 3 | *p* |
|---|---|---|---|---|
| Resection type | | | | 0.072 |
| Right Hemicolectomy | 4 (9.3%) | 9 (34.6%) | 5 (45.5%) | |
| LeftHemicolectomy | 9 (20.9%) | 3 (11.5%) | 2 (18.2%) | |
| LAR | 27 (62.8%) | 11 (42.3%) | 3 (27.3%) | |
| APR | 3 (7%) | 3 (11.5%) | 1 (9.1%) | |
| Operation Method | | | | 0.327 |
| Laparoscopy | 20 (46.5%) | 13 (50%) | 4 (46.3%) | |
| Open | 19 (44.2%) | 7 (26.9%) | 6 (54.5%) | |
| Conversion | 4 (9.3%) | 6 (23.1%) | 1 (9.1%) | |
| Morbidity | 13 (30.2%) | 7 (26.9%) | 4 (36.4%) | 0.848 |
| Mortality | 1 (2.3%) | 1 (3.8%) | 1 (9.1%) | 0.574 |

LAR=Low Anterior Resection, APR=Abdominoperineal Resection

A similar frequency of morbidity in the postoperative period was seen in all three groups. The most common cause of morbidity in all groups was wound infection. Anastomotic leak was observed in only 1 (2.3%) patient in Group 1. There was no anastomotic leak in Group 2 or in Group 3, which consisted of octogenarian patients. However, ischemia developed in the end part of the ostomy in two (18.2%) patients from Group 3 and revision was needed. Overall, no statistically significant difference was detected among the three groups regarding morbidity ($p$=0.848).

Mortality rates for the 30-day postoperative period were also evaluated. In total, we lost three patients. One (2.3%) patient from Group 1 with preoperative liver metastasis died due to postoperative liver failure; one (3.8%) patient from Group 2, due to development of necrotizing fasciitis, and one (9.1%) patient from Group 3, following anterior myocardial infarction. Statistically, no significant difference was detected between the groups in terms of mortality ($p$=0.574).

Lymph node metastases and their stages were evaluated by analyzing pathological samples from the three groups following resection (Table 3). Although lymph node metastases were seen in 72.7% of Group 3, there was no statistically significant difference between the groups ($p$=0.264). When disease stages were evaluated, it was seen that patients in all three groups were most commonly in Stage 3 (41.9%, 46.2%, and 54.5%, respectively). However, there was no statistically significant difference ($p$=0.187).

Average follow-up time, disease recurrence during the follow-up period and general survival rates are given in Table 4. Median follow-up time was 30 months in Group 1, 25 months in Group 2 and 20 months in Group 3; hence, there was no statistically

**Table 3.** Lymph node metastases and stages

| | Group 1 | Group 2 | Group 3 | *p* |
|---|---|---|---|---|
| Lymph node metastasis | 20 (46.5%) | 15 (57.7%) | 8 (72.7%) | 0.264 |
| Stage | | | | 0.187 |
| 1 | 9 (20.9%) | 7 (26.9%) | - | |
| 2 | 14 (32.6%) | 3 (11.5%) | 3 (27.3%) | |
| 3 | 18 (41.9%) | 12 (46.2%) | 6 (54.5%) | |
| 4 | 2 (4.7%) | 4 (15.4%) | 2 (18.2%) | |

**Table 4.** Recurrence during follow-up and average follow-up time

| | Group 1 | Group 2 | Group 3 | *p* |
|---|---|---|---|---|
| Recurrence rate | 4 (9.3%) | 1 (3.8%) | 2 (18.2%) | 0.363 |
| Follow-up (month) | 30 | 25 | 20 | 0.168 |
| 1-year survival (%) | 97.1 | 100 | 87.5 | 0.237 |
| 2-year survival (%) | 87.4 | 100 | 87.5 | 0.237 |

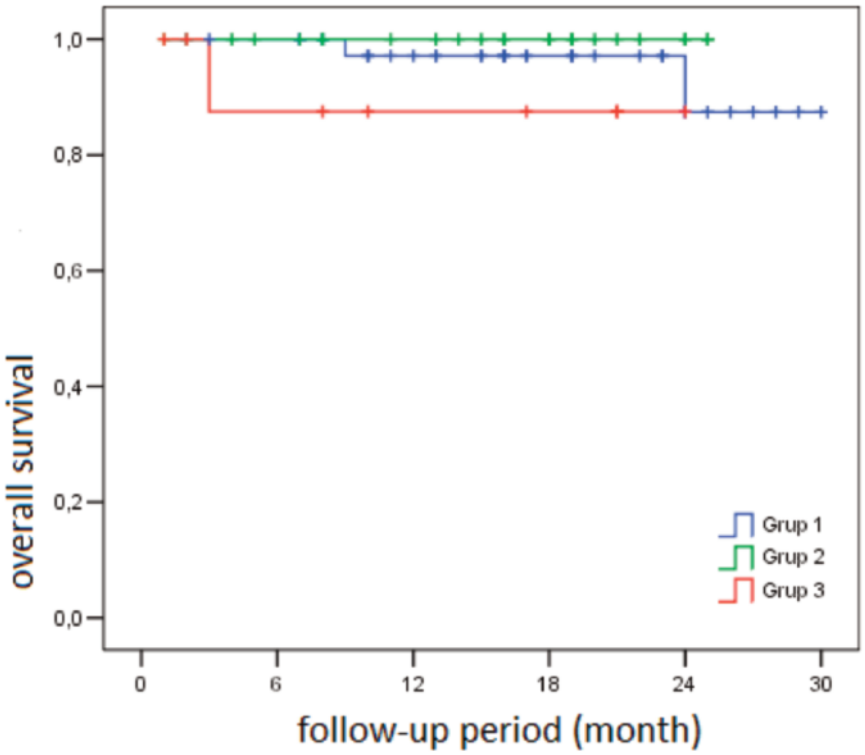


**Figure 1.** General survival rates in the groups

significant difference ($p$=0.168). Recurrence during the follow-up period was seen in 4 (9.3%) patients in Group 1, in 1 (3.8%) patient in Group 2 and in 2 (18.2) patients in Group 3. Although a proportionally higher level was detected in Group 3 (octogenarian patients), statistically there was no significant difference between the groups ($p$=0.363). During the follow-up, two patients in Group 1 died from myocardial infarction and cerebrovascular bleeding; and one patient in Group 3 died due to myocardial infarction. One and two-year general survival data were assessed among the groups: statistically, no significant difference was detected. Survival rates for the groups are shown in Figure 1.

## Discussion

Despite publications asserting that colorectal cancers at an advanced age are seen more frequently in females and in the right colon (by up to 40%), in our study the incidence was higher in male patients and right colon involvement was more frequent [8, 9]. It should be emphasized that mechanical bowel obstruction occurs in older patients with colorectal malignancy at a rate of 16%, compared to a rate of only 4% in younger patients and efforts should be made to perform these operations under elective conditions rather than as an emergency [10, 11]. Although patients with obstructive pathology such as an ileus were excluded from our study, the rate of technical difficulty in using the colonoscope in patients aged 80+ undergoing elective resection was 81.8%.

The presence of comorbidities is accepted as a significant predictor of postoperative morbidity and mortality, affecting the prognosis negatively [12-14]. One study that included 34,194 patients, divided into age-related groups, showed that the presence of accompanying comorbidities at age 80+ lowered the general survival rate, but not cancer-specific survival. Moreover, the survival rate of elderly patients is reported to increase significantly following surgery [15]. The postoperative complication rate specific to surgery in 2,932 patients aged 80 and over was found to be similar to that of younger patients [16]. However, more cardiovascular and pulmonary complications are seen with advanced age. While the presence of accompanying comorbidity was 51.2% in the group aged 65 and below in our study, this rate climbed to 76.9% in 65-79 year olds but dropped to 63.6% in the group aged 80 and over. Therefore, in our study, there was no particular increase in comorbidity in octogenarian patients or a statistically significant

difference between the age groups.

The most efficient treatment alternative for local disease control, reduction of symptoms and long-term survival in the presence of colorectal malignancy in octogenarian patients is surgical resection. Laparoscopic approaches are recommended as the best alternative for protecting and improving functional status and allowing rapid recovery in elderly patients [17]. Laparoscopic colorectal surgery has been shown to reduce the length of hospital stay; increase recovery by reducing postoperative pain; causeless blood loss and result in lower morbidity and mortality in octogenarian patients [18, 19]. Laparoscopic approaches are also shown to cause much less systemic stress [20]. Bowel movements and functional results after rectal surgery are better in elderly patients than in young patients [21]. No significant difference in postoperative complications was found between laparoscopic surgery and open surgical interventions [22]. In our study, age was not taken into consideration when selecting resection type and method. Laparoscopic surgery was applied in 36.4% (n=4), open surgery in 54.5% (n=6) and conversion surgery in 9.1% (n=1) of the patients over 80 years old. In published studies, conversion rates vary from 0.7% to 25% [23-26]. In these studies, it was reported that the rate of anastomosis leak varied between 4.7% and 10% (3% in the elective resection group, 6.3% in the emergency operation group), and the rate of stoma creation was 24% [26, 27]. In our study, anastomosis leak was seen in only 1 (2.3%) patient from the group aged 65 and below. End colostomy was formed in two (18.2) patients over 80 years old, and no leak was detected after anastomosis in the other nine patients. Our relatively high rate of conversion was thought to be linked to the learning curve of the single surgeon concerned. The most common reasons for conversion were severe intra-abdominal adhesions and invasion of the tumor into neighboring tissues.

Discussion of any possible increase in morbidity and mortality in elderly patients caused by colorectal resections is ongoing. While in previous studies age was indicated as an independent parameter increasing postoperative morbidity and mortality [28, 29], current studies show that morbidity and mortality rates among young and old patients are, in fact, similar [30, 31]. In older patients who undergo resection for colorectal cancer, age is not thought to be an independent predictor of morbidity and mortality, but existing co-morbidities and ASA value are useful as independent predictors [32]. In various studies of elderly patients, morbidity rates vary between 25% and 81%, and mortality between 5% and 30% [33-35]; with a nine-fold greater risk after colorectal surgery in older patients [36]. In our study, we saw that the score most similar for patients 80+ and those inother age groups was the ASA 2 score (81.8%). Rates of postoperative morbidity were also similar (36.4%) in both 80+ patients and the other age groups. The incidence of mortality in the 80+ group was 9.1%; it seems likely that the small number of patients in this group caused the high morbidity and mortality rates as a percentage. While locally advanced tumor presence is more frequently detected in elderly patients through tumor diameter, pT stage, etc.; distant metastases are seen less than in younger patients [37]. Despite this tendency to locally advanced tumor, no difference in RO curative surgical resection has been reported between age groups [38]. In our study, the frequency of constricting lesions was high (81.8%), lymph node metastasis rate was higher than the other age groups (72.7%), and there was a presence of distant metastases (18.2%) in our 80+ group. However, there was no statistically significant difference between the ages.

One study has reported a median survival rate of 74 months in a young patient group and 73 months in an older group, witha 5-year survival rate of 66% in the young group and 53.1% in the patients of advanced age [39]. In the literature, the five-year survival rate in octogenarian patients varies between 24% and 51% [6, 32, 40]. In our study, the patients below age 65 were followed-up for a median of 30 months, patients between ages 66-79 were followed-up for a median of 25 months, and our octogenarian patients were followed-up for 20 months on average. Local recurrence was seen at low rates during the follow-up period for each of the three groups (9.3%, 3.8% and 18.2%, respectively). No statistical difference was found between one and two-year general survival values of the three groups.

*The Limitations of the Study*

In our study, there are some restrictive conditions to the interpretation of the data. The small number of patients aged 80 years and over; lack of randomization among the groups and the retrospective nature of the evaluation are examples of these constraints. Moreover, date regarding physical condition and quality of life were not recorded.

## Conclusions

Certainly, locally advanced tumor presence can be seen more frequently in patients aged 80 and over, from difficulty in carrying out the colonoscopy and lymphatic metastasis positivity. However, it is thought that patients whose overall physical condition is good and who are at a suitable stage of the disease with good life expectancy can safely undergo colorectal resections in experienced centers without considering an upper age limit, even if they have accompanying co-morbidities. As morbidity and mortality rates are acceptable, laparoscopic surgical attempts should be preferred to open surgery.

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# Bactericidal efficacies of nebulized non-thermal atmospheric plasma-treated liquids

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## ABSTRACT

***Objectives.*** Antimicrobial activities of non-thermal atmospheric plasma-treated liquids on various bacterial and fungal strains in their planktonic and biofilm forms have been widely reported. Since most of the plasma-treated liquids are water based, they might be washed off certain surfaces and they cannot be applied for the infections of respiratory tract. In the present study we have tested antimicrobial activities of plasma-treated N-acetyl cysteine solution (NAC), phosphate buffered saline (PBS) solution and deionized water when nebulized over planktonic forms of *E. coli* and *S. aureus*. ***Methods.*** Antimicrobial activities of nebulized plasma-treated liquids were evaluated with zone of inhibition test and colony counting assay. Moreover pH of NAC, PBS solution and deionized water were measured before, after plasma treatment and during nebulization since low pH is well known consequence observed in plasma-treated liquids. ***Results.*** Our results have revealed that pH of plasma-treated NAC, PBS solution and DIW decreases after plasma treatment consistent with previous reports and does not change during nebulization. Moreover, antimicrobial activity assessment indicates that nebulized plasma-treated NAC shows the strongest antimicrobial activity, which leads complete inactivation of bacteria for $10^3$ to $10^6$ CFU/ml initial bacterial load and 5-log reduction for $10^7$ CFU/ml initial bacterial load on both *E. coli* and *S. aureus*. ***Conclusions.*** Plasma-treated liquids could retain their antimicrobial activity during nebulization and nebulization could be considered as a future alternative method for delivery of plasma-treated liquids for respiratory tract infections.

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***Keywords:*** Non-thermal plasma, plasma medicine, nebulization, antimicrobial, biomedical engineering

## Introduction

Even though the word "plasma" defines the liquid component of blood in medical terminology, Irving Langmuir used the same word in order to define physical plasma since the complex structure of physical plasma reminded him the blood plasma [1]. Basically, plasma is defined as the ionized gas and the fourth state of the matter next to solid, liquid and gas. When a gas is subjected to an electric field and when the voltage reaches the breakdown voltage of the gas, an electric discharge will form which then leads formation of ionized gas (or plasma) via removal of electrons from gas atoms and/or molecules. Plasma is

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the most abundant state of the matter and comprises more than 99% of the universe. Sun, lightning, aurora borealis are examples of natural plasmas [2]. Plasmas are classified as thermal (hot or equilibrium) and non-thermal (cold or non-equilibrium) plasmas according to gas and electron temperature. In all plasmas generated under electric field electron temperature reaches thousands of Kelvin. In thermal plasmas, the gas temperature is in equilibrium with electron temperature and also reaches thousands of Kelvins. In non-thermal plasmas, cooling of heavy particles (uncharged molecules and ions) is more effective that the energy flux from electrons and gas remains in room temperature [2]. Therefore non-thermal plasmas can be used for the treatment of temperature sensitive materials including cells and living tissue [3]. During generation of plasma, formation of ultraviolet (UV) light, free electrons, electric field, reactive oxygen species (ROS) such as hydroxyl radical, superoxide, atomic oxygen, hydrogen peroxide, singlet oxygen and reactive nitrogen species (RNS) such as nitric oxide, nitrite, nitrate, peroxynitrite make plasma a reactive medium [4].

Plasma can be utilized in various modalities. In direct plasma treatment modality, the material that is being treated serves as a counter electrode and comes in direct contact with plasma discharge. In indirect plasma treatment, plasma is generated remotely and plasma products are carried to the material that is being treated via gas flow. Therefore in indirect plasma treatment effects of UV light, electric field and free electrons can be excluded [5]. Moreover, Ercan *et al.* [6] have defined fluid mediated plasma treatment in which a particular fluid is first treated with plasma and then the treated fluid is exposed to material that is being treated.

The use of non-thermal plasmas on cells and living tissues created a new multidisciplinary, emerging area called as plasma medicine that investigates therapeutic effects of physical non-thermal plasmas [7]. Non-thermal atmospheric plasma technology found itself vastly growing various application areas in biomedicine such as disinfection, cancer therapy, wound healing, blood clotting, tooth whitening, biomaterial modification [8-13]. Broad range antimicrobial activity of non-thermal plasmas on planktonic and biofilm forms of bacteria - including multi drug resistant (MDR) strains -, fungi, viruses and even prions have been reported [6, 14-17]. In addition to antimicrobial activity of non-thermal plasmas, also various materials such as liquids, gels may acquire antimicrobial activity when treated with plasma [6, 18-20]. Poor *et al.* [18] have shown that calcium alginate gel wound dressings gains strong antimicrobial activity over several bacterial and fungal strains upon non-thermal plasma treatment. Furthermore, different groups have shown strong antimicrobial activity acquisition of fluids such as water, phosphate buffered saline (PBS) solution, saline solution, N-acetyl cysteine (NAC) solution against biofilm and planktonic forms of bacteria and fungus when treated with non-thermal plasma [6, 21-23]. Antimicrobial activity of plasma-treated liquids was attributed to presence of ROS and RNS in plasma-treated liquids that are generated during plasma treatment [24, 25]. Besides, plasma-treated liquids can maintain acquired microbial activity up to several years [6]. Therefore, plasma-treated liquids are considered as novel antimicrobial solutions that may be conveyed to clinical practice. Despite broad range and strong antimicrobial activities of plasma-treated liquids, their use for eradication of certain infections such as respiratory tract infections is not applicable since presence of liquid in respiratory tract leads suffocation. Thus, a delivery method of plasma-treated liquids should be established and investigated for possible future applications. Nebulization is a drug delivery method that is used for administration of medications in liquid phase towards respiratory tract. Nebulization is a process in which a liquid is converted to form of mist that can be inhaled directly and therefore leads to medications to reach higher concentration in respiratory tract [26].

Present proof of concept study aims to investigate whether plasma treated liquids could still exert their antimicrobial activity when nebulized for possible future applications such as eradication of respiratory tract infections. In the present study, we have chosen three liquids - deionized water (DIW), PBS solution and NAC solution - that are previously reported to gain antimicrobial activity following plasma treatment [6]. Those liquids were nebulized over gram-negative and gram-positive model organisms (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) after treatment with non-thermal dielectric barrier discharge (DBD) air plasma to evaluate antimicrobial activity.

## Methods

*Bacterial Cultures*

*E. coli* (ATCC 25922) and *S. aureus* (ATCC

25923) strains were generously donated by Ege University School of Medicine, Department of Microbiology.

Frozen stocks of microorganisms were thawed and cultures were grown on trypticase soy agar (TSA) plates. Single isolated colony of each organism was collected from TSA plate using a 10 µl loop and transferred into 10 ml of trypticase soy broth (TSB) medium and incubated in shaker incubator for overnight at 120 rpm and 37°C. After overnight incubation, suspended cultures of each strain was diluted appropriately in 1X sterile PBS solution in order to achieve desired number of bacteria by measuring absorption of each diluted culture using a spectrophotometer.

*Plasma Treatment of Liquids*

In the present study, DIW, PBS solution and 5 mM of NAC solution were treated separately with non-thermal atmospheric air DBD plasma in order to evaluate antimicrobial activities after nebulization on planktonic forms of *E. coli* and *S. aureus*. DIW was collected from a water purification system. 100 mM stock solution of NAC solution was prepared appropriately by weighing and dissolving NAC powder in PBS solution, and stored at -20°C until used. 5 mM of working solution of NAC solution was prepared by diluting NAC stock solution in 1X PBS solution.

An alternating current (AC) microsecond power supply was operated at 31 kV of voltage, 1.5 kHz of frequency with 10 µs of pulse duration, which yields 0.29 W/cm$^2$ power distribution. A custom-made glass fluid holder was used to maintain liquids during plasma treatment, which provides 1mm long liquid column. 1 ml of each liquid was treated with non-thermal atmospheric air DBD plasma for 3 minutes separately by fixing the discharge gap as 2 mm (Figures 1a and 1b).

*pH Measurements of Plasma-Treated Liquids*

Each liquid was transferred into microcentrifuge tubes after 3-minute plasma treatment. An ultrasensitive pH probe that is connected to a pH meter was immersed in plasma-treated liquids for pH measurement. Moreover, during nebulization, pH probe was fixed about 1cm away from outlet of nebulizer to allow proper contact of mist with pH meter, and pH of nebulized liquids were measured during the course of nebulization. pH measurement were repeated at least 9 times for each liquid.

*Nebulization of Plasma-Treated Liquids*

After 3-minute plasma treatment, total 2 ml of each plasma-treated liquid was transferred into reservoir of the nebulizer. 2 ml of each plasma-treated liquid was nebulized over bacteria for ZOI and inactivation tests (Figure 1c). Required time for the nebulization 2 ml of each liquid was determined as approximately 7 minutes.

*Zone of Inhibition (ZOI) Experiments:*

1 ml of $10^7$ CFU/ml *E. coli* and *S. aureus* cultures were transferred over TSA plates and spread using disposable spreader. Then, plates were kept in biological safety cabin in order to let excess liquid to be evaporated. Then, 1 ml of each liquid was separately nebulized over *E. coli* and *S. aureus*. After nebulization, plates were incubated in stationary incubator for 24 hours at 37°C. After incubation, plates were visually examined to determine zone of inhibition. Plates without nebulization, with nebulization of untreated liquids and with nebulization of 3% $H_2O_2$ solution were used as internal, positive and negative controls respectively. Examined plates were incubated further 48 hours in stationary incubator

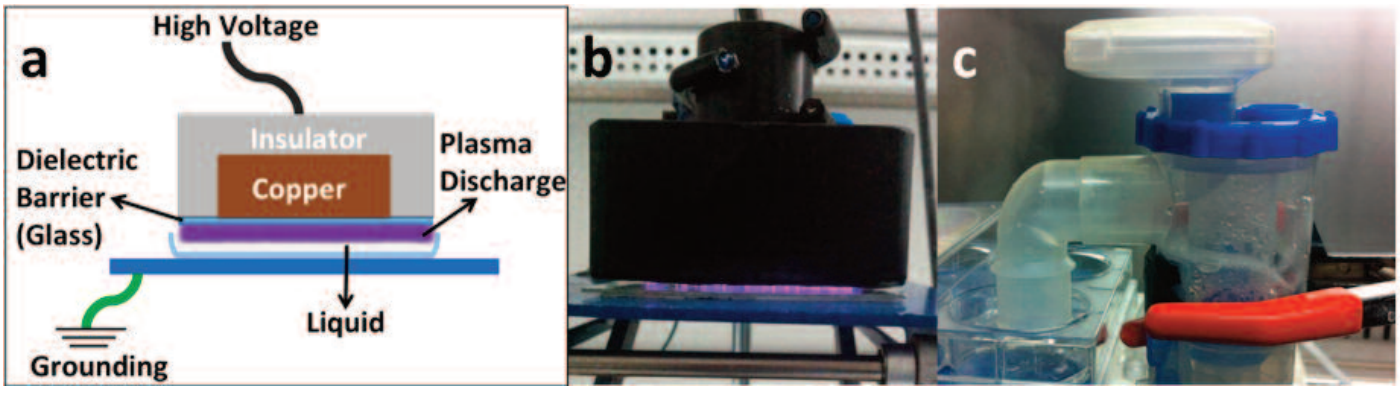


**Figure 1.** Plasma treatment set up and nebulization of plasma-treated liquids. Schematic view (a), and image of plasma treatment procedure of liquids (b). Plasma discharge (in violet color) is clearly visible in between DBD electrode and liquid surface (b). Plasma-treated liquids were nebulized over planktonic forms of bacteria that are kept in wells of 6-well plate (c).

at 37°C to rule out any possible dormancy of microorganisms. Each experiment was carried out thrice in triplicate.

*Bactericidal Effect of Nebulized Plasma-Treated Liquids*

Bactericidal activity of nebulized plasma-treated liquids were tested on $10^3$, $10^4$, $10^5$, $10^6$ and $10^7$ colony forming unit /ml (CFU/ml) of initial bacterial numbers of *E. coli* and *S. aureus*. After overnight incubation of *E. coli* and *S. aureus* cultures, concentration of each bacterial culture was set to $10^7$ CFU/ml using spectrophotometer. Afterwards, cultures of *E. coli* and *S. aureus* were further diluted to obtain desired initial concentration ($10^3$, $10^4$, $10^5$, and $10^6$ CFU/ml) using sterile 1X PBS solution.

100 µl of each initial concentration of *E. coli* and *S. aureus* cultures were transferred into wells of 6-well plate and held in biological safety cabin to allow evaporation of excess liquid. Afterwards, 1 ml of each plasma treated liquid was nebulized over various initial concentrations of *E. coli* and *S. aureus*. Following completion of nebulization, samples were held for 15 minutes to allow complete interaction of bacteria and nebulized plasma treated liquids. Then, samples were homogenized using 1 ml of sterile 1X PBS and serially diluted and plated on TSA plates to carry out colony counting assay. Plated samples were transferred into a stationary incubator and incubated for 24 hours at 37°C. After 24 hours of incubation, surviving colonies on plates were counted to determine bactericidal activity of plasma treated liquids. Plates were incubated further 48 hours in stationary incubator at 37°C to exclude possible dormancy. Nebulization of untreated liquids and nebulization of 3% $H_2O_2$ were used as positive and negative controls respectively. Each experiment was carried out thrice in triplicate.

*Statistical Analysis*

Each experiment was performed thrice in triplicate unless otherwise stated. Statistical analysis was performed using Prism software v4.03 for Windows. Student's t-test was used for pair comparisons and one-way analysis of variance was used for multiple comparisons. A $p$ value of $<0.05$ was considered as statistically significant.

## Results

*pH Measurements of Plasma-Treated Liquids*

As presented in Figure 2, pH of NAC, PBS solutions and DIW were measured as 6.7, 7.1 and 6.1 respectively. All liquids have had acidic pH following non-thermal atmospheric plasma treatment. After plasma treatment, pH of NAC, PBS solutions and DIW were dropped to 2.5, 2.8 and 2.2 respectively. The pH drop of each liquid after plasma treatment was statistically significant. Furthermore, acidic pH of all tested liquids was not significantly changed during nebulization and measured as 2.6, 2.8, and 2.3 for NAC, PBS solutions and DIW respectively (Figure 2).

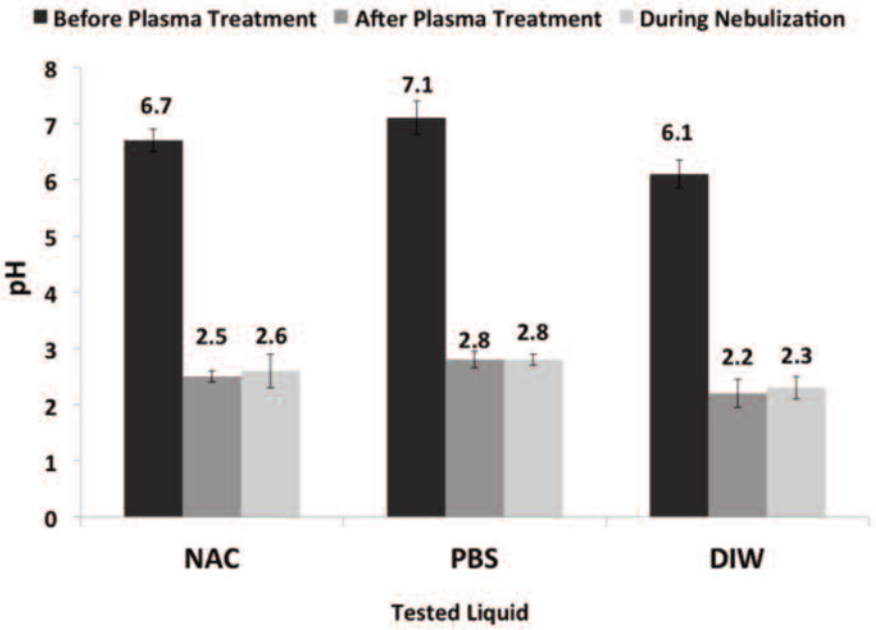


**Figure 2.** pH of tested liquids before, after and during nebulization. pH of all liquids were close to neutral before plasma treatment. Following plasma treatment pH of all liquids dropped to acidic pH and has not significantly changed during nebulization. ).

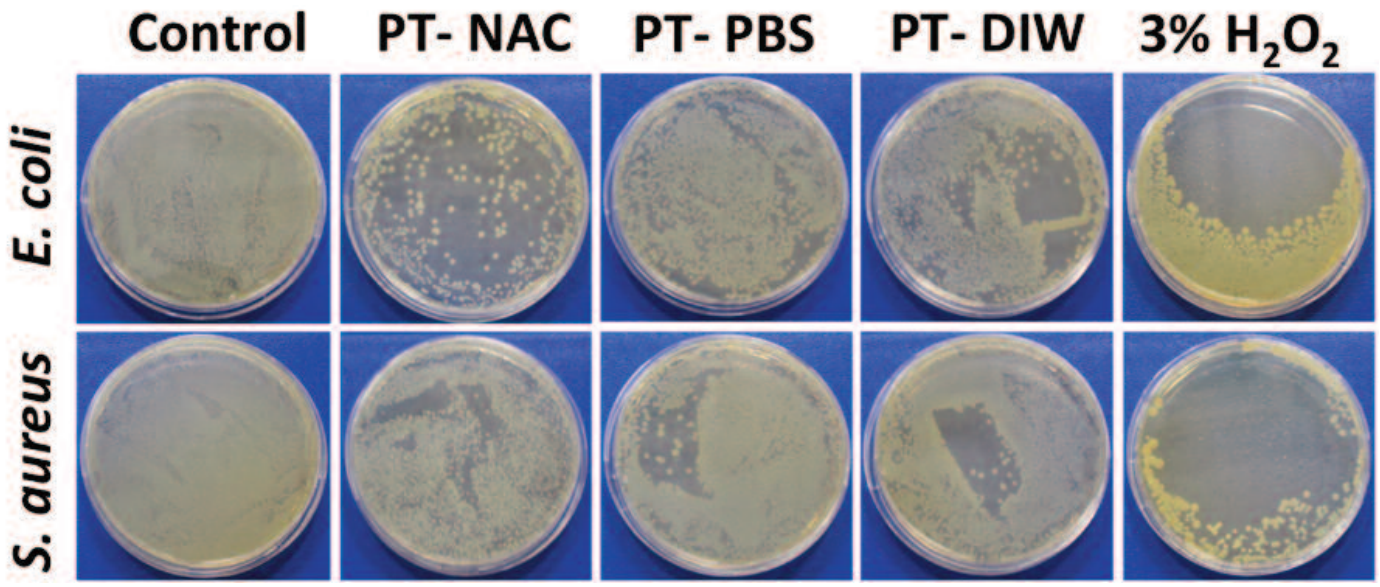


**Figure 3.** Zone of Inhibition Test of nebulized plasma-treated liquids. Nebulization of plasma-treated NAC solution led the strongest antimicrobial activity for both E. coli and S. aureus. More diluted and separated colonies were grown after nebulization of plasma-treated NAC solution, which corresponds nebulized plasma-treated NAC solution shows its antimicrobial activity over a larger area while nebulized plasma-treated PBS solution and DIW showed inhibition zone on the area where they came in contact first. 3% H2O2 was used as negative control.

*Zone of Inhibition Experiments*

Antibacterial activities of nebulized NAC, PBS solutions and DIW following non-thermal atmospheric plasma treatment were qualitatively determined on *E. coli* and *S. aureus* and represented as shown in Figure 3. In positive control groups (in which no plasma-treated liquids were nebulized) bacterial lawn growth on TSA plates were observed. 3% $H_2O_2$ was nebulized over *E. coli* and *S. aureus* on TSA plates as negative control and a clear bacterial inhibition zone was observed on TSA plates for both *E. coli* and *S. aureus* where nebulized hydrogen peroxide solution came in contact with bacteria. For the tested plasma-treated liquids, the most significant inhibition zone was obtained after nebulization of plasma-treated NAC solution over *E. coli* and this effect was dispersed all over the plate in which single colonies with spaces between them were observed. However similar effect following nebulization of plasma-treated NAC solution over *S. aureus* was less pronounced and even though, presence of less frequent bacterial colonies also, inhibition zone was observed on the plate in which nebulized NAC solution first came in to contact with bacteria. After nebulization of plasma-treated DIW, inhibition zones were clearly observable on both *E. coli* and *S. aureus* which also were limited in areas where nebulized plasma-treated DIW first contacted to bacteria. Moreover, nebulization of plasma-treated PBS solution was resulted as a similar inhibition zone on *S. aureus* that was limited to contact area. The inhibition zone over *E. coli* was less pronounced and remained limited on the edges of the TSA plate along with little dilution of bacterial lawn following nebulization of plasma-treated PBS. Furthermore, untreated NAC, PBS solutions and DIW were also nebulized over bacteria to ensure that the flow of mists of liquids generated during nebulization were not responsible for removal of bacteria and for generation of inhibition zone. Following nebulization of untreated NAC, PBS solutions and DIW no inhibition zone was observable on TSA plates for both *E. coli* and *S. aureus* (data not shown).

*Bactericidal Effect of Nebulized Plasma-Treated Liquids*

Colony counting assay was performed for determination of bactericidal efficacy of nebulized plasma-treated liquids at different initial concentrations of *E. coli* and *S. aureus*. As depicted in the Figures 4a and 4b, for $10^3$, $10^4$, $10^5$ and $10^6$ CFU/ml initial bacteria number, after nebulization of plasma-treated NAC, PBS solutions and DIW on both *E. coli* and *S. aureus* complete inactivation was achieved. However, after nebulization of all plasma-treated liquids over $10^7$ CFU/ml initial bacterial number of *E. coli* and *S. aureus*, complete inactivation was not observed. After nebulization of plasma-treated liquids over $10^7$ CFU/ml initial bacterial number of *E. coli*, NAC solution, PBS solution and DIW led >5-log,

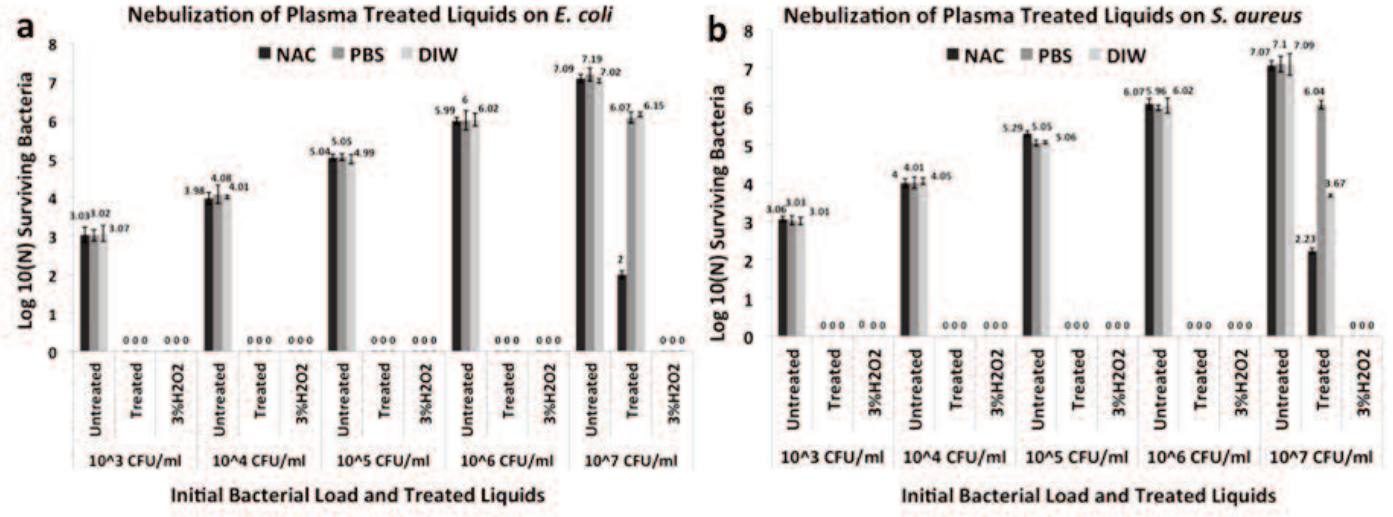


**Figure 4.** Bactericidal effects of nebulized plasma treated liquids were quantified with colony counting assay on E. coli (a) and S. aureus (b). All tested liquids have led complete inactivation of *E. coli* (a) and S. aureus (b) for the initial bacterial load of $10^3$, $10^4$, $10^5$ and $10^6$ CFU/ml. However, when initial bacterial load was increased to $10^7$ CFU/ml nebulized plasma-treated liquid have shown the strongest antimicrobial activity with around 5-log reduction over E. coli (a) and S. aureus (b). Nebulized plasma-treated PBS solution and water led around 1-log reduction on *E. coli* (a) and around 1-log and 3-log reduction on *S. aureus* (b) respectively when the bioburden was $10^7$ CFU/ml.

around 1-log and <1-log inactivation respectively. Moreover, after nebulization of plasma-treated liquids over $10^7$ CFU/ml initial bacterial number of S aureus, NAC solution, PBS solution and DIW led about 5-log, 1-log and 3.5-log inactivation respectively. Bactericidal effects of all nebulized plasma-treated liquids for each various initial bacterial load of *S. aureus* and *E. coli* were found to be statistically significant. 3% $H_2O_2$ was used as negative control and for all initial bacterial numbers of both *E. coli* and *S. aureus*, it led complete inactivation.

## Discussion

As discussed in the previous reports in detail, not only non-thermal atmospheric plasma by itself, but also liquids modified by it exerts strong antimicrobial activity over wide range of gram positive and negative bacteria - including antibiotic resistant strains - and fungus in their both planktonic and biofilm forms [6, 14, 22]. Strong antimicrobial activities of non-thermal plasma and non-thermal plasma-activated solutions have drawn attention for their possible future clinical use including for control and prevention of hospital acquired infections [27]. However, utilization of non-thermal plasma-treated liquids in respiratory tract is not applicable since presence of liquids in respiratory tract may cause asphyxia. In the present study, possible use of non-thermal atmospheric plasma-treated liquids was investigated through nebulization.

During plasma treatment of various liquids, different plasma generated ROS and RNS may react and/or diffuse into liquid, which then make plasma-treated liquids strong antimicrobial solutions. Presence of various ROS such as hydrogen peroxide, superoxide, hydroxyl radical and various RNS such as nitrate, nitrite, nitric oxide, peroxynitrite, in plasma-treated liquids were reported [4, 25, 28]. Even though plasma-treated liquids are complex mediums containing ROS and RNS and synergetic effects of those species were pronounced, researchers attempted to determine the most dominant species in plasma-treated liquids for antimicrobial activity [24, 25, 29, 30]. Dominant species for antimicrobial activity of plasma-treated liquids depends on the type of plasma and the treated liquid [25, 31]. Ercan *et al.* [25] have shown that peroxynitrite as the dominant species for bactericidal efficacy in NAC solution when treated with an atmospheric pressure non-thermal air DBD plasma [25]. Wu *et al.* [31] have detected various ROS in He/$O_2$ microjet plasma treated water and indicated singlet oxygen ($^1O_2$) as contributing the most of the inactivation.

Besides formation of ROS and RNS in plasma-treated liquids, also acidification of liquids after non-thermal atmospheric plasma treatment is one of the most common finding reported in various studies [32]. Decreased pH in plasma-treated liquids was mainly attributed to formation of nitric acid, nitrous acid, hydrogen cation and superoxide anion during plasma treatment [23]. Ercan *et al.* [6] have reported

pH drop in PBS, NAC solutions and DIW from 6.48, 6.24 and 6.80 to 2.58, 2.35 and 2.00 respectively after 3-minutes non-thermal atmospheric DBD plasma treatment. Also in the present study, we have shown statistically significant pH drop for all three liquids, which are consistent with the literature. However, low pH was not attributed to antimicrobial activity. In a previous study Machala *et al.* [29] have correlated reduced pH of plasma treated water with antimicrobial activity. In that study, low pH was found to be leading rapid oxidation of nitrites - formed during plasma treatment - to nitrates, which then associated as dominant species for antimicrobial activity. However, when buffers were used to prevent drastic pH drop, a weaker antimicrobial activity was observed instead of complete loss [29].

Moreover, in another study, antimicrobial activity of plasma treated NaCl solution was tested. Following surface DBD plasma treatment, pH of NaCl solution was decreased to acidic range along with formation of other chemical species. Treatment of NaCl solution led formation of nitrite, nitrate and hydrogen peroxide along with reduction of pH to around 3 [23].

As reported previously, acidification of nitrate and nitrite leads strong antimicrobial activity on various bacterial strains [33-35]. Formation of nitrates and nitrites and their diffusion to liquids during plasma treatment could enlighten the role of low pH for antibacterial effects of plasma treated liquids. In previous studies, effect of pH was investigated to clarify its contribution to antimicrobial activity. pH of various acid solution were set to pH values determined after plasma treatment and then exposed to bacteria. However, significant antibacterial effect of low pH was not observed In summary, as it was mentioned previously, low pH could play a supportive role instead of being main reason for antimicrobial activity arising from plasma treated liquids since low pH is not sufficient for microbial inactivation by itself [23, 25, 36].

In the present study antimicrobial activities of nebulized plasma-treated NAC, PBS solutions and DIW were evaluated on *E. coli* and *S. aureus*. As depicted in the Figure 2, inhibition zones on which nebulized plasma-treated liquids came in contact were presented. Zone of inhibition test was conducted to evaluate if plasma-treated liquids whether loose or could conserve antimicrobial activity during nebulization. Moreover, even though zone of inhibition test is not a quantitative method, depending on the obtained area of inhibition zone the antimicrobial power of plasma-treated liquids could be compared qualitatively [37]. As shown in Figure 2 when plasma treated NAC solution was nebulized over *E. coli*, the obtained inhibition zone was more generalized and bacterial growth turned out as separated single colonies as oppose to inhibition zones and lawn like bacterial growth obtained from nebulization of plasma-treated PBS and DIW. Moreover, nebulized plasma-treated PBS solution led inhibition on a smaller are at the edges of the plate, compared to nebulized plasma treated NAC solution and DIW. Inhibition zone results on *S. aureus* were similar to results obtained from *E. coli*. Effect of nebulized plasma-treated NAC solution over *S. aureus* was not prominent as observed over *E. coli* however this effect was spread over the plate leading separated single colony growth on the plate. Similarly effects of nebulized plasma-treated PBS and DIW on *S. aureus* mostly remained on the area where the nebulized mist first contacted on the plate.

As represented on Figures 4a and 4b bactericidal effects of plasma treated NAC, PBS solutions and DIW both on *E. coli* and *S. aureus* were initial bacterial load dependent. All nebulized plasma-treated liquids led complete statistically significant inactivation on tested bacterial strains for the initial bacterial load from $10^3$ to $10^6$ CFU/ml. However when initial bacterial load was increased to $10^7$ CFU/ml, even though all liquids led statistically significant inactivation, the strongest bactericidal effect was exerted by nebulized plasma-treated NAC solution. The nebulized plasma-treated NAC solution led about 5-log reduction both on *E. coli* and *S. aureus*. Results of zone of inhibition test and colony counting assay were consistent and both showed nebulized plasma-treated NAC solution as the strongest antimicrobial solution among all plasma-treated liquids. Also, our findings were consistent with literature in which plasma-treated NAC solution was presented as a stronger antimicrobial solution compared to plasma-treated PBS solution and DIW [6]. Previously, chemical modifications in the NAC solution during plasma treatment, was reported and stronger antimicrobial activity mainly attributed to formation of RNS and especially peroxynitrite [25].

In consequence of nebulization size of mist particles were reduced to the order of micrometers and whole liquid had a bigger surface area to react with ambient air, which then would lead loss of antimicrobial effect. However, our results indicates that, plasma-treated liquids could retain their antimicrobial effect when

they were nebulized.

Best of our knowledge, this study is the first report, which shows antimicrobial activity of nebulized plasma-treated liquids and presents nebulization as a delivery method for plasma-treated liquids for their possible future use on respiratory tract infections. Moreover, sterilization of endoscopes is a common challenge due to presence of lumen and associated with infection outbreaks [38]. Thus, nebulization of plasma-treated liquids could be considered as an alternative method for sterilization of endoscopes.

## Conclusions

In the present study we have tested the antimicrobial activities of plasma-treated NAC, PBS solutions and DIW when nebulized over *E. coli* and *S. aureus* gram positive and negative model organisms respectively. Zone of inhibition test and colony counting assay consistently showed that nebulized plasma-treated NAC solution exerted the strongest effect over two model organisms. Taken together, nebulization of plasma-treated liquids could lead novel applications of plasma treated liquids for the respiratory tract infections and sterilization of endoscopes. Experiments regarding the efficacy of nebulized plasma-treated liquids on biofilm forms of bacteria and fungus and cytotoxic effects of nebulized plasma-treated liquids are underway.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Uterine artery embolization: is it reliable for myoma treatment?

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## ABSTRACT

***Objectives.*** We aimed to share the long-term technical and clinical success results, complications and radiologic follow-up findings in our myoma cases treated with uterine artery embolization (UAE) ) and to make a contribution to the literature data on this subject. ***Methods.*** The study was retrospective and the results of 70 patients who underwent UAE for myoma treatment at our institute between January 2012 and January 2015 were analyzed. ***Results.*** The age range was 22 to 46 years and the mean age 34 years. The postprocedural follow-up duration was 6 to 24 months and the mean follow-up duration was 14 months. The technical success rate was 100% and the clinical success rate was 84.7%. On postprocedural follow-up, fibroid passage was seen in 2 patients (2.85%), severe hypermenorrhea in 4 (5.7%) and postembolization syndrome in 6 (8.5%). Our myoma recurrence rate was 11.36% (n=5). The UAE procedure did not need to be repeated in any of the patients. ***Conclusions.*** UAE is a reliable alternative to hysterectomy and myomectomy. We believe that UAE should be preferred in patients who are recommended hysterectomy or are predicted to potentially need hysterectomy during myomectomy.

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***Keywords:*** Uterine artery embolization, myoma, myomectomy

## Introduction

Uterine myomas are the most common gynecological problem that is seen in women of reproductive age [1]. Since most of the myomas are asymptomatic, their exact prevalence cannot be known. In symptomatic myomas, hysterectomy and myomectomy are traditional treatment methods [2]. According to literature data, myomectomy is recommended as the first option especially for patients who expect to conceive. Today, women prefer uterine myoma embolization (UME) more over abdominal myomectomy, even though they expect to get pregnant [3-6]. The reasons of this preference are that UME has less invasive, shorter hospitalization time and providing quick healing.

In our country, in accordance with the decision of the consensus established with obstetrician and gynecologists, we suggest patients who expect to conceive to undergo myomectomy. Likewise, we

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recommend hysterectomy to patients above 42 years who do not expect to conceive, since it has a protective role against cancer. However, we implement UME process on patients whom hysterectomy is recommended even though they expect to conceive, and also in cases that may require hysterectomy during the operation. Our short-term results were published in previous studies [7]. In this study, we share our long-term technical success, clinical success and complications.

## Methods

Our study was retrospective and the results of 70 patients who were treated with UAE at the unit between January 2012 and January 2015 were analyzed. All the patients had undergone pelvic computed tomography (CT) angiography for pelvic vessel mapping before the procedure (Figure 1) and pelvic magnetic resonance imaging (MRI) before and 3 months after the procedure.

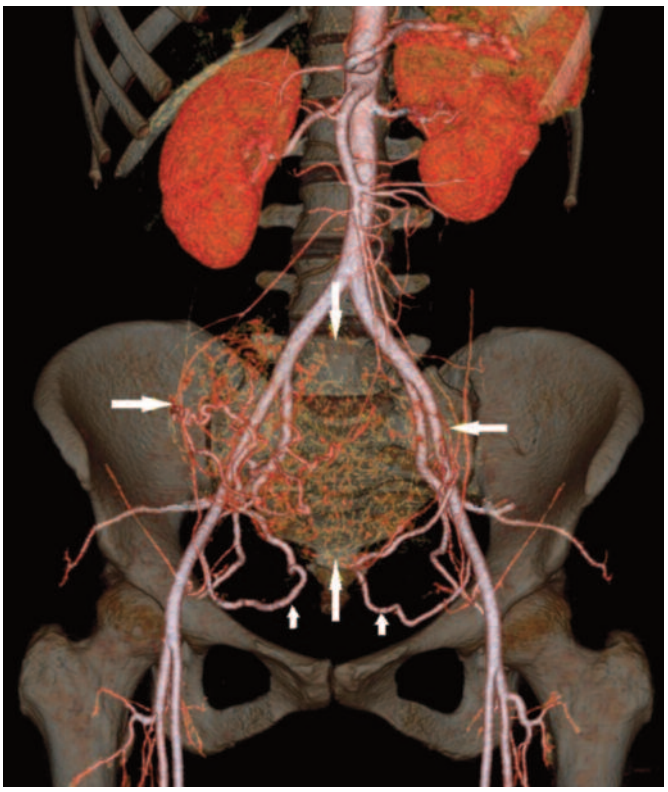

**Figure 1.** Pelvic CT angiography image of a 35-year-old patient with intramural hypervascular myoma 12 cm in diameter. The branches of the uterine arteries separating from the internal iliac artery (small arrow) and feeding the myoma (big arrow) are clearly seen.

Pelvic MRI investigations included T2W sagittal and axial, T1W sagittal, axial and coronal, and T1W sagittal, axial, and coronal sequences after an i.v. injection of 0.1 mmol/kg Gadabutrol (Gadovist; Bayer). The patients were called for a follow-up at the postprocedural period in the 1st week and at 3rd, 6th, 9th and 12th months. Imaging was performed with a pelvic MRI at the third month follow-up and pelvic ultrasonography (US) was performed at the other follow-ups. Pelvic MRIs of the patients were analyzed in terms of uterus volume, total myoma volume (total volume of all myomas in the uterus), T2W image signal intensities, contrast enhancement of myomas, and accompanying pathologies.

According to literature data, in patients with pregnancy expectancy, myomectomy is accepted as the first option in myoma treatment [1-6]. Therefore, we directed all patients to surgery who we thought can benefit from myomectomy in preoperative MRI. Since we thought that hysterectomy would also be a cancer protection method, we also directed patients to surgery who are over 42 years old and have no pregnancy expectancy. We operated on only two patients who are over 42 years. Both patients were recently married and haven't got any children. Gynecology consultation was required from all patients. All patients had been recommended a hysterectomy or had been told that hysterectomy could be needed during myomectomy before they were presented at our unit.

Technical success was defined as both uterine arteries being selectively catheterized and all arcuate arteries being embolized at the end of the procedure. Clinical success criteria were a decrease of more than 50% in total myoma volume at the third month follow-up, the disappearance of contrast enhancement of myomas in the MRI, and a decrease in myoma signal intensity in T2W images after the procedure (Figure 2).

All patients were informed on how the myoma embolization procedure would be performed, the expected results, complications, and the alternative treatment methods before the procedure. This was followed by obtaining written consent.

*Technique*

As it was stated in our previous study [7], all patients received i.v. analgesia (100 mg Tramadol HCl; Contramal) starting half an hour before the procedure and lasting until the postprocedure 12th hour. Local anesthesia and a single femoral artery entry were used in all patients. A 5F Cobra (Cordis, USA) catheter was placed into the uterine arteries with the help of a 0.035" hydrophilic guidewire (Terumo,

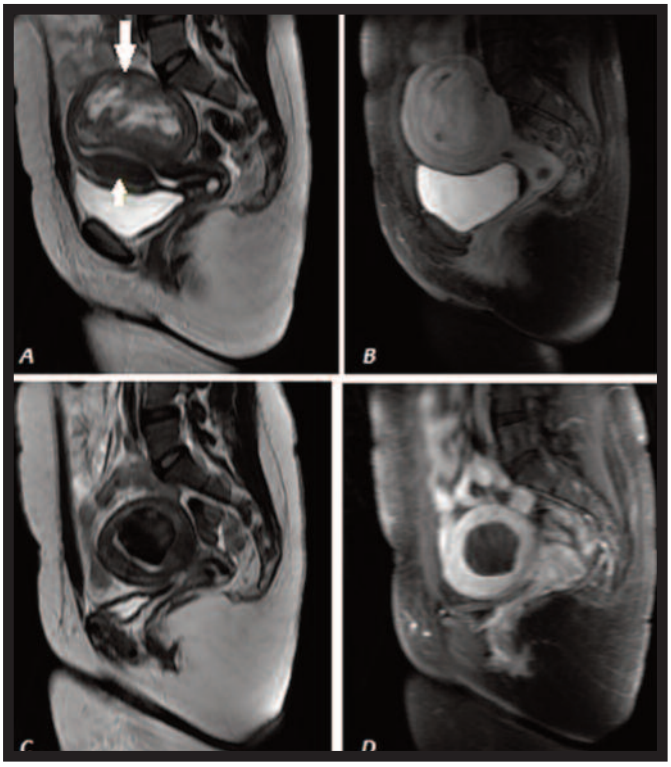


**Figure 2.** **(A)** Intramural myomas 4x2 cm in size (big arrow) in the anterior uterus and 8 cm in diameter (small arrow) in the posterior uterus are observed on preoperative sagittal T2W MRI investigation. **(B)** The myoma is seen to enhance more intensively than the myometrium on preoperative sagittal T1W contrast MRI investigation. **(C)** The diameter of the posterior myoma has regressed to 3.5 cm and has a homogenous hypointense appearance at the postoperative third-month sagittal T2W MRI investigation. The myoma and myometrium borders are sharpened. The myoma observed anteriorly at the previous investigation has disappeared completely. **(D)** The myoma has become hypointense due to devascularization at the postoperative third-month sagittal T1W contrast MRI investigation.

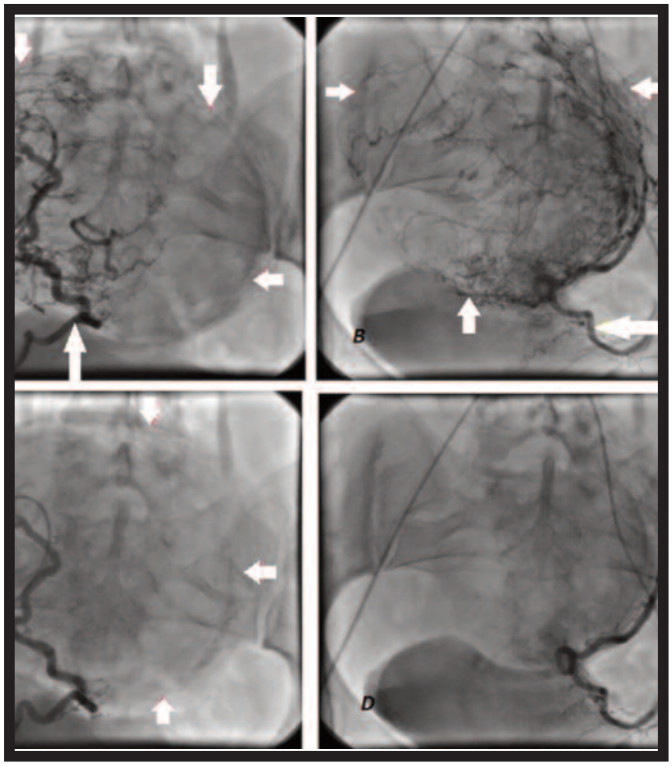


**Figure 3.** **(A)** Preoperative angiographic image of the branches of the myoma supplied by the right uterine artery. **(B)** Preoperative angiographic image of the branches of the myoma supplied by the left uterine artery. **(C, D)** The branches feeding the myoma from the right and left sides are seen to be completely embolized on postoperative angiographic images (uterine arteries: big arrow, myoma borders: small arrows).

Japan). The transverse segment of the uterine artery was accessed using a microcatheter (EmboCath Plus, Biosphere Medical) and 0.018" guidewire (Sequitor, Biosphere Medical) in cases that were challenging due to tortuosity or diameter. After the arcuate arteries were imaged by administering a contrast substance to the uterine artery transverse segment, microparticles (500-700 micron; Embosphere, Biosphere Medical) were injected. If an arterio-arterial shunt was observed, the procedure was started with microparticles 700-900 micron in diameter. The injection was continued until all arcuate arteries were embolized. Later, the same procedures were performed at the contralateral uterine artery so that all arcuate arteries were embolized on both sides. Care was taken to preserve the cervicovaginal artery during the procedure (Figure 3).

All patients were discharged after fourteen hours. Vaginal bleeding similar to menstruation with occasional particles started a few hours after the procedure and continued for 3 to 6 days in all patients. All patients were administered painkillers (75 mg/day; Diclofenac sodium; Voltaren) orally for one week. None of our cases received prophylactic antibiotics. None of the cases had been presented for infertility treatment.

*Statistical Analysis*

Mean or median with minimum an maximum values were determined for the demographic data, uterine volume, and amount of embolic agent used. Changes in uterine volume were calculated the Wilcoxon test for paired samples. Statistical significance was accepted at a $p$ value less than .05.

## Results

The age range of the cases was 22 to 46 years and the mean age was 34 years. All patients were symptomatic and the most common symptom was

menstrual irregularities (n=56, 80%). Other symptoms were anemia (n=46, 65.7%), abdominal pain (n=28, 40%), and mass-related symptoms (palpable mass, frequent urination, constipation, etc.) (n=14, 20%). Most patients had multiple symptoms. All patients had been recommended hysterectomy or had been told that hysterectomy could be needed during myomectomy before they were presented at our unit. A history of myomectomy was present in 9 (12.8%) patients. The postprocedure follow-up duration was 6 to 24 months and the mean follow-up duration was 14 months.

Both uterine arteries were catheterized successfully and all arcuate arteries were embolized in all patients (technical success rate 100%). According to the clinical success criteria provided above, 59 of 70 UAE procedures were accepted as successful after the third month follow-up (clinical success rate of 84.3%). All patients that were clinically successful stated at the third month follow-up that the initial symptoms had disappeared. The procedure was considered a failure in 11 patients as the size of the myoma did not decrease as much as desired, total or partial contrast enhancement continued, and image intensity did not decrease in T2W images (clinical failure rate of 15.7%). Although some of the cases accepted as clinical failure at third month follow-up stated that their symptoms had decreased, none stated that all symptoms had disappeared. When patients' pre-operation MRIs that were accepted as unsuccessful were examined, it was found that myoma sizes of 6 of them were bigger than 10 cm (average: 13.6 cm), myomas of 4 of them were dyed weak with contrast agent, and the myoma of 1 patient was has intense calcification centers [5, 6].

Mean preprocedure uterus volume on MRI was 404 $cm^3$, and mean preprocedure myoma volume was 280 $cm^3$, while at the third month follow-up the mean uterus volume was 182 $cm^3$ and mean myoma volume was 116 $cm^3$. The largest myoma size in pre-operation MRI examinations of every operated patient is between 3 and 16 cm. The reduction in the total uterus volume was 52.47% and reduction in total myoma volume was 62.14% (Table 1).

The passage of fibroids was seen in two patients (2.85%). Both patients were presented at the third postprocedural week. The first patient had symptoms of malodorous vaginal discharge, pieces of tissue coming from the vagina, cramping abdominal pain, fever, and nausea, while the second patient had symptoms of pieces of tissue coming from the vagina and cramping abdominal pain. Our first patient was hospitalized for intravenous antibiotics and anti-inflammatory treatment administration following hysteroscopy and curettage (Figure 4). Our second patient was only followed-up with oral antibiotics and anti-inflammatory treatment. The symptoms of both patients disappeared during the follow-up. Myomas of both of these patients were submucosal located. In comparative assessment, there was no difference observed in terms of pre-operation fibroid passage formation between other patients with myomas who are successfully operated upon and these patients' pre-operation MRI examinations.

**Table 1.** Preprocedural and postprocedural changes

| | Mean Uterine Volume (ml) | Mean Myoma Volume (ml) |
|---|---|---|
| Preoperative | 404 | 280 |
| Postoperative | 182 | 116 |
| Change (decrease percentage) | 52.47% | 62.14% |

Four patients were hospitalized due to severe hypermenorrhea before the postoperative third month (5.7%). All four were treated with curettage and oral hormonal support treatment. Post-embolization syndrome was seen in six patients (8.5%). Supportive treatment was used for all six. Hysterectomy was not necessary in any of the patients during the postoperative period. Evaluation of the 44 patients with a follow-up duration over 12 months from the 59 patients accepted as clinically successful revealed myoma recurrence and related symptoms in 5. Our myoma recurrence rate at the end of the first year of follow-up in clinically successful patients was therefore 11.36% (n=5). In these patients' examinations, new myomas that weren't seen in their pre-operation MRIs were detected. The UAE procedure was not repeated in any of the patients.

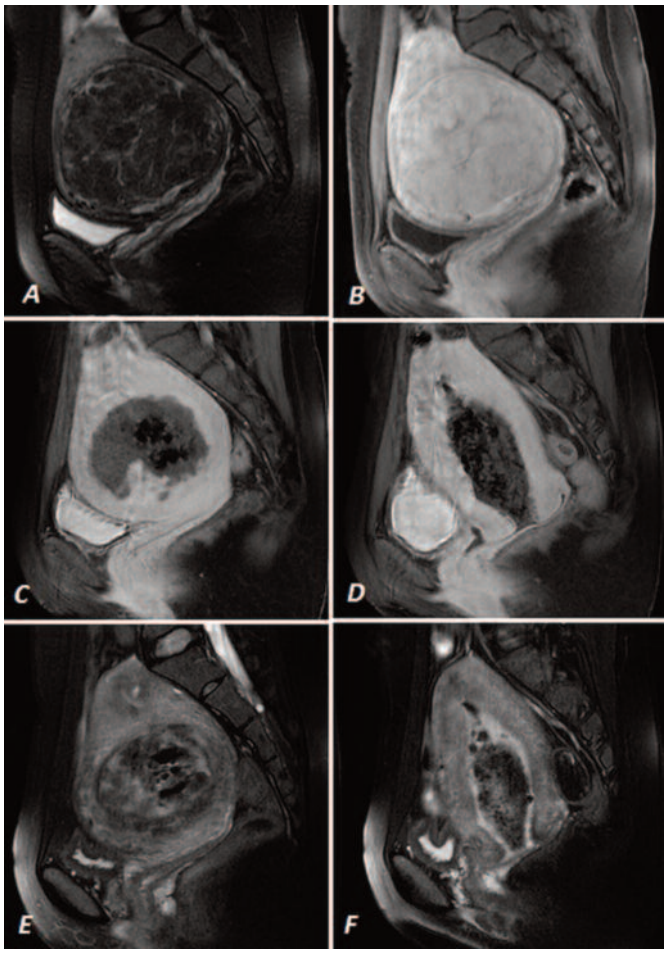


**Figure 4.** **(A)** Preoperative T2W and **(B)** T1W contrast sagittal images of a 41-year-old patient with a myoma 10 cm in diameter. Fibroid passage is observed at **(C, D)** T1W contrast and **(E,F)** T2W sagittal images of the case that presented with symptoms of malodorous vaginal discharge, pieces of tissue coming from the vagina, cramping abdominal pain, fever and nausea 3 weeks after the procedure. The air appearance inside the necrotic tissue was interpreted as infection.

## Discussion

Uterine myomas are the most common benign tumors of the uterus. Momas can be asymptomatic [7-10]. Symptoms develop according to the localization, number, and size of the myomas. Symptomatic myomas usually cause symptoms of bleeding (irregular, increased or decreased menstrual bleeding, dysmenorrhoea), pain (pelvic area or the back), an abdominal mass (bladder or bowel compression), or infertility [8, 9]. Frequent and severe bleeding (hypermenorrhea) is the most common symptom with a rate of 60%. The hypermenorrhea often causes anemia. Myoma is the accepted cause of only 1% to 2.4% of infertility cases [9]. The symptoms of our patients were similar to those reported in literature and consisted of abdominal pain, mass symptoms, and most commonly bleeding.

Hysterectomy and myomectomy are the conventional treatment methods for symptomatic myomas [10]. Hysterectomy is the most commonly used myoma treatment method with rates differing between countries. Myomectomy is the second most commonly used method [9, 11]. Observation, medical treatment, endometrial ablation, UAE, and surgical occlusion of the uterine artery and high intensity focused ultrasound (HIFU) are other current treatment options [12]. The number, size, and location of the myomas, the age, number of children, child expectation, previous history of abortion, the severity of the symptoms, the patient's medical condition, the risk of malignancy and proximity to menopause are some of the factors influencing the treatment decision [11]. Hysterectomy is thought to be too radical by most patients because of the loss of fertilization. Myomectomy is recommended in cases where fertility wishes to be preserved [2]. There has been an increasing interest in UAE since 1995, when it began to be used, as it is a minimally invasive procedure [1-8, 13]. Its advantages are lack of an abdominal incision, no need for general anesthesia, short hospitalization duration, and fast recovery time. Obesity, past abdominal surgery, and the number and localization of the myomas do not prevent the UAE procedure, unlike surgery [14].

The aim in UAE is to disrupt the branches feeding the hypervascular myoma with selective embolization of both uterine arteries [3]. The procedure causes devascularization infarction and consequently a reduction in the size of the myoma and a disappearance of the symptoms [8].

Some authors recommend preprocedure pelvic CT angiography to be performed on patients before UAE and the procedure to be planned on the 3D images obtained. The pelvic vascular network, vessel diameters, and the rotation of the digital subtraction angiography tube can be calculated before the procedure and an easier access to the uterine artery ensured [15]. We planned our procedures using 3D images from the preoperative pelvic CT angiogram in all of our cases.

The uterine arteries are characteristically U-shaped, and descending, transverse, and ascending parts are present [3, 6]. The cervicovaginal artery, a major branch of the uterine artery, should be protected during embolization when possible [3]. Particle injection is administered after a catheter is advanced to the transverse segment of the uterine artery for the

UAE procedure [3, 6].

A large number of different materials have been used for embolization. Polyvinyl alcohol (PVA), Gelfoam, and Trisacryl microparticles were the first materials to be used. Microspherical microparticles produced by using various materials are now used more often [16]. We also used microspherical microparticles (Embosphere, Biosphere Medical) in all of our cases. Particles larger than 500 micron are usually recommended as the diameter of the utero-ovarian anastomoses are less than 500 microns. Injection is continued until all branches going to the myoma are obstructed. When the endpoint is reached, the main uterine artery branch appears like a pruned tree [3, 6]. Another endpoint criterion is starting to see the anastomoses between the right and left branches of the uterine artery or between the uterine artery and the ovarian artery that were not observed at the beginning of the procedure (Figure 5).

The reasons for failure of the UAE procedure are the inability to create full blockage in the arteries feeding the myoma due to the anatomy- or operator-related reasons and consequently a lack of infarction in the myoma. The inability to reach the distal part of the uterine artery, using insufficient amount of embolic agent, the presence of large shunts, and the presence of vascular structures other than the uterine artery feeding the myoma (from the abdominal aorta, cervicovaginal artery, etc.) can also be listed as causes of failure [6, 16, 17]. The most common cause of failure is unilateral embolization [3, 6]. Very large myomas (over 10 cm) respond poorly to embolization due to potential collateral feeding. Multiple myomas respond better to embolization than single myomas. UAE is more effective in small submucosal and intramural myomas [3].

Complications of the UAE procedure are quite rare. The expected complications can be classified into 3 groups as angiography-related (contrast agent allergies, contrast nephropathy, etc.), infection, and embolization-related complications (ovary embolization) [18-20]. One must consider that patients are exposed to radiation during the procedure. The most common complication requiring an intervention

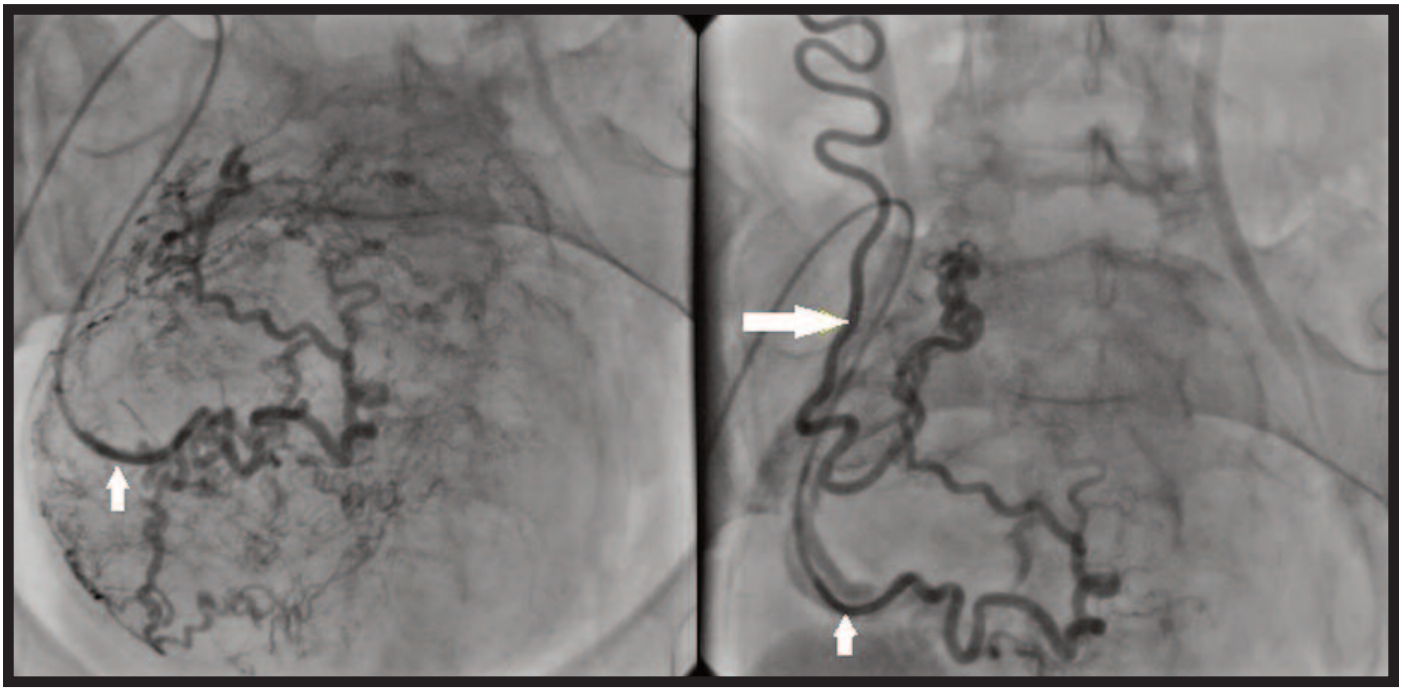

**Figure 5.** **(A)** An utero-ovarian anastomosis that was not observed in pre-embolization images of the right uterine artery (small arrow), which was selectively catheterized, **(B)** has become visible after embolization (big arrow).

is a fibroid passage with an incidence of 4.7% (3.9-5.7%). This condition is due to the migration of intramural and submucous myomas towards the endometrial cavity in the post-embolization period. Ovarian dysfunction characterized by temporary or permanent amenorrhea has been reported at a rate of 3.9% (2.7%-5.3%). Ovarian dysfunction is usually seen in cases that were older than 45 years at the time they underwent UAE [5, 6, 21]. Non-targeted embolization of organs other than the ovary is a rare complication and the most common examples are vaginal damage and sexual dysfunction, due to embolization of the cervicovaginal branch, and vulvar pain and labia necrosis, due to the embolization of the

internal pudendal artery [6]. Uterine necrosis, endometritis, septic shock, pulmonary embolism, deep vein thrombosis, posterior reversible encephalopathy, and death are very rarely reported complications [3, 6, 22]. In addition to the complications reported in the literature, four of our patients were hospitalized due to severe hypermenorrhea in the first 3 postoperative months (5.7%). All four patients were treated with curettage and oral hormonal support therapy. We did not find any publication regarding this complication in literature. Myomas can cause severe hypermenorrhea even when no procedure is performed so whether our observation is related to the myoma itself or to the procedure is controversial. However, we thought it was as a complication.

The major complication rate of UAE has been reported as 2.9% (2.2%-3.8%) and the postprocedural hysterectomy rate as 0.7% (0.5%-0.9%) in a meta-analysis including 8,159 patients. The clinical-symptomatic improvement rate was 78% to 90% in the 3 months to 2 years of follow-up in that study [21]. No significant difference was found between surgery and UAE in terms of major complication rates in another meta-analysis comparing UAE, hysterectomy, and myomectomy. The 2$^{nd}$ and 5$^{th}$ year patient satisfaction rates were similar with UAE and surgery in this meta-analysis. However, a reintervention was required within 2 years in 7% of surgery patients and 15%-32% of UAE patients [23].

The most common side effect of the procedure is the postprocedural pain described by the patients as uterine cramps. The reason for one night of hospitalization is pain management [24]. We observed that the severity and duration of the pain varied significantly from patient to patient. Another common side effect is vaginal bleeding that starts within hours after the procedure and continues for 1 to 6 days. The amount of vaginal bleeding was from spots to menstrual bleeding in the style of parts dropping. The described vaginal bleeding was seen in all of our patients. Another common side effect of embolization is the post-embolization syndrome. We treated the post-embolization syndrome we observed in 6 of our patients with supportive methods.

Although an increase in the rates of conception has been reported following UAE, obstetric complications such as spontaneous abortion, premature delivery, placental implantation abnormalities, cesarean delivery, and post-partum hemorrhage have also been reported to increase [3, 12, 25]. However, a recent meta-analysis has reported that the evidence supporting a positive effect of myomectomy on fertility to be more pronounced than with UAE is low quality [23]. The data on performing UAE in patients with an expectation of fertility is controversial [3, 12, 25]. However, considering that hysterectomy causes loss of all fertility, we believe that UAE should be preferred in particular in patients who are recommended hysterectomy or who are predicted to potentially require hysterectomy during myomectomy and who also have an expectation of fertility.

*The Limitations of the Study*

Though no complications have been identified in our short-term results except for process failure [7], we have observed different complications in this study. This shows that the complications of the process can be identified better with increasing number of patients. The results belong to a limited number of patients undergoing the procedure by the same clinician in a single center. We believe that performance of this procedure in more than one center and with higher number of patients will provide more efficient assessment of the process.

## Conclusions

UAE is a reliable alternative to hysterectomy and myomectomy. We believe that UAE should be preferred in particular in patients recommended hysterectomy or predicted to potentially require hysterectomy during myomectomy and who have an expectation of fertility.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Correlation of serum C-reactive protein and procalcitonin levels in infections of kidney transplant recipients

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## ABSTRACT

***Objectives.*** Procalcitonin is a propeptide of calcitonin and has been increasingly used as a biomarker of infection. The aim of this study was to evaluate correlation of serum C-reactive protein (CRP) and procalcitonin (PCT) levels of kidney transplant patients hospitalized due to infection. ***Methods.*** There were 121 patients who had kidney transplant in our center between September 2012 and February 2017 and patients with a diagnosis of infection or rejection were included in the study. Simultaneous 106 serum CRP and PCT levels at the beginning or during any time of treatment for post-transplant infection, cytomegalovirus (CMV) positivity, BK viremia and rejection were evaluated. ***Results.*** Median and interquartile ranges of CRP and PCT serum levels were 40 mg/l [24.7-64.9] and 0.19 ng/ml [0.1-0.61], respectively. A significant positive correlation between serum CRP and PCT levels of the patients were observed (r=0.490, $p<0.001$). When serum CRP levels were grouped as <50 mg/l, 50-100 mg/l and >100 mg/l, correlations with serum PCT levels were as r=0.461 ($p<0.001$), r=-0.52 ($p=0.860$) and r=0.488 ($p=0.153$), respectively. Serum levels of PCT did not increase in CMV and BK virus infections and rejection. ***Conclusions.*** Serum CRP and PCT levels were correlated as a whole in the study, whereas serum CRP levels of 50-100 mg/l and >100 mg/l did not show a statistically significant correlation. Stability of PCT levels in viral infections and rejections might be an advantage for the follow-up of solid organ transplants. We need prospective trials of PCT measurements for the evaluation of post-transplant infections.

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***Keywords:*** C-reactive protein, procalcitonin, correlation, kidney transplantation

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## Introduction

Systemic infection is a very common complication after solid organ transplantation and is associated with increased morbidity and mortality [1, 2]. Infections that develop after kidney transplantations might be associated with acute cellular rejection, graft failure, graft loss and even death [3-5]. Mediators like serum C-reactive protein (CRP), cytokines and interleukins increase with systemic infections. Among those CRP is the most widely used clinically and serum levels of <5 mg/l are accepted as normal. But serum CRP levels increase in vascular occlusive diseases, malignancies, trauma and bacterial, viral, fungal and protozoal infections [6]. Procalcitonin (PCT), a species-specific propeptide of calcitonin, is a glycoprotein of 116 amino acids with a molecular weight of 13 kD. Its origin and function are unknown. Gene structure and gene-locus are described by Le Moullec *et al.* [7]. PCT is believed to be secreted by peripheral monocytes, liver and other tissues in response to cytokines such as tumor necrosis factor, interleukin 6, granulocyte colony stimulating factor and endotoxins from bacterial wall [8, 9]. PCT usually does not increase with viral infections. Because interferon γ that is secreted due to viral particles prevents PCT production. Serum PCT levels below 0.5 g/l is usually accepted as normal. PCT has come to use in solid organ transplants recently [10, 11]. The purpose of this study is to evaluate correlation between synchronous serum PCT and CRP levels at the beginning and during treatment of kidney transplant patients hospitalized for infections.

## Methods

There were 121 patients (108 living kidney donor, 13 cadaveric) who had kidney transplant in our center, University of Health Sciences, Gazi Yasargil Training and Research Hospital, Diyarbakir, Turkey between September 2012 and February 2017. Patients with a diagnosis of infection or rejection were included in the study. The study is retrospective and compatible with the Helsinki declaration in 2008. All patients gave informed consent. Synchronous serum CRP and PCT levels were measured before and/or during treatment of hospitalized patients for infection. A total of simultaneous 106 serum samples were taken. Correlation between 97 serum CRP and PCT levels were evaluated. These cases were hospitalized for post-transplant infections. Nine cases were rejection, cytomegalovirus (CMV) and BK virus (BKV). Three subgroups of serum CRP levels, <50 mg/l, 50-100mg/l and>100mg/l, were correlated with serum PCT levels. Demographic data, immunosuppressive protocols, presence of rejection, loss of graft and patient, postoperative infections, BK nephropathy (BKN), CMV infection were all evaluated retrospectively (Table 1-3).

**Table 1.** Descriptive data of the kidney transplant cases.

| **Number of patients** | **n=121** |
|---|---|
| **Gender M/F** | |
| Recipient | 68/53 |
| Donor | 47/71 |
| **Age (years)** | |
| Recipient | 34.9 (12-68) |
| Donor | 42.3 (23-72) |
| Follow up period (months) | 26 [6-46] |
| **Donor type** | |
| Live | 108 (89.2%) |
| Cadaveric | 13 (10.7) |
| Preemptive | 43 (35.5%) |
| **Operation of donor** | |
| Open nephrectomy | 79 |
| Laparascopic nephrectomy | 32 |
| **Induction** | |
| None | 15 (13%) |
| ATG | 65 (53.7%) |
| Basiliximab | 41 (33.8%) |

ATG=anti-thymocyte globulin, M=male, F=female

**Table 2.** Clinical results of the kidney transplant patients.

| **Patient number** | **n=115** |
|---|---|
| **Rejection** | |
| Humoral | 2 (1.6%) |
| Cellular | 4 (3.2%) |
| Humoral+Cellular | 1 (0.8%) |
| **Graft loss** | |
| CAN | 1 (0.8%) |
| BKN | 1 (0.8%) |
| RAP | 1 (0.8%) |
| Rejection | 1 (0.8%) |
| **CMV** | |
| Infection | 2 (1.6%) |
| Disease | 0 |
| **BKV** | 4 (3.2%) |
| **BKN** | 1 (0.8%) |

CAN= chronic allograft nephropathy, BKN= BK nephropathy, RAP=renal artery pseudoaneurysm, CMV= serum C-reactive protein; BKV=BK viremia

*Immunosuppression and Prophylaxis*

**Table 3.** Post-transplant hospitalization rates due to infection.

| Cause of hospitalization | Number of hospitalization (n) | Number of CRP and PCT samples (n) |
|---|---|---|
| Urinary tract infection | 91 | 35 |
| Pneumonia | 11 | 4 |
| Gastroenteritis | 32 | 11 |
| Herpes Zoster | 2 | 0 |
| Abscess of breast | 1 | 0 |
| Tuberculosis | 2 | 2 |
| Brucellosis | 1 | 1 |
| Invasive aspergillosis | 1 | 1 |

CRP= serum C-reactive protein; PCT= procalcitonin

Basiliximab (20 mg at days 0 and 4 of operation) or anti-thymocyte globulin (ATG; for high risk patients, 3 mg/kg during operation and 1.5 mg/kg at postoperative days 1 and 2) were used as induction treatment. Methylprednisolone 1000 mg was given intraoperatively. Methylprednisolone dose was decreased by half everyday and 20 mg oral prednisolone was started on the 6th postoperative day for daily use. Oral prednisolone dosage was reduced gradually to reach 5 mg a day at the first year after transplantation. Calcineurin inhibitors (tacrolimus or cyclosporin) and mycophenolate mofetil (MMF; 2 g a day in two divided doses) or mycophenolate sodium (MMF; 1440 mg a day, in two divided doses) were used as maintenance immunosuppression. MMF was used as 600 mg/m$^2$ in two divided doses in children. We considered both mycophenolate mofetil and mycophenolate sodium in doses described above as the same drugs in our study. Everolimus was used in only one case (plasma level of the drug was targeted as 8-10 mg/dl). Trimethoprim/sulfamethoxazole and valganciclovir (450 mg a day) was prescribed for pneumocytis jirovecii and CMV prophylaxis for 6 months after the transplantation. Acute rejection was diagnosed by kidney biopsy. Acute cellular rejection was treated with intravenous pulse methylprednisolone or ATG depending on the severity of the rejection.

*Determination of PCT and C-Reactive Protein*

A total of 106 serum samples were measured for CRP and PCT levels simultaneously. CRP and PCT measurements were performed by immuno turbidimetric method on Cobas c702 (Roche Diagnostics, Germany) instrument and immunoassay method on Cobas c702 (Roche Diagnostics, Germany) instrument, respectively. Interassay coefficients of variation values were below 3% for both tests.

*Diagnostic Criteria and Follow-Up*

All patients were followed-up closely for renal functions, clinical infection, BKV and CMV after kidney transplantation. Clinical infection was diagnosed on the basis of positive culture or serology combined with the use of appropriate antibiotic treatment or in the absence of microbiological confirmation, on the basis of fever (above 38°C) combined with the use of appropriate treatment. Urinary tract infection was established by clinical symptoms: fever and chills, flank pain, and irritative voiding symptoms (e.g., urgency, frequency, and dysuria), nausea or vomiting, inflammatory status, and positive urine culture [12, 13]. Enterocolitis was established through diarrheal syndrome (symptoms: fever, abdominal swelling, nausea, vomiting, diarrhea, rectal bleeding, sluggishness) with inflammatory status and/or positive culture [14]. Rejection was diagnosed by biopsy. First BKV tests were done on 1st postoperative month. All transplanted patients were tested for BKV from their serum by polymerase chain reaction (PCR) monthly in the first year after transplantation. Cases with >500 copies/ml by two or more consecutive measurements were accepted as having viremia. Tests for CMV were performed every 3 months after 6th postoperative month. Viremia detection was accepted as CMV infection, presence of symptoms were considered as CMV disease.

*Statistical Analysis*

Statistical analyses were performed using the SPSS software version 15. The variables were investigated using visual (histogram, probability plots) and analytical methods (Kolmogorov-Simirnov/ Shapiro-Wilk'stest) to determine whether or not they are normally distributed. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A 5% type-I error level was used to infer statistical significance.

## Results

One hundred and twenty-one patients (108 living kidney donor, 13 cadaveric) who had kidney transplant in our center betweenSeptember 2012 and February 2017 were evaluated, retrospectively. Average age of recipients and living donors were 34.9 (12-68) and 42.3 (23-72), respectively. Ten cases were at pediatric age. Male/female ratios of recipients and living donors were 68/53 and 47/71, respectively. Median and interquartile range follow-up time were 26 [6-46] months. Average duration of hospitalization was 8 (5-32) days. Postoperative discharge median and interquartile range creatinine was 1.04 [0.55-1.48] mg/dl. The most frequent reason for hospitalization was urinary tract infection. One case who used intermittant clean urinary catheterization was hospitalized 24 times for recurrent urinary tract infection due to neurogenic bladder. Simultaneous serum CRP and PCT levels were measured before and/or during treatment of hospitalized patients for infection. So total synchronous serum samples for CRP and PCT was 97. Median and interquartile ranges of CRP and PCT serum levels were 40 mg/l [24.7-64.9] and 0.19 ng/ml [0.1-0.61], respectively. Simultaneous CRP and PCT levels were measured in 3 patients with BKV and 2 patients with CMV infection. Simultaneous CRP and PCT levels were measured four out of seven cases with a diagnosis of rejection. Serum CRP levels of hospitalized renal transplant patients due to infection correlated with serum PCT level (r=0.490, $p$<0,001).When serum CRP levels were grouped as <50 mg/l, 50-100 mg/l and >100 mg/l, correlations with serum PCT levels were as r=0.461 ($p$<0.001), r=-0.52 ($p$=0.860) and r=0.488 ($p$=0.153), respectively (Table 4). Four cases with biopsy proven acute rejection had normal serum PCT levels. Serum levels of PCT and CRP did not increase in CMV infection and BKV viremia patients. CMV disease was not observed in any patient. One case had CMV infection, BKV nephropathy and invasive aspergillosis at the same time. The patient had graft loss and CRP and PCT level of the patient was as high as 250 mg/l and 47 g/l respectively. Four cases of graft loss were observed. Chronic allograft rejection (from cadaveric donor), renal artery pseudoaneurysm (from cadaveric donor) and humoral rejection were the causes of graft loss.

## Discussion

Synchronous serum CRP and PCT levels at the beginning or during any time of hospitalization for post-transplant infection were evaluated retrospectively in our study. Retrospective nature of the study and insufficient number of PCT measurements due to nonroutine use of PCT comparing to CRP measurements made sensitivity, specifity and cut-off value analysis impossible. But CRP and PCT levels showed a significant positive correlation (r=0.490, $p$<0.001) when all cases were considered. Cooper *et al.* [15] did not find a significant difference between serum PCT, serum amyloid protein and CRP levels in 43 heart, 34 lung and 33 liver transplanted patients. Serum PCT levels do not usually increase in viral infections [9]. In our study 2 CMV positive and 3 BKV patients were found. These 5 cases had normal serum PCT and CRP levels. But a patient with BKV, CMV infection and invasive aspergillosis had high serum PCT and CRP levels. Roques *et al.* [16] did not observe high PCT levels despite increased CRP levels in leukemia patients with invasive aspergillosis. But Cooper *et al.* [15] found that serum CRP, serum amyloid protein and PCT levels increased significantly in solid-organ transplant patients with fungal infections. Sometimes it is difficult to differentiate acute allograft rejection and infection clinically. In a retrospective study, Hammer *et al.* [17] observed that serum PCT levels did not increase in rejection cases of heart and lung transplants. In a prospective study by Eberhard *et al.* [18], procalcitonin values for patients with rejection were not significantly different from those of the healthy

**Table 4.** Correlation of serum C-reactive protein (CRP) and procalcitonin (PCT) levels.

| CRP level | Number of samples | r | *p* |
|---|---|---|---|
| <50 mg/L | 52 | 0.461 | <0.001 |
| 50-100 mg/L | 23 | -0.52 | 0.860 |
| >100 mg/L | 22 | 0.488 | 0.153 |
| All samples | 97 | 0.490 | <0.001 |

transplant recipients. During postoperative follow up, 7 cases had rejection. Four of the cases had PCT and CRP levels measured and PCT levels were found to be normal and 3 of these cases had higher serum CRP levels.

*The Limitations of the Study*

Our study was retrospective, serum PCT measurements did not routinely measured as serum CRP levels. So that only synchronous serum PCT and CRP levels were used for correlation analysis. Synchronous measurements were done any time during treatment, so there were no standardized or planned time for laboratory testing of these parameters. We only made correlation analysis, design of the study was not suitable to determine the usefulness of PCT measurements for the response of the therapy. In ability to determine reliability and specifity of PCT levels and retrospective nature of the study were the limiting factors of the study.

## Conclusions

In our study, serum CRP and PCT levels were significantly correlated; but when CRP levels were grouped, CRP >100 mg/l and 50-100 mg/l subgroups were not significantly correlated. Stability of PCT levels in viral infections and rejections might be an advantage for the follow-up of solid-organ transplants. We need prospective trials of PCT measurements for the evaluation of post-transplant infections.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Biometric and intraocular pressure changes after Nd:YAG laser capsulotomy

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## ABSTRACT

***Objectives.*** We aimed to investigate the influences of Nd:YAG (neodymium-doped yttrium aluminum garnet) laser capsulotomy on ocular biometric parameters. ***Methods.*** In this prospective study, 117 eyes of 117 pseudophakic patients were included. Full ocular examination, the best-corrected visual acuity (BCVA), and intraocular pressure (IOP) measurements were performed before the procedure as well as one week, one month, and three months after Nd:YAG laser capsulotomy. The axial length (AL), central corneal thickness (CCT), anterior chamber depth (ACD), and pupil diameter (PD) were measured by LenStar 900 optical biometry. The measurements were repeated one week, one month, and three months. ***Results.*** The BCVA improvements at preoperative, one week, and one month were statistically significant, but no significant changes in the BCVA were found at one month and three months ($p$=0.345). No association was found between the capsulotomy and mean IOP changes during the follow-up period ($p$=0.300). No significant changes were found in the CCT ($p$=0.059). The ACD changes occurred at preoperative; the first week and the first month were statistically significant ($p$<0.001). No statistically significant differences were found between the first month and the third month ($p$=0.365). No significant changes in the pupil size ($p$=0.200) and AL ($p$=0.112) were found after Nd:YAG laser capsulotomy. ***Conclusions.*** Our study demonstrated that the BCVA parameter changed after Nd:YAG laser capsulotomy. The BCVA and ACD values follow-up period should be at least one month after Nd:YAG capsulotomy.

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***Keywords:*** Ocular biometry, Nd:YAG laser capsulotomy, LenStar, posterior capsular opacification

## Introduction

Posterior capsular opacification (PCO) is the most common complication that occurs after extracapsular cataract extraction or phaco-emulsification [1]. It develops as a consequence of the proliferation of remaining epithelial cells and their migration to the space between the intraocular lens (IOL) and the posterior capsule [2, 3]. Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser capsulotomy is an effective technique and the so-called gold standard for treating visually significant PCO in pseudophakic eyes [4]. A number of complications can occur after YAG laser capsulotomy, such as an increase in intraocular

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pressure (IOP), the rupture of the anterior vitreous face, damage to the IOL, hyphema, acute iritis, and cystoid macular edema (CME) [5, 6]. Unusual complications include corneal endothelial damage, a macular hole, vitreous hemorrhage, retinal detachment, macular hemorrhage, and endophthalmitis [7-11]. To detect any of these complications and to take possible precautions, it is important to evaluate both the anterior and posterior segments before and after Nd:YAG laser capsulotomy. Conventional imaging of the anterior segment was applied with slitlamp biomicroscopy; however, the objective quantitative assessment of anterior segment parameters was limited. Recently, LenStar LS 900 optical biometry (Haag-Streit AG) has become useable in the clinical setting. The technology is based on optical low-coherence reflectometry with an 820 mm superluminescent diode. This noninvasive and noncontact examination offers the rapid collection and easy analysis of ocular biometric parameters. This optical biometer measures the axial length (AL), central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), pupil diameter (PD), and keratometry (K) values of the eye in a single step [12]. The evaluation of the changes in the ocular biometric parameters after Nd:YAG laser capsulotomy may enable us to understand the dynamic mechanism of the eye and related complications.

In this prospective study, we aimed to investigate the influences of Nd:YAG laser capsulotomy on the best-corrected visual acuity (BCVA), IOP, CCT, ACD, AL, and pupil size in patients with PCO. To the best of our knowledge, the alterations in these parameters as measured with LenStar LS 900 optical biometry have not been previously reported.

## Methods

This prospective study was performed in the Department of Ophthalmology at Adiyaman University Hospital in Turkey between February 2015 and December 2015. This study was performed in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient before the study. Adiyaman University Institutional Ethics Committee approval was obtained. In this prospective study, 117 eyes of 117 pseudophakic patients were included. All of the patients had undergone non-complicated small-incision phaco-emulsification surgery with three-piece hydrophobic acrylic intraocular lens (Sensar, Advanced Medical Optics, Santa Ana, CA, USA) implantation at least six months earlier. All patients had significant vision loss (at least two lines compared to the most recent visit) and hazy fundus appearance. Exclusion criteria were complications related to cataract surgery, corneal pathology, pseudoexfoliation, glaucoma, uveitis, glaucomatous optic neuropathy, previous ocular surgery or trauma, and posterior segment pathology. Ocular examination with slitlamp biomicroscopy, BCVA, IOP measurements, and fundoscopy were performed before the procedure as well as one week, one month, and three months after Nd:YAG laser capsulotomy. The BCVA was measured with an office-based Snellen system, and the IOP was measured with a Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). The IOP measurements were repeated three times, and the average values were used in the analysis.

The posterior capsulotomies were performed by the same surgeon (AS) in a single session with an Nd:YAG laser, Visulas YAG III (Carl Zeiss Meditec AG), and a contact lens (Abraham capsulotomy lens) with an average diameter of 4.0 mm with a crisscross pattern. The Nd:YAG laser was posterior defocused by 0.50 mm in every eye. The spot energy level, total spot count, and total energy use of each patient were recorded. After the Nd:YAG laser, inflammation was controlled with diclofenac (four times/day for one week) given to all patients. No procedure-related complications were detected.

All LenStar 900 measurements were obtained under standard dim-light conditions. The measurements were repeated three consecutive times, and the average measurement value was recorded. After the daily calibration of the biometer, measurements were performed in the same room conditions and without topical medication. At each measurement, the patient was fixated to a flashing red light during the measuring process. All measurements were repeated five consecutive times. The average measurement value was recorded. The AL (the anterior corneal surface to the central retinal pigment epithelium), CCT, ACD (the corneal endothelium to the anterior lens surface), and PD were measured. The pupil diameter was measured using the instrument's inbuilt edge-detection software. The measurements were repeated one week, one month, and three months after Nd:YAG laser capsulotomy. All measurements were performed by the same operator, who was masked to the subject's eye condition.

*Statistical Analysis*

Statistical analysis was performed with SPSS for Windows Version 15.0 (SPPS Inc, Chicago, Illinois, USA). All data were reported as averages and standard deviations. The Kolmogorov-Smirnov test was used to test for normality. In addition, non-parametric tests were used according to the results. Wilcoxon and Friedman were further used to compare the changes in the BCVA and ocular biometric parameters (CCT, ACD, PD, AL). A value of p<0.05 was considered to be statistically significant.

## Results

We evaluated a total of 134 patients with PCO during the study period. Nine patients did not appear for the quarterly examination, three missed the monthly examination, and five were lost immediately after the treatment. After these exclusions, 117 patients with PCO remained as participants in the study. The mean follow-up was about 3.41±0.49 (range; 3-4) months. All patients underwent posterior capsulotomy with the cross technique.

Of the 117 patients, 45 were female (38.5%), and 72 were male (61.5%). The mean age was 62.82±7.40 (range; 45-79) years. Sixty-five (55.6%) right eyes and 52 (44.4%) left eyes were included in the study. The PCO type in 26 (22%) patients was the pearl type, was fibrous in 27 (23%), and was a combination of the two in 64 (55%). The mean applied energy was 14.91±2.79 mj, the mean spot count was 13.40±3.36, and the mean capsulotomy size was 3.53±0.82 mm.

The BCVA and IOP values before the procedure prior to the capsulotomy and at week one, month one, and month three are illustrated in Table 1. Significant changes occurred in the mean BCVA ($p$<0.001). The BCVA improvements at preoperative, one week, and one month were statistically significant, but no significant changes in the BCVA were found at one month and three months ($p$=0.345). No association was found between the capsulotomy and mean IOP changes during the follow-up period ($p$=0.300).

A comparison of the repeated measurements and repeatability analyses of the CCT, ACD, pupil size, and AL are shown in Table 2. No significant changes were found in the CCT ($p$=0.059). The ACD was found to be decreased in the follow-up period, but no change was found one month and three months later. The ACD values changes were statistically significant at preoperative, the first week and the first month measurements ($p$<0.001). No statistically significant differences were found between the first month and the third month ($p$=0.365). Also, no significant changes in the pupil size ($p$=0.200) and AL ($p$=0.112) were found after Nd:YAG laser capsulotomy.

**Table 1.** Visual acuity and intraocular pressure values in the study group (n=117 eyes)

| | Before the procedure | 1 week | 1 month | 3 month | *p*-value | Test |
|---|---|---|---|---|---|---|
| **BCVA** | 0.30±0.06 | 0.76±0.05 | 0.84±0.06 | 0.85±0.05 | <0.001* | Friedman |
| **IOP** | 14.13±1.83 | 14.29±1.85 | 14.26±1.85 | 14.23±1.83 | 0.300 | Friedman |

BCVA=best corrected visual acuity, IOP=intraocular pressure (mmHg), *Significant changes

**Table 2.** The values of ocular biometric parameters in the study group (n=117 eyes)

| | Before the procedure | 1 week | 1 month | 3 month | *p*-value | Test |
|---|---|---|---|---|---|---|
| CCT (µm) | 533.80±30.96 | 534.96±31.15 | 534.22±31.07 | 534.24±31.07 | 0.059 | Friedman |
| ACD (mm) | 4.31±0.73 | 4.23±0.72 | 4.22±0.72 | 4.22±0.72 | <0.001* | Friedman |
| PD (mm) | 2.86±0.38 | 2.85±0.39 | 2.84±0.37 | 2.84±0.39 | 0.120 | Friedman |
| AL (mm) | 22.89±0.82 | 22.88±0.81 | 22.87±0.83 | 22.88±0.81 | 0.067 | Friedman |

ACD=anterior chamber depth, PD=pupil size, CCT=central corneal thickness, AL=axial length, *Significant changes (repeated measures analysis of variance)

## Discussion

Nd:YAG laser capsulotomy, which is the main treatment for PCO, developed after cataract extraction. So far, Nd:YAG laser has been used as the gold standard for the treatment of PCO. A number of studies have used different devices to examine the effects of Nd:YAG laser capsulotomy on ocular biometric parameters. These devices, such as optical coherence tomography, ultrasonic biomicroscopy, Orbscan scanning slit topography, the scanning peripheral anterior chamber depth analyzer, the Pentacam, and LenStar LS 900 optical biometry have been used for the qualitative and quantitative evaluation of the ocular biometric parameters of the eye [13, 14]. LenStar LS 900 optical biometry, which provides for noninvasive and noncontact examination, offers the rapid collection and easy analysis of the ocular biometric parameters [14].

Nd:YAG laser capsulotomy is usually a safe procedure but may sometimes cause complications [9]. Nd:YAG laser capsulotomy after the evaluation of the ocular biometric parameters may help us to understand the dynamics, and thus, it provides us with the support needed for taking precautions against associated complications [15]. The ocular biometric parameter effects of Nd:YAG laser capsulotomy used for PCO depend on the size of the capsulotomy, the amount of energy involved, and the intraocular lens types [15]. In our study, the sizes of the capsule opening and the intraocular lens were standard in all patients. The patients who could be measured with LenStar LS 900 optical biometry were added into the study. In addition, we applied the cross technique for the posterior capsule opening, and the posterior capsule opening was obtained with a lower number of shots. Therefore, Nd: YAG laser capsulotomy was performed at low energy in the study.

After Nd:YAG laser application, visual acuity is increased significantly. Oztas *et al*. [14] showed that the BCVA increased up to the first-month follow-up. Ruiz-Casas *et al.* [17] showed a significant increase in the BCVA up to the third-month control. We found a significant improvement in the BCVA ($p<0.001$). The BCVA improvements at preoperative, one week, and one month were statistically significant, but no significant changes were found in the mean BCVA at one month and three months ($p=0.345$). After Nd:YAG laser treatment, an increase in IOP is usually temporary [17]. The frequency of increased IOP after Nd:YAG laser capsulotomy is highly variable, ranging from 0.8% to 82% in different studies [18]. In some studies, the IOP was found to be unchanged [13, 16]. In our study, no association was found between the capsulotomy and IOP changes during the follow-up period ($p=0.300$).

Wroblewska-Czajka *et al.* [19] reported that postoperative CCT values increased compared to CCT values before the procedure. Oztas *et al.* [14] suggested that a statistically significant 10 µm decrease in CCT was detected after Nd:YAG capsulotomy. Ruiz-Casas *et al.* [17] reported thatno association was found between the capsulotomy and CCT values. In our study, no significant change was found in the CCT.

Several trials reported that they did not find significant changes in the ACD after Nd:YAG posterior capsulotomy [20-22]. Eliacik et al. [24] reported that postoperative ACD values increased compared to ACD values before the procedure. Zaidi and Askari [25] reported a significant decrease in the ACD after Nd:YAG capsulotomy. In our study, a significant change in the ACD was found. In the ACD values before the procedure, compared to after the first week and the first month, statistically significant changes were found ($p<0.001$). No statistically significant differences were found between the first month and the third month ($p=0.365$). Oztas et al. [14] suggested that a decrease in the ACD is related to the anterior displacement of the IOL, and possible mechanisms for the anterior displacement of the IOL include positive vitreous pressure, the disruption of capsule capsulotomy, and PD changes during the follow-up period. In our study, no significant change occurred in the PD. As far as we know, no studies have evaluated the AL in patients of Nd:YAG laser capsulotomy used for PCO. In our study, no significant change was found in the AL.

### *The Limitations of the Study*

A limitation of this study is the lack of further correlations between the techniques type, capsulotomy size and IOL types (one piece, three-piece, et cetera. Moreover, the effects of Nd:YAG capsulotomy on the ocular biometric parameters might be further evaluated. Additional studies to investigate the association of these factors with increased number of patients are required to substantiate our results.

## Conclusions

The BCVA and ACD values were found to be unchanged after one month. In other words, the follow-up period should be at least one month after Nd:YAG capsulotomy. The data from our study demonstrated that ocular biometric parameters, such as CCT, ACD, PD, and AL, measured by LenStar LS 900 optical biometry may change after Nd:YAG laser capsulotomy. To the best of our knowledge, this is thefirst study to demonstrate the effects of Nd:YAG laser posterior capsulotomy onocular biometric parameters with LenStar LS 900 optical biometry.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Incidence of the genetic mutations in patients with coronary artery disease

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## ABSTRACT

***Objectives.*** Coronary artery disease (CAD) is the leading cause of mortality in the world. It is a complex disorder resulting from the interaction between environmental risk factors and hereditary predisposition. The role of the factor V Leiden (FVL), protrombin gene (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in the development of CAD is controversial. In this study, we investigated the incidence of these polymorphisms in order to delineate their roles in the development of CAD in a tertiary University hospital. ***Methods.*** This study included 58 consecutive CAD patients. Diabetic and hypertensive patients were excluded. FVL, PT G20210A, and MTHFR (C677T, A1298C) mutations were investigated in all patients. Polymerase chain reaction and the amplification refractory mutation system were used to identify these polymorphisms. ***Results.*** Thirty-six men and 22 women were enrolled with an age ranging between 41 to 85 (mean age: 62.75±9.18 years). The heterozygous PT G20210A genotype was identified in 5 (8.6%) patients (2 males, 3 females). The heterozygous FVL genotype was found in 8 (13.8%) patients (6 males and 2 females). The incidence of homozygous MTHFR C677T and homozygous MTHFR A1298 carriers was found to be 17.2% and 8.6%, respectively. There were no significant differences in the distribution of polymorphisms according to gender ($p>0.05$). ***Conclusions.*** The FVL and PT G20210A polymorphisms most likely play a contributory role in the development of CAD. In contrast, the MTHFR C677T and MTHFR A1298C genotypes were not associated with a predisposition to the development of CAD. However, in compound MTHFR C677T/A1298C carriers, the presence of FVL or PT G20210 polymorphism may contribute the development of CAD. Further studies are needed to support these findings.

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***Keywords:*** Methylenetetrahydrofolate reductase, polymorphism, genetic mutation, atherosclerosis, coronary artery disease

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## Introduction

Coronary artery disease (CAD) is the leading cause of mortality in the world. CAD is a complex disorder resulting from the interaction between environmental risk factors and hereditary predisposition [1]. In addition to well-known conventional risk factors, increased evidences support that coagulation may be involved in the pathogenesis of atherosclerosis [2]. Myocardial infarction (MI) usually develops as a result of thrombotic occlusion of the coronary artery, which is triggered by the rupturing of atherosclerotic plaque followed by clotting processes [3]. However, MI can develop in patients with normal coronary arteries due to defects of the clotting system [4]. Studies focusing of the prevalence of MI in families suggest a possible genetic basis for the development and progression of CAD [1]. The molecular mechanisms causing coronary artery disease has not been fully elucidated [2].

The factor V Leiden (FVL) and protrombin gene (PT G20210A) polymorphisms have been known as the two most common thrombophilic risk factors leading to venous thrombosis but their roles in the development of CAD remain controversial [5].

FVL is a missense mutation in the factor V gene, which result in the replacement of arginine at amino acid position 506 by glutamine [1]. This polymorphism is known as the most common genetic thrombophilic risk factor of the coagulation system and leads to a reduced effect of activated protein C (APC) known as APC resistance [3]. The FVL polymorphism may also be an important inherited risk factor for the development of thrombotic complications leading to MI and/or digital ischemia [6].

In 1996, a variant of prothrombin gene (PT G20201A) was discovered and associated with increase synthesis and secretion of prothrombin [7]. This mutant allele has a guanine to adenine substitution at the nucleotide 20210 locus in the 3'-untranslated region of the prothrombin gene [4].

Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, a thymidine-cytosine change at nucleotide position 677, especially in the homozygote carriers, leads to decreased enzymatic activity and mild hyperhomocysteinemia. The MTHFR A1298C polymorphism presents a less well-defined effect, with a lesser decrease of the enzyme function [8]. The role of the MTHFR polymorphism in the development of CAD is also controversial [5].

In this study, we investigated if the incidence of the FVL, PT G20210A and MTHFR C677T polymorphisms plays a role in the development of CAD.

## Methods

This study was conducted in the Bozok University Hospital, in Yozgat, Turkey between April 2015 and October 2016. An informed consent was obtained from all the study participants. The study was approved by the ethic committee of Bozok University and conducted in accordance with the principles of the Declaration of Helsinki.

This study included 58 consecutive patients with angiographically diagnosed CAD. Diabetic and hypertensive patients were excluded from the study. FVL, PT G20210A, and MTHFR (C677T, A1298C) mutations were determined in all patients.

*Laboratory Studies*

Venous blood (5-8 ml) was drawn from each patient by venipuncture into the vacutainer tube containing ethylenediaminetetraacetic acid (EDTA). Peripheral blood (200 µl) was used to isolate DNA with QIAamp DNA Blood Mini Kit (Qiagen Inc. Germany). The extracted DNA was stored at -200 C. Polymerase Chain Reaction (PCR) and the amplification refractory mutation system were used to identify FVL, PT G20210A, MTHFR C677T, and MTHFR A1298C. Polymorphism screening was carried out with a SNaPshot® multiplex system (Applied Biosystems Inc., Switzerland). Wild, heterozygous, and homozygous genotypic distributions of these polymorphisms were defined as numbers and percent frequencies.

*Statistical Analysis*

Data were expressed as the mean ± standard deviation (SD). The incidence of polymorphisms was shown as percent (%). Student t-test was used for statistical analysis. A $p$ value of less than 0.05 was considered statistically significant.

## Results

Thirty-six males and 22 females with an age ranging from 41 to 85 and a mean age of 62.75±9.18 years were enrolled in the study.

**Table 1.** Distribution of polymorphisms in patients with coronary artery disease

| Polymorphism | Male | Female | Total and % |
|---|---|---|---|
| HET PT G20210A | 2 (5.5%) | 3 (13.6%) | 5 (8.6%) |
| HET FVL | 6 (16.6%) | 2 (9.1%) | 8 (13.8%) |
| HET MTHFR C677T | 15 (41.6%) | 7 (31.8) | 22 (37.9%) |
| HOM MTHFR C677T | 7 (19.4%) | 3 (13.6%) | 10 (17.2%) |
| HET MTHFR A1298C | 22 (61.1%) | 8 (36.3) | 30 (51.7%) |
| HOM MTHFR A1298C | 4 (11.1%) | 1 (4.5%) | 5 (8.6%) |
| No Polymorphism | 1 (2.77%) | 4 (18.1%) | 5 (8.6%) |

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET= heterozygous, HOM= homozygous

**Table 2.** Distribution of co-existence PT G20210A polymorphism and other thrombophilic mutations in patients with coronary artery disease

| PT G20210A | FVL | MTHFR C677T | MTHFR A1298C |
|---|---|---|---|
| HET | WT | HET | HET |
| HET | WT | WT | HET |
| HET | WT | HET | HET |
| HET | WT | HET | WT |
| HET | WT | HET | HET |

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, WT=wild type

**Table 3.** Distribution of co-existence heterozygous FVL polymorphism and other thrombophilic mutations in patients with coronary artery disease

| FVL | PT G20210A | MTHFR C677T | MTHFR A1298C |
|---|---|---|---|
| HET | WT | HET | HET |
| HET | WT | HET | HET |
| HET | WT | HET | HET |
| HET | WT | WT | HOM |
| HET | WT | WT | HET |
| HET | WT | WT | HOM |
| HET | WT | HET | HET |
| HET | WT | HET | WT |

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, HOM=homozygous, WT=wild type

There were no homozygous carriers for either PT G20210A or FVL polymorphisms (Table 1) and no significant differences in the distribution of polymorphisms according to gender ($p$>0.05). The heterozygous PT G20210A genotype was identified in 5 (8.6%) patients (2 males, 3 females). All of them had additional defects. A combination of heterozygous PT G20210A and heterozygous MTHFR C677T polymorphisms were detected in 4 (6.9%) patients (Table 2).

The heterozygous FVL genotype was found in 8 (13.8%) patients (6 males and 2 females). Furthermore, 5 (8.6%) patients had a combination of the heterozygous FVL and heterozygous MTHFR C677T polymorphisms (Table 3).

The homozygous and heterozygous MTHFR C677T polymorphisms were found in 10 (17.2%) and 22 (37.9%) patients, respectively, giving an overall incidence of 55.1%. The homozygous MTHFR A1298C polymorphism was detected in 8.6% of patients (n=5) and heterozygous MTHFR A1298C polymorphism in 51.7% (n=30) of the patients,

**Table 4.** Distrubution of the combined heterozygous MTHFR 677/1298 carriers according to additional genetic mutations

| MTHFR C677T | MTHFR A1298C | PT G20210A | FVL |
|---|---|---|---|
| HET | HET | WT | WT |
| HET | HET | WT | HET |
| HET | HET | WT | HET |
| HET | HET | WT | HET |
| HET | HET | WT | WT |
| HET | HET | HET | WT |
| HET | HET | WT | WT |
| HET | HET | WT | WT |
| HET | HET | WT | WT |
| HET | HET | WT | HET |
| HET | HET | WT | WT |
| HET | HET | WT | WT |
| HET | HET | WT | WT |
| HET | HET | HET | WT |

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, WT=wild type

revealing an overall incidence of 60.3%. There were 14 (24.1%) patients with double heterozygous for the MTHFR C677T and the MTHFR A1298C polymorphisms. Hence, 28.5% (n=4) of them had additional FVL polymorphism and 14.2% (n=2) had additional PT G20210A polymorphism (Table 4). On the other hand, 8.6% (n=5) of patients carried none of these polymorphisms.

## Discussion

FVL polymorphism could be related to the development of CAD [9] by two different mechanisms. First, FVL polymorphism causes thrombosis on inner arterial walls leading to a reduction in blood flow. Second, FVL polymorphism can contribute to the enlargement of atherosclerotic plaque due to development of thrombus. Female FVL carriers with of one or more cardiovascular risk factors (smoking, hypertension, diabetes, or dyslipidemia) had increased risk of MI as compared to females with neither FVL nor other risk factor [10]. Risk of MI increased 32-fold in smoking women carrying FVL polymorphism as compared to non-smoking non-carriers women [10]. The FVL polymorphism may also be an important inherited risk factor for the development of thrombotic complications leading to myocardial infarction and/or digital ischemia [6].

Boroumand *et al.* [11] suggested that FVL polymorphism is an important risk factor for the development of CAD and linked it to disease severity. Thus, FVL mutation status may be useful to predict probability of CAD [11]. Gurlertop *et al.* [9] suggested that combinations of FVL mutation and cardiovascular risk factors (hypertension, diabetes mellitus, etc.) might be associated with an enhanced risk of CAD, especially in inhabitants in the northeast part of Turkey. Similarly, Hobikoglu *et al.* [3] detected a higher prevalence of FVL polymorphism in young Turkish males with MI than healthy controls. In contrast, Donmez *et al.* [4] reported that the FVL and PT G20210A polymorphisms are not risk factors for the occurrence of MI in younger patients.

Dunn *et al.* [12] suggested that the heterozygous FVL genotype may not be an independent risk factor for CAD, but its homozygous genotype could play a role in the development of CAD. The prevalence of heterozygous FVL genotype was found in 10.9% of patients undergoing CABG [13]. The rate of heterozygous FVL genotype was found in 13.8% of our patients, which was higher than the prevalence rate of 7.9% in healthy Turkish individuals [14].

It has been speculated that FVL polymorphism might be associated with an increased thrombotic risk and a decreased bleeding risk in patients that undergoing cardiac operations [15]. The incidence of thromboembolic complications during perioperative and postoperative period in cardiac surgical patients was related with heterozygous FVL mutation [16]. In

contrast, Emiroglu *et al.* [13] did not find a statistically significant difference with postoperative thromboembolic complications, which may be due to routine heparin administration postoperatively. In contrast to the report of Emiroglu *et al.* [13], Donahue *et al.* [15] reported that postoperative bleeding drainage was statistically lower in patients with FVL mutation compared with non-carriers.

Franco *et al.* [17] confirmed that the heterozygous PT G20210A polymorphism was associated with 25% increase in prothrombin levels and increased thrombin formation. In addition, the increased levels of prothrombin could lead to an increase in thrombin-active fibrinolysis inhibitor (TAFI), which is an inhibitor of the fibrinolysis and therefore allows for increased clotting [18]. Thrombin is also involved in the regulation of endothelial cell proliferation and fibroblast mutagenesis. Thus, it may attribute the development of atherosclerotic plaques [19]. Based on this, the PT G20210A polymorphism may lead to increased risk of CAD [1]. Additionally, PT G20210 mutation in association with other major risk factors may amplify the risk of CAD [19].

The prevalence of PT G20210 polymorphism was reported as 7.3% in the patients with MI as compared to 1.8% in healthy controls [4]. Similarly, Rahimi et al. [5] found a high prevalence of PT G20210A polymorphism (3.1%) in CAD patients with diabetes mellitus. In our study, a high rate (8.6%) of PT G20210A polymorphism was detected, suggesting a relationship with coronary artery disease. However, the association of CAD with high prothrombin activity may be a biomarker for disease development of CAD [19].

A number of genetic polymorphisms and nutritional factors can affect the homocysteinemia levels [20]. Increased homocysteinemia levels were associated with progressive changes of atherosclerotic coronary plaques [21]. The MTHFR C677T polymorphism has a clear effect on homocysteinemia levels, however contradicted results are reported regarding the association with CAD. Yilmaz *et al.* [22] reported that MTHFR C677T polymorphism was not associated with the development of CAD in the Turkish people.

Unlike homozygous MTHFR C677T carriers, homozygous MTHFR A1298C carriers are not associated with increased homocysteinemia levels [23]. The frequency of homozygous MTHFR A1298C carriers is approximately 9% in Canadian and Dutch populations, and 10% in Turkish populations [24]. In our current study, the frequency of the homozygous MTHFR A1298C mutation in patients with CAD was 8.6%.

Hyperhomocysteinemia may cause oxidative DNA damage leading to coronary artery lesions [25]. Despite the influence on homocysteinemia levels, the role of MTHFR C677T polymorphism as a genetic risk factor of CAD is still controversial [25]. Yenilmez *et al.* [26] suggested that FVL and MTHFR A1298C polymorphisms could play important roles in the progression of coronary lesions. The MTHFR C677T polymorphism was reported as an important risk factor for early fatal coronary occlusion in Hungarian people [27]. Furthermore, the MTHFR C677T polymorphism was associated with hyperhomocysteinemia and CAD in Asian patients but not in European counterparts [28]. Kawashiri *et al.* [29] reported a relation between MTHFR C677T mutation and the risk of CAD in male patients with heterozygous familial hypercholesterolemia. In contrast, Kim *et al.* [30] did not found associations among the MTHFR C677T polymorphism, the risk of CAD, and homocysteinemia level in Koreans. They suggest that MTHFR gene is related to homocysteine metabolism but is not a predictor for the risk of CAD [30]. Furthermore, meta-analysis did not support the hypothesis that the MTHFR C677T mutation is an independent predictor of developing CAD [31].

The frequency of MTHFR C677T genotype for homozygous and heterozygous carriers was reported 9.6% and 47.4%, respectively in healthy Turkish people [24]. Similarly, the rate of C677T variant for homozygous and heterozygous carriers in this study was 17.2% and 37.9%, respectively. The prevalence of compound MTHFR C677T/A1298C genotypes was 21.6% in healthy Turkish population [24], similar to the rate (24.1%) our study. Based on this data, we speculate that these genotypes are not playing an important role in the development and progression of CAD.

## Conclusions

FVL and PT G20210A polymorphisms could contribute to the development of CAD. MTHFR C677T and MTHFR A1298C genotypes were not associated with the predisposition of the development of CAD. However, in compound MTHFR C677T/A1298C carriers, the presence of FVL or PT

G20210 polymorphism may contribute to the development of CAD. Further studies are needed to confirm the importance of these combinations as hereditary risk factors for the development of CAD.

*Conflict of interest*

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# Outcomes of routine surgical exploration in children who admitted to the emergency service with acute scrotum

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## ABSTRACT

***Objectives.*** The acute scrotum frequently presents a diagnostic and therapeutic challenge. In cases where the testicular torsion cannot be excluded, a surgical exploration is recommended. The aim of the study was to present the results of our management in the patients with acute scrotum. ***Methods.*** We reviewed the medical records of 33 patients suffering from acute scrotal pain underwent scrotal exploration between 2011 and 2016. Anamnestic data (age of patient, duration of anamnesis prior to admission and the side that was involved), scrotal color Doppler ultrasound (SCDUS) results, intraoperative findings, mode of operative treatment, and the clinical outcomes were analyzed. ***Results.*** Patients aged from several hours up to 17 years of age (average 11.9 years). The duration of symptoms prior to admission ranged from 3 h to 4 days, (average 32 h). Acute scrotum was found 18 in right side, 15 in left side. SCDUS was diagnostic for testicular pathologies in 19 (51%) of 30 patients. In patients who underwent scrotal exploration, 18 (54%) had a testicular torsion, 11 (33%) had a torsion of the appendix testis, and 4 (12%) had an orchio-epididymitis. Three patients who found testicular torsion required an orchidectomy immediately because of necrosis. The rest had detorsion and ipsilateral fixation of testis. Three patients underwent second look, and orchiectomy was performed in 2 of them. In 2 of detorsioned testes, two testicular atrophies developed on follow-up period. ***Conclusions.*** Regardless of the etiology, the management of the patients with acute scrotum by urgent surgical intervention allows the salvage of a possible testicular torsion.

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***Keywords:*** C-reactive protein, procalcitonin, correlation, kidney transplantation

## Introduction

Acute scrotum or acute scrotal pain is a common clinical situation that present with pain, hyperemia and swelling of the hemiscrotum. The most common causes of acute scrotum include inflammation of the testicle and/or epididymis (acute orchio-epididymitis), testicular torsion, torsion of the appendix testis. Among of them, the testicular torsion is of major concern because it requires urgent surgical intervention to avoid testicular loss [1]. Since decades, the patients with acute scrotum have underwent

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emergency surgery to treat all testicular torsions as soon as possible. On the other hand, an approach to the patient with acute scrotum might be more challenging in recent years, especially when available more different imaging facilities, such as color coded duplex sonography, scintigraphy, magnetic resonance, etc [2]. In this study, we aimed to determine the results of surgical management of children with acute scrotal pain suspicious for testicular torsion.

## Methods

Between 2011 and 2016, we reviewed the medical records of 50 patients suffering from acute scrotal pain with/without swelling and redness. Seventeen patients who managed conservatively but were not operated were excluded from the study. Clinical features (age of patient, duration of symptoms prior to admission and the side of scrotum that was involved), physical examination findings, scrotal color Doppler ultrasound (SCDUS) results, duration between admission and operation, operative diagnosis, mode of surgical management, and the postoperative outcomes were noted.

The following summarizes our approach to initial investigation in a patient with acute scrotum. The onset, site, duration, character, severity, precipitating and relieving factors of symptoms have been noted. History of trauma and other accompanying symptoms and signs such as nausea, vomiting and fever have been questioned. On physical examination, redness or edema of the scrotum, location of testis, tenderness, trans-illumination and a “blue dot sign”, and result of Prehn’s sign were recorded. A white blood cell and urine analysis has routinely obtained prior to surgery. If available, a SCDUS has been requested in an emergency setting in all cases with acute scrotal pain to evaluate of suspected testicular torsion. Finally, the operative decision was made by evaluating the history, symptoms, laboratory tests and SCDUS together with the physical examination. Manual detorsion was not attempted when testicular torsion is suspected in the patients.

Scrotal exploration was made via an incision in midline raphe of scrotum, and inguinal incision under 1 year old babies. When a testicular torsion was detected during surgery, if it is live, the testis was made detorsion and ipsilateral fixation also. If it was found that the testis had undergone torsion and was gangrenous, it was applied heat by warm saline soaked sponges for at least for five minutes. Then, if there was no improvement in the testis color, the testis capsule was incised. If there was a hope that the testis can recover, it was left in place and a second-look operation performed a 24-48 hours later. If no bright bleeding was seen during surgery, or if it is still dark at second-look surgery, the affected testis was removed, and the contralateral testicle was fixed to the dartos fascia in three points. If exploration is revealed torsion of the appendix testis, the appendage was simply excised and no orchidopexy was done. The broad spectrum antibiotic therapy was started in the patients who diagnosed epididymo-orchitis at surgery, and operative debridement was done, if needed.

*Statistical Analysis*

Statistical data are presented as the mean ± the standard deviation of the mean or number (percent) when necessary.

## Results

Thirty-three patients with acute scrotal pain underwent scrotal exploration for a high clinical suspicion of testicular torsion and were included in the study. The postoperative diagnoses were confirmed as orchio-epididymitis (n=4, 12%), torsion of the appendix testis (n=11, 33%), or testicular torsion (n=18, 54%). Demographic and clinical features of the patients are summarized in Table 1. Pain and tenderness of the hemiscrotum were the most common findings in all patients. Positive Prehn's test was diagnostic in adolescents with testicular torsion. Hyperemia was frequently detected in orchio-epididymitis patients. “A blue dot sign” was visible in only in 2 cases with torsion of the appendix testis.

The mean age in the patients with testicular torsion was high, although the extent of the age gap varies widely. While testicular torsion occurred predominantly on the left side, other causes were mostly on right. Although, some of the patients with testicular torsion have applied late period to emergency service, a shorter onset of symptoms in the most of patients was identified. A SCDUS was applied in 16 (87%) of cases, and 13 (81%) of those with testicular torsion facilitated the preoperative diagnosis; however torsion of the appendix testis diagnosed in only one case, and orchio-epididymitis in one case. Of the 14 cases with SCDUS reports compatible with normal vascularization, all of them underwent surgical

**Table 1.** Demographic and clinical features of the patients with acute scrotum according to the postoperative diagnoses summarized.

| | TT | TAT | OE | Total |
|---|---|---|---|---|
| **Number** | 18 | 11 | 4 | 33 |
| **Age (Mean, Average)** | 13.2 (3 mo-16 y) | 11.8 (8-15 y) | 9.6 (6 mo-17 y) | 11.9 (3 mo- 17 y) |
| **Affected side** | 8 R, 10 L | 8 R, 3 L | 2 R,2 L | 18 R, 15 L |
| **Duration of symptoms (h)** | 18 h (3-72 h) | 53 h (12-96h) | 36 h (24-72 h) | 32 h (3-96 h) |
| **SCDUS (accuracy)** | 16 (81%) | 9 (9%) | 4 (25%) | 29 (51%) |

OE=orchio-epididymitis, SCDUS=scrotal color Doppler ultrasound, TAT=torsion of the appendix testis, TT=testicular torsion

exploration due to a strong testicular torsion suspicion on physical examination and the testicular torsion was diagnosed in 2 cases, torsion of the appendix in 8 and orchio-epididymitis in 3. When the torsion of the appendix testis is also considered a surgical disease, the negative exploration rate was 12% in our series. On scrotal exploration, three patients who found testicular torsion required an orchidectomy because of necrosis. The time between onset of symptoms and admission to the hospital was 6 hours or more in these patients. It had been done detorsion and ipsilateral fixation of testis in the remaining patients. Three patients underwent second look, and orchiectomy was performed in 2 of them. In two of detorsioned testes, a testicular atrophy developed on follow-up period. Results of surgical management in children with acute scrotal pain suspicious for testicular torsion are depicted in Figure 1.

## Discussion

In addition to testicular torsion, torsion of the appendix testis and orchio-epididymitis, other conditions include idiopathic scrotal edema, trauma, hernia, hydrocele, varicocele and Schonlein-Henoch purpura, however, most of them do not usually require immediate surgical intervention [1]. The incidence of testicular torsion among the patients with acute scrotum ranges from 18% to 45% depending on the age of subjects, type of clinical institution and the diagnostic method [3]. Testicular torsion is the most important because if it is diagnosed within a short time, testis can be salvaged. If testicular torsion occurred in less than 6 hours, testicular salvage rates of 90%, if detorsion occured in after 24 hours testicular salvage less than 10% [4]. Therefore, the surgeon must move quickly to identify or exclude this condition in any patient who presents with an acute scrotum. In our clinic, testicular torsion incidence was identified in 36% of all acute scrotum, and this rate was 54% in the operated patients.

The appendages of testis and epididymis are normal remnants of embryonic tissue and are usually located at the superior testicle. Torsion of the appendix testis is benign situation that necrotic appendix testis usually resorbs without any problem. The classic presentation of torsion of the appendix testis is “blue dot sign”, where the inflamed and ischemic appendage can be visualized from scrotal skin in the upper pole [1, 3]. In a series of 119 males with acute scrotum, more than one-half had torsion of the appendix [5]. Actually, in cases due to torsion of the appendix testis, emergency operation is not necessary, scrotal pain resolve spontaneously within a few days and do not require any intervention. It has been putted forward that nonsurgical approach to torsion of the appendix testis have admitted that 22% of cases need emergency surgery for differential diagnosis and 14% of conservatively treated patients develop persistent pain [6]. The overlap in clinical findings between testicular torsion and torsion of the appendix testis was so great that we could not reliably make this differentiation; and urgent scrotal exploration performed for these cases.

Although some are viral origin, but the most of orchio-epididymitis occur for bacterial reasons. Most cases, when clinically not diagnosed before, are therefore diagnosed after surgical exploration [1]. Nevertheless, clinical findings can suggest a diagnosis of orchio-epididymitis, once again, it must be taken together with physical examination and diagnostic testing to guide conservative management. In our study, four patients were carried out an emergency scrotal exploration because of there is a strong clinical suspicion of testicular torsion. As our mention is that

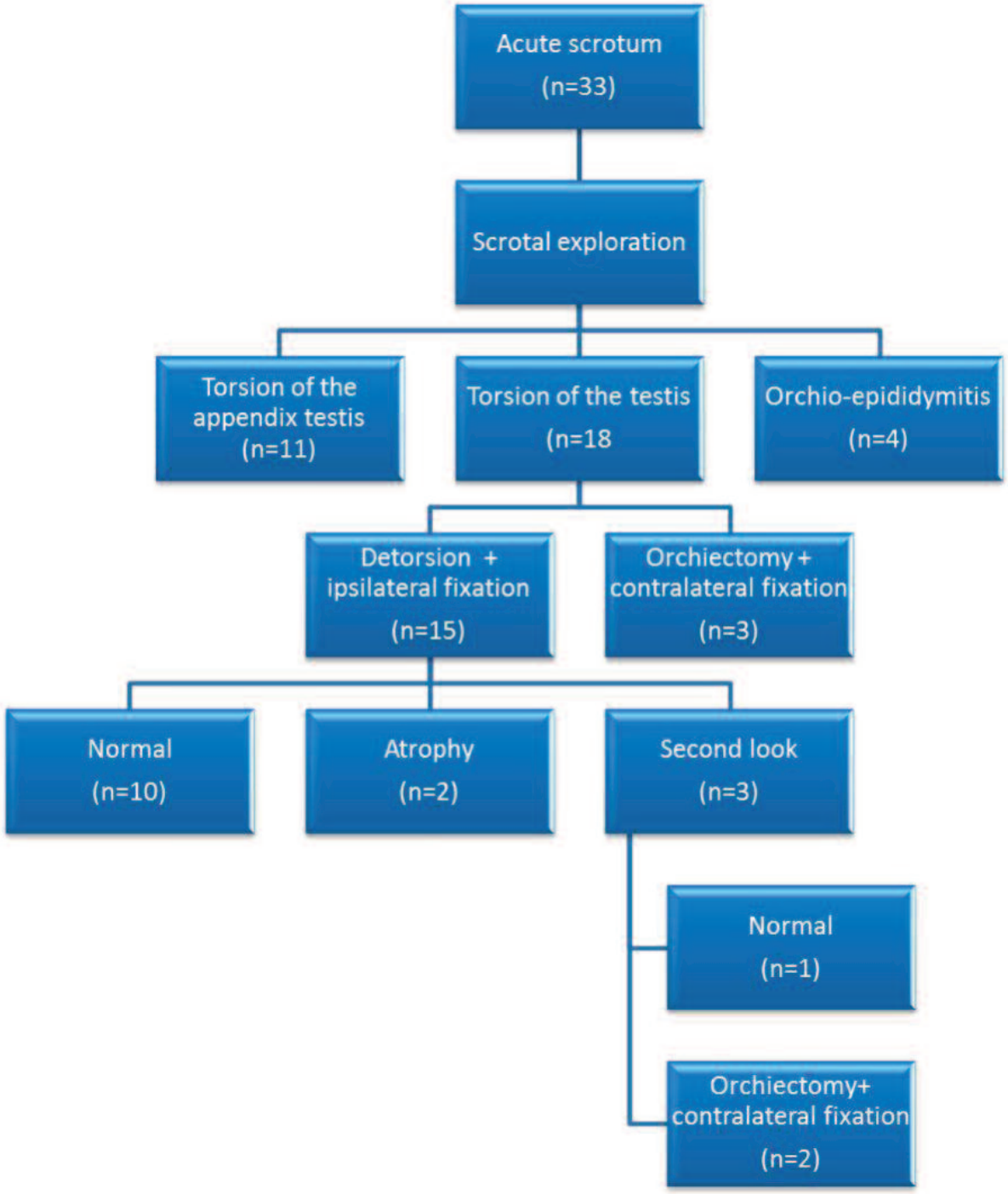


**Figure 1.** Flow chart of present study.

since over most of the patients with acute scrotum will have either testicular torsion or torsion of the appendix testis that would be treated at the time of scrotal exploration, we believed that scrotal exploration was the most reliable diagnostic approach.

Imaging studies are unlikely as a result, since the sensitivity or specificity of a single test in diagnosing testicular torsion is not high. However, imaging studies (eg, SCDUS, nuclear scans) may be useful when testicular torsion is a low suspicion. SCDUS is often used to complete physical examination findings and clinical history in acute scrotum. Recently, a prospective study of the accuracy of SCDUS in testicular torsion suspected or referred patients found sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 75.2%, 80.4% and 100%, respectively [2]. Even though US is a valuable diagnostic tool, correct diagnosis could not be ensured

in every case and the method is highly dependent on the expertise and technique of the investigator. If a mismatch exists between the clinical suspicion of testicular torsion and the US findings, the patient should undergo surgical exploration [7]. Our study has shown that the SCDUS is not a good diagnostic tool to detect testicular torsion in the patients with acute scrotum due to its low diagnostic accuracy. Nuclear scans, or other tests, may have a role in the management of the acute scrotum today; additionally, complete blood count and urinalysis may helpful to evaluate other reasons of acute scrotum, but waiting for these outcomes should not postpone scrotal exploration. The management of the patients with acute scrotum in that study was based mainly on clinical findings and all of them had been operated. In our series, successful testicular salvage was succeeded in 11 cases with testicular torsion, and we had only 4 negative explorations as considering that torsion of the appendix testis is surgical problem. Orchiectomy was required in 5 patients because of necrosis. Results in an orchiectomy rate are ranging from 39% to 71% in most series in boys undergoing surgery for testicular torsion [6, 8]. Our low rate of orchiectomy (15%) suggests that we are not selective in approach to the patient with acute scrotum suspicious of testicular torsion.

## Conclusions

In conclusion, patients with symptoms suggestive of testicular torsion, who admitted to the emergency due to acute scrotum, should be evaluated for prompt surgical intervention regardless of the onset of symptoms or the result of SCDUS, unless there are definite findings suggesting epididymitis or other reasons of acute scrotum. Although we identified non-testicular torsion diseases on scrotal exploration, we salvaged a large number of the torsioned testis with our approach method to the acute scrotum.

*Conflict of interest*

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# The analysis of the patients taken to emergency service by 112 emergency healthcare services: a prospective clinic study

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## ABSTRACT

***Objectives.*** The aim of this study was to analyze the cases transferred by 112 emergency healthcare services (EHS) according to the triage criteria of Turkish Ministry of Health. ***Methods.*** All of the patients brought to adult emergency service unit of our hospital between April 01, 2015 and May 01, 2015 by 112 EHS have been involved in this study. The triage tags of the patients have been determined according to the criteria specified in T.R. Ministry of Health's "Procedures and principles of emergency health services in inpatient healthcare facilities" dated October 16, 2009. The end of emergency service process has been classified as discharge, hospitalization in service, transfer to another healthcare center, and excitus. ***Results.*** A total of 1,101 patients have been involved in this study. The mean age was 45.6 years. The age range was 1-94 years. While 545 (49.50%) patients were female, 556 (50.49%) were male. The portion of Red area was 15.16% (n=167), that of Yellow 1 area 34.60% (n=381), that of Yellow 2 area 23.34% (n=279), and that of Green area 24.80% (n=274). The distribution of the patients by treatment results was as follows; discharge 86.46% (n=952), hospitalization in service 10.08% (n=111), hospitalization in intensive care 2.27% (n=25), transfer to another healthcare center 0.63% (n=7), excitus 0.5% (n=6). The total share of the 65 year-old or older cases was 23.88% (n=263). The distribution of the red area patients by triage was as the following; 47.30% (n=79) hospitalization in service, 10.7% (n=18) hospitalization in intensive care unit, 33.53% (n=56) discharge, 3.59% (n=6) exitus and 4.19% (n=7) transfer to another healthcare service. ***Conclusions.*** The inappropriate use of 112 EHS is very common. Majority of the patients taken into emergency service by 112 EHS does not require urgency. After the triage in the ambulance, the patients can be directly transferred to an appropriate field without creating a time and work load on emergency service. Moreover, after the triage in ambulance, the patients requiring the hospitalization may be transferred to the appropriate facility according to the available bed capacity.

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***Keywords:*** Emergency healthcare services, emergency department, ambulance

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## Introduction

Emergency ambulance services are the important public health care service that helps individuals with surviving in life-threatening or emergency-service cases and allows them to be transferred to emergency services as soon as possible [1]. In our country, the pre-hospital emergency healthcare service (EHS) has been started in year 1980. Offered firstly since 1986 in certain metropolitan cities, this service has been provided by 112 Emergency Healthcare Services Directorate of the Ministry of Health via 112 EHS teams since year 1994 [2]. The number of patients transferred by ambulances has shown increase over the years. Even though not all of the calls are expected to be a life-threatening case, the frequent use of 112 EHS for non-urgent injuries or complaints makes it very difficult for urgent patients to make use of ambulances [3, 4]. For appropriate and efficient use of resources, it is important to carefully select the cases taken by ambulances and to transfer them to appropriate hospitals [2]. Otherwise, the patients requiring no serious treatment or examination are transferred to intensive hospitals and they also increase the actual intensity and the waste of resources [2]. Our aim in this study was to analyze the cases transferred via 112 EHS according to the triage criteria of the Ministry of Health. We will reveal if the patients transferred by 112 EHS to emergency services are in emergency class and if the use of 112 EHS and emergency services is efficient and appropriate. These data may also be used as base for transferring the patients to appropriate hospitals after the triage.

## Methods

All of the patients taken to adult emergency service unit of our hospital between 01.04.2015 and 01.05.2015 by 112 EHS have been involved in this study. Firstly ethics committee permits was taken from Bursa Yuksek Ihtisas Training and Research Hospital Ethics Committee. The adult emergency service of our hospital accepts all of the trauma patients in any age group and >16 year-old patients having internal diseases (>16 year-old internal diseases and trauma patients and <16 year-old only trauma patients). As a routine, the patients taken into emergency service of our hospital by 112 EHS are examined in red area. This process has been continued routinely in our study. After taking our patients in red area, their examinations and treatments have been routinely continued. For the patients involved in this study, the 112 EHS ambulance registration forms and patient files prepared in emergency service have been examined, and the complaint, age, gender, final diagnosis, and triage area have been determined and then recorded into data form. The triage area determination of the patients has been accomplished according to the Republic of Turkey Ministry of Health's "Procedures and Principles of Emergency Healthcare Services in Inpatient Healthcare Facilities" dated 16.10.2009 (Table 1). As a difference, the red area wasn't divided into 1st and 2nd section, and shown as a single red area. The end of emergency service process has been classified as discharge, hospitalization in service, transfer to another healthcare center, and excitus.

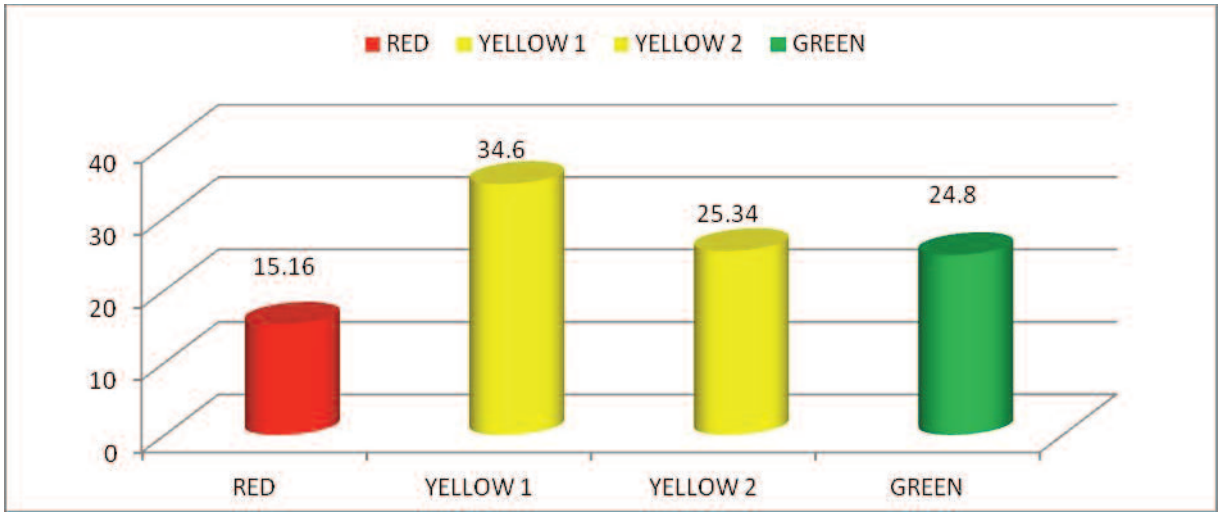


**Figure 1.** Triage distribution percentage of the patients brought by 112 Emergency Health System

**Table 1.** Triage codes according to "Procedures and Principles of Emergency Health Services in Inpatient Healthcare Facilities-Turkey Ministry of Health" dated 16.10.2009

| TRIAGE CODES | |
|---|---|
| **RED 1** | The cases threatening the life and requiring aggressive approach and simultaneous examination and treatment urgently. In these cases, the patient is taken into red area without immediately. |
| **RED 2** | The cases involving high life-threatening risk and requiring the diagnosis and treatment within 10 minutes. |
| **YELLOW 1** | The cases involving the possibility of threatening the life, loss of an organ and involving high morbidity rate. |
| **YELLOW 2** | The cases with medium and long-term symptoms and involving severity potential. |
| **GREEN** | The cases applying by themselves, being generally stable, and involving health problems that can be treated without hospitalization. |

*Statistical Analysis*

Study data has been analyzed with SPSS 22 software.

## Results

A total of 1,101 patients have been involved in this study. The mean age was 45.6 years. The age range was 1-94 years. While 545 (49.50%) patients were female, 556 (50.49%) were male. The portion of Red area was 15.16% (n=167), that of Yellow 1 area was 34.60% (n=381), that of Yellow 2 area was 25.34% (n=279), and that of Green area was 24.80% (n=274) (Figure 1). The distribution of the patients by diagnosis was as follows; trauma 24.79% (n=273), neurological cases 16.25% (n=179) and gastrointestinal system diseases 10.99% (n=121) (Table 2).

The distribution of the cases by the results of treatment was as follows; discharge 86.46% (n=952), hospitalization in service 10.08% (n=111), hospitalization in intensive care 2.27% (n=25), transfer to another healthcare center 0.63% (n=7), excitus 0.54% (n=6) (Figure 2). The total share of 65 year-old and older cases was found to be 23.88% (n=263).

Given the triage distribution of the patients; red

**Table 2.** Distribution of the patients by final diagnoses.

| Final Diagnosis | Number | % |
|---|---|---|
| **Trauma** | 273 | 24.78 |
| **Neurology** | 179 | 16.25 |
| **Gastrointestinal system** | 121 | 10.99 |
| **Respiratory system** | 84 | 7.62 |
| **Psychiatry** | 79 | 7.17 |
| **Cardiovascular system** | 75 | 6.81 |
| **Urology** | 75 | 6.81 |
| **Toxicology** | 57 | 5.17 |
| **General surgery** | 40 | 3.63 |
| **Others** | 118 | 10.71 |
| **Total** | 1,101 | 100 |

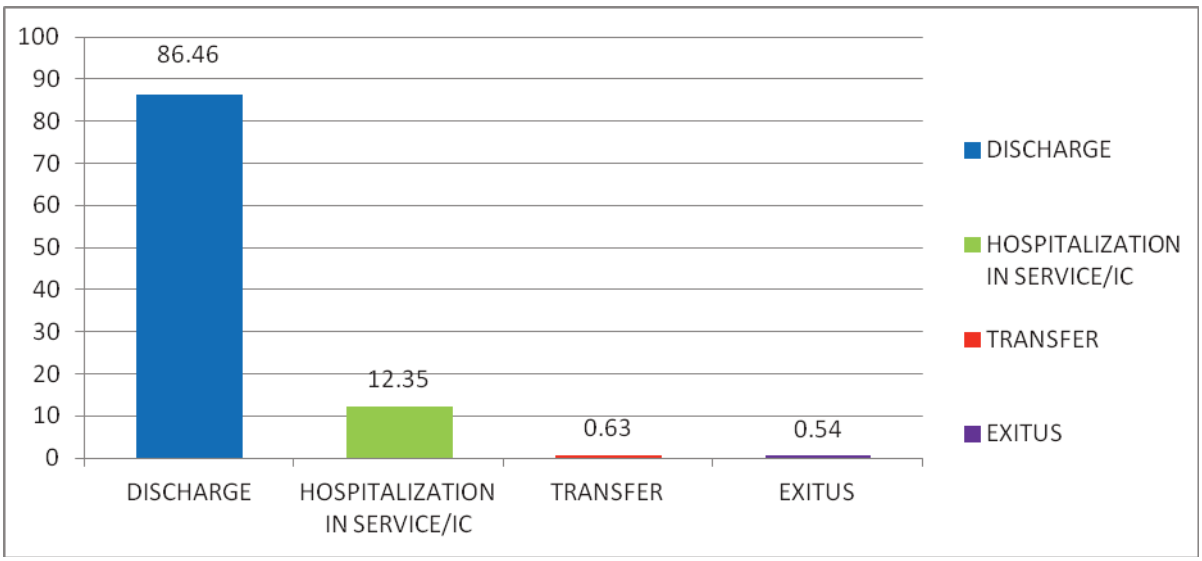


**Figure 2.** Percentage of the patients brought by 112 Emergency Health System

area patients have 47.30% (n=79) hospitalization in service, 10.7% (n=18) hospitalization in intensive care unit, 33.53% (n=56) discharge, 3.59% (n=6) excitus and 4.19% (n=7) transfer to another healthcare service (Figure 3).

## Discussion

The rate of ambulance use is affected from many factors such as age, gender, socioeconomic status, severity of disease, geographical factors, and social security [3, 5, 6]. The inappropriate use increases the workload and economic costs of both pre-hospital and in-hospital emergency service [7]. The high population density in metropolitans, the irregular urbanization, and the traffic problems increase the importance of appropriate use of EHS [7]. In our study, it has been seen that 50.49% of the cases were male, while 49.51% were female. Considering the cases, it is seen that the portion of the male patients transferred by 112 EHS was 63% in 1996, 61.1% in 2001, 58.1% in 2008,

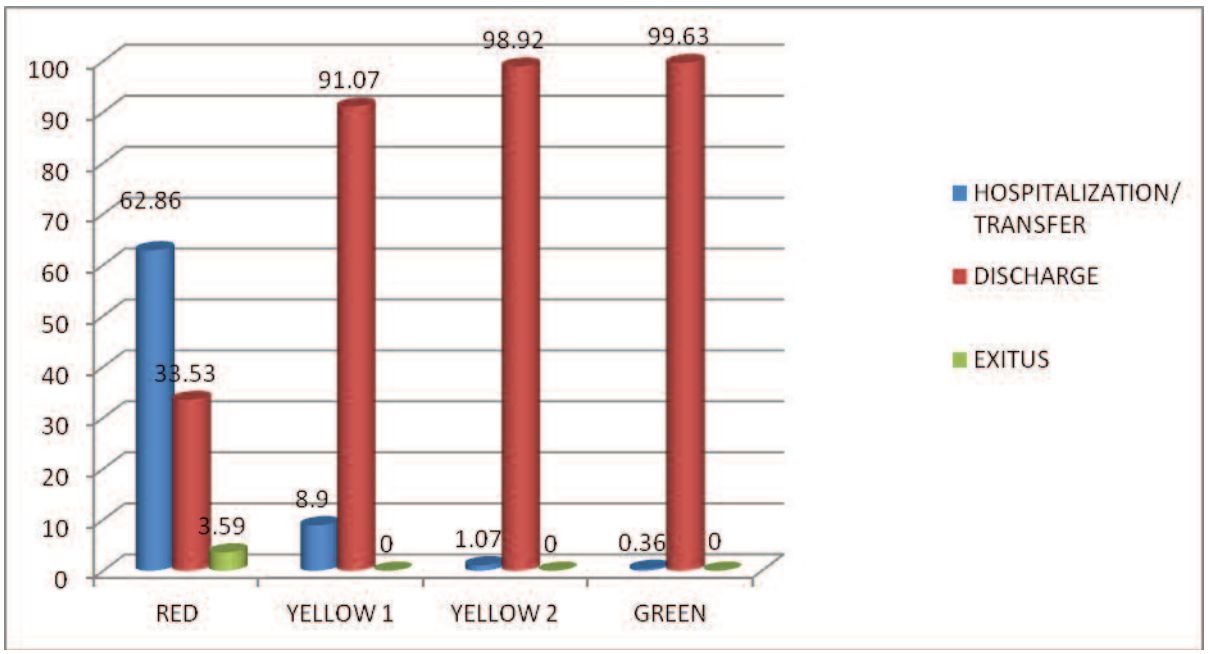


**Figure 3.** Results of the patients by triage areas

58.1% in 2010 and 53.5% in 2013 [2-4, 8, 9]. The portion of male patients has been found to be 50.49% in our study. The increasing activity of women in social life in course of time, the increase in the number of female drivers, and making 112 EHS wider decreased the domination of the number of male patients using 112 EHS.

The appropriate use of 112 EHS by the patients is of significant importance for the ones really needing this service to be able to make use of it. The use of ambulance in northern Europe countries is about 0.77-0.110% annually [10]. In a study carried out in Adana city, the same value has been found to be 0.54% [2]. Our percentage of ambulance service use is still lower than the European standards. But, the inappropriate usage, however, increases the risk of medical-legal problems, besides the interruptions in services. It also limits the time that can be allocated to really emergency patients [11]. In study of Morris *et al.* [12], it has been seen that 51.7% of the patients transferred by ambulance were inappropriate and 10.2% were suspicious. In the study of Gardner *et al.* [13], the rate of inappropriate use of ambulance service has been found to be 61.9%. In another study, where the ambulance calls in London have been analyzed, the rate of inappropriate use has been found to be 53.7% [14]. It has been reported that 40-50% of the total ambulance use in Canada, USA, Sweden, and England was inappropriate [15]. In a similar study carried out by Yaylaci *et al.* [7] in a private hospital, the patients taken by ambulance have been examined and the rate of inappropriate use has been found to be 37.7%. This rate found by Yaylaci *et al.* [7] is lower than the values in literature, and they have attributed this result to that the hospital has been newly opened and the use of ambulance hasn't become prevalent. In our study, among the patients brought by 112 EHS to our emergency service, in accordance with the declaration of the Republic of Turkey Ministry of Health, the triage results are as follows; Red area of 15.16%, Yellow 1 area of 34.60%, Yellow 2 area of 23.34% and Green area of 24.80%. While only 15.16% of the cases were in position requiring red area triage, it has been observed that 1 out of 4 patients brought by 112 EHS has been triaged to green zone that doesn't require urgency. Considering that the green and yellow 2 areas contain the patients that don't medically require the ambulance service, it can be seen that the rate of inappropriate use of ambulance is 48.14%. According to the data of Disease Control and Prevention Center (2005), the rate of real emergency patients has been reported to be 5.5% [16] .The distribution of the patients by treatment results was as follows; discharge of 86.46% (n=952), hospitalization in service of 10.08% (n=111), hospitalization in intensive care of 2.27% (n=25), transfer to another healthcare center of 0.63% (n=7), excitus of 0.5% (n=6). As it can be seen, 86.46% of the cases have been discharged from the emergency service, even though they have been brought to the hospital by an ambulance. According to Snooks *et al.* [17], the high rate of discharge among the patients brought by ambulance has been reported to be one of the criteria for inappropriate use of ambulances. In a study of Yurteri *et al.* [8], the rate of discharge from emergency service has been found to be 30.2%. The same value has been found to be a rate of 53.6% by Atilla *et al.* [5], 74.9% by Ongel *et al.* [2], and 81.7% by Karakus *et al.* [18]. Even though only the patients transferred by 112 EHS have been involved, a higher value (86.46%) has been found in our study. The fact that our results were higher than those of other studies has been attributed to the absence of any low-level hospital in neighborhood (despite that our hospital is a 3rd step one) and the factors such as high population density in region and low socio-cultural status. These results are significant findings indicating the inappropriate use of 112 EHS transfers. Free- and easy-to-achieve service of EHS and unconsciousness of the public increase the inappropriate use. The data, which indicate the inappropriate use of EHS and emergency services in our country, also reveal that the consciousness level of our society about this issue should be improved.

The 112 EHS units are the units consisting of healthcare professionals. For this reason, besides transporting the patient, also the fast transfer of patient to the appropriate hospital is an important criterion for EHS. Otherwise, the recurrent transfers of the patients between the hospitals would be necessary and this leads to loss of time, interruptions in treatment, and decrease in general patient satisfaction. In our study, while the percentage of hospitalization in service and intensive care unit among red area patients were 47.30% and 10.7%, respectively, the hospitalization was 8.9% in Yellow 1 and lower than 1% in Yellow 2 and Green areas. In a study carried out in Istanbul, it has been determined that 84.9% of the patients requiring hospitalization have been transferred to other hospitals [18]. In same study, it has also been found that 45.3% of the patients planned to hospitalize in service have been transferred to other hospitals due to the lack of appropriate bed capacity. At this point, we

strongly believe that, by considering the hospitalization rates, the personnel working in 112 EHS should perform the triage of the patient during the transportation by asking the available bed capacity before transferring to the hospital.

Among the emergency case pre-diagnoses in Turkey, first 2 ranks belong to trauma (25.7%) and cardiovascular system diseases (19.5%) [19]. In study of Oktay *et al.* [17], first 3 ranks belonged to trauma (33.1%), cardiovascular system diseases (18.5%), and neurological diseases (14.2%). In study of Zenginol et al. [6] in year 2008, first 3 ranks belonged to trauma (29.80%), cardiovascular system (16.14%) and neurological cases (9.50%). In study of Ongel *et al*. [2], the same ranks were found to belong to trauma of 28.4%, neurological cases of 16.4%, and cardiovascular system diseases of 14.2%. In our study, the distribution of the patients by their final diagnoses in emergency service was as follows; trauma (24.78%), neurological cases (16.25%) and gastrointestinal system-related diseases (10.99%). Our results seem to be in harmony with literature information. The reason for cardiovascular system cases to be at lower ranks in our study is thought to be that there is a cardiovascular branch hospital near our hospital and those cases are generally transferred to that hospital.

In study of Kidak *et al.* [1], where they examined the use of 112 EHS, they have reported the rate of applications from individuals older than 65 years to be 26.7%. In study of Nur *et al.* [20], the percentage of the individuals older than 65 years among the cases transported by 112 in Sivas in year 2006 has been reported to be 21.9%. In our study, the percentage of >65 year-old cases transported by ambulance has been found to be 23.88%. These results show similarity with other studies. One out of 4 cases transported by 112 EHS ambulance is 65 year-old or older. Considering the increase in mean age of population, we believe that the use of 112 EHS will be higher in future. For this reason, it would be better to make the 112 EHS ambulances and healthcare teams ready for the patients in this age group.

## Conclusions

In our country, the 112 EHS has become easier-to-use since it has been established. But the inappropriate use of ambulance service is still at significant level. A significant portion of the patients taken to emergency service by the 112 EHS has no urgency. By making the triage of patients in ambulance, they can be directly taken to the appropriate area without leading to time and labor load in emergency service. Moreover, by triaging during the transportation in ambulance, the patients requiring hospitalization can be transferred in accordance with the available bed capacity of the hospital. It is obvious that the >65 year-old population's use of the 112 EHS will increase along with the growth in this population. In this parallel, the 112 EHS should be organized and the required equipment and the training of teams would be important for the service quality.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# The value of cervical mediastinoscopy in the diagnosis of mediastinal lymphadenopathy

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## ABSTRACT

***Objectives.*** This study retrospectively evaluated mediastinoscopic interventions performed for diagnostic purposes in cases with mediastinal lesions remained undiagnosed by other diagnostic methods. ***Methods.*** We retrospectively evaluated the medical information of 218 patientss that underwent diagnostic cervical mediastinoscopy for mediastinal lymphadenopathy apart from lung cancer staging between January 2011 and December 2015. The patients were evaluated for age, sex, distribution of sampled lymph node stations detected to have lymphadenopathy, intraoperative and postoperative mortality and morbidity, and histopathological diagnostic parameters. The gender-based distribution of the disease types diagnosed by cervical mediastinoscopy were analyzed with the Chi-Square test. ***Results.*** Two hundred and six (94.5%) of 218 patients were diagnosed by cervical mediastinoscopy. The most common diagnosis was sarcoidosis in women and bronchogenic carcinoma in men. Nineteen (8.7%) patients suffered minor complications, with pain being the most common; no major complication occurred. ***Conclusion.*** The present study shows that mediastinoscopy is still an invasive diagnostic method with high diagnostic accuracy that can be safely used with low rates of mortality and morbidity in a large proportion of patients with mediastinal lymphadenopathy of undetermined origin.

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***Keywords:*** Cervical mediastinoscopy, mediastinal lymphadenopathy, mediastinum, videomediastinoscopy

## Introduction

Histopathological tissue diagnosis is regarded essential to determine the most appropriate treatment approach for disorders characterized by mediastinal lymphadeopathy. For this purpose, a diagnostic intervention should provide adequate amount of tissue samples for histopatological and immunological studies. Cervical mediastinoscopy is an effective minimally invasive diagnostic method that can be safely used with low mortality and morbidity rates and that effectively harvests adequate amount of tissue in cases that remain undiagnosed by other methods [1, 2].

Cervical mediastinoscopy is primarily used for the diagnosis and staging of primary lung cancer. In addition to this common indication, it also provides highly valuable information, albeit rarely, for some

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other disorders such as sarcoidosis, tuberculosis, lymphoma, and mediastinal mases. Although many large-scale studies have been conducted for staging lung cancer so far, there are only limited number of studies that explored the role of mediastinoscopy in the diagnosis of disorders with mediastinal lymphadenopathy [3-6].

In this retrospective study it was aimed to establish the efficacy, safety, and feasibility of cervical mediastinoscopy used for the diagnosis of disorders characterized by mediastinal lymphadenopathy apart from lung cancer staging.

## Methods

We retrospectively reviewed the medical records of 218 patients with mediastinal lymphadenopathy who underwent diagnostic cervical mediastinoscopy between January 2011 and December 2015 after remaining undiagnosed by other diagnostic methods. In addition to demographic characteristics such as age and gender, we also recorded symptoms, mediastinoscopy indication, and pathology results. All patients underwent a posteroanterior chest X-Ray imaging, thoracic computerized tomographic imaging, complete blood count, and routine biochemistry studies. In addition to symptoms (e.g; chronic cough, dyspnea, pain), a mediastinal lymphadenopathy with a short axis diameter of greater than 10 mm and a central localization on thoracic computerized tomographic was regarded as an indication for carrying out mediastinoscopy. The mediastinal lymph node map developed by Mauntain and Dresler [7] in 1997 was used to determine lymph nodes' localization. Apart from mediastinal lymphadenopathy, the presence of lesions in lung parenchyma and lymphadenopathies in other body sites were also investigated.

*Operative technique*

Following single-lumen intubation under general anesthesia in supine position, neck was brought to hyperextension and turned right, and a 3-cm cut was made one finger width above the jugular notch. After passing downwards through the subcutaneous tissue and the platysma muscle, pretracheal area was accessed through the strep muscles. Thyroid gland was retracted upward, pretracheal fascia was opened up, and a video mediastinoscope with an openable tip (Richard Wolf, Germany) was placed. Both bronchi were visualized through a blunt dissection with the help of an aspirator tip above the trachea. Biopsy samples were taken from paratracheal, subcarinal, and

**Table 1.** General characteristics of the study group

| Variables | Patients (n=218) |
|---|---|
| Age (years) | |
| Mean | 52.7±14.7 |
| Min-Max | 16-85 |
| Duration of stay (days) | |
| Mean | 2.9±1.0 |
| Min-Max | 2-5 |
| Sex | |
| Male | 102 (46.8%) |
| Female | 116 (53.2%) |
| Smoking | |
| Smoker | 105 (48.2%) |
| Non-smoker | 113 (51.8%) |
| Symptoms | |
| Pain and cough | 31 (14.2%) |
| Dyspnea and cough | 187 (85.8%) |
| Level of mediastinal lesion | |
| Hilar | 70 (32.1%) |
| Paratracheal | 81 (37.2%) |
| Subcarinal | 67 (30.7%) |

The values were presented as mean ± SD or number and %. Min=minimum, Max=maximum

hilar lymph nodes. After achieving hemostasis, the layers were closed on anatomic plane. The patients were extubated on operating table and transferred to their wards.

*Statistical Analysis*

Statistical analyses were done using SPSS v21.0 (IBM) software package. Number and percentage were used for qualitative variables and mean ± standard deviation for quantitative variables. Chi-square and Fisher's exact test were used to compare the frequency differences of observed and expected qualitative variables. A *p* value of less than 0.05 was considered statistically significant.

## Results

This study included a total of 218 patients with mediastinal lesions who had a mean age of 52.7±14.7 years. One hundred and two (46.8%) patients were male and 116 (53.2%) patients were female. One hundred and five (48.2%) patients were smokers and 113 (51.8%) were non-smokers. The most common symptoms were cough, dyspnea, and pain. Among those with mediastinal lesions, the most common lesions localization was the paratracheal region (n=81, 37.2%), followed by the hilar region in 70 (32.1%) patients and the subcarinal region in 67 (30.7%) patients. Parenchymal infiltrates were present in 8 (3.6%) patients and they remained undiagnosed by bronchoalveolar lavage and transbronchial lung biopsy (Table 1). A definitive diagnosis could be made in 206 (94.5%) of 218 patients. In the present study, the most common diagnosis was sarcoidosis (n=89, 40.8%). This was followed by, in descending order, bronchogenic carcinoma in 54 (24.8%) patients, tuberculous adenitis in 43 (19.7%) patients, and lymphoma in 20 (9.2%) patients. A specific diagnosis could not be made and a non-specific diagnosis of reactive adenitis was made in 12 patients (Table 2).

A total of 19 patients developed minor postoperative complications including retrosternal pain in 8 patients, minor hemorrhage in 5, wound infection in 4, and temporary hoarseness in 2. Major vessel, tracheal injury or death did not occur (Table 3).

The majority of patients with sarcoidosis were women (27/62; 70%) while the majority of patients diagnosed with bronchogenic carcinoma were men (42/54; 65.6%). Twenty-six (60.5%) of 43 patients diagnosed with tuberculosis were women (Table 4).

**Table 2.** Disorders diagnosed by mediastinoscopy

| Diagnosis | Number | % |
|---|---|---|
| Sarcoidosis | 89 | 40.8 |
| NHL | 20 | 9.2 |
| Bronchogenic carcinoma | 54 | 24.8 |
| Tuberculosis | 43 | 19.7 |
| Reactive lymph node | 12 | 5.5 |
| Total | 218 | 100.0 |

The values were presented as number and %. NHL=non-Hodgkin's lymphoma The values were presented as number, %

**Table 3.** Complications of mediastinoscopy

| Complications | Number | % |
|---|---|---|
| Wound infection | 4 | 1.8 |
| Pain | 8 | 3.7 |
| Minor hemorrhage | 5 | 2.3 |
| Hoarseness | 2 | 0.9 |
| None | 199 | 91.3 |
| Total | 218 | 100.0 |

The values were presented as number and %

**Table 4.** Gender distribution of the diagnosed disorders

| | Male (n=102) | | Female (n=116) | | *p* value |
|---|---|---|---|---|---|
| **Diagnosis** | n | % | n | % | |
| Sarcoidosis | 27 | 26.4 | 62 | 53.5 | **0.000*** |
| NHL | 9 | 8.8 | 11 | 9.5 | |
| Bronchogenic carcinoma | 42 | 41.2 | 12 | 10.3 | |
| Tuberculosis | 17 | 16.7 | 26 | 22.4 | |
| Reactive lymph node | 7 | 6.9 | 5 | 4.3 | |

The values were presented as number (n) and % and analyzed with Chi Square test. NHL=non-Hodgkin's lymphoma

There was a significant difference between both genders with respect to the disorders diagnosed by cervical mediastinoscopy.

## Discussion

Cervical mediastinoscopy is primarily used for diagnosing and staging lung cancer. It is a minimally invasive diagnostic method that provides quite valuable information about disorders characterized by mediastinal lymphadenopathy which remain undiagnosed by other methods [1, 2, 8, 9]. Although valuable information about a lesion's main radiological properties and metabolic activity can be obtained by non-invasive diagnostic methods such as computerized tomography and positron emission tomography in a case with mediastinal lymphadenopathy with or without accompanying parenchymal lesions, a pathological tissue diagnosis is primarily required to determine the best treatment for a particular patient [2, 3, 10-12]. To achieve this goal, it is essential to perform firstly less invasive interventions such as transbronchial needle biopsy (TBNB) or transthoracic needle biopsy (TTNB) and then proceed with more invasive diagnostic methods such as cervical mediastinoscopy or thoracotomy when these methods fail. Studies on the efficacy of TBNB in patients with mediastinal lymphadenopathy showed a definitive tissue diagnosis rate of 50-78%, and suggested that TBNB should be primarily used for these patients [10, 12-14]. However, TBNB cannot always reach a final diagnosis. On the other hand, despite being a less invasive method compared with cervical mediastinoscopy, TBNB under radiological guidance has been reported to carry a significant risk for central vascular injury, and the incidence of penumothorax with this method has been reported to be 25-30% [11].

Diagnostic mediastinal lymph node aspiration with endobronchial or endoesophageal approaches under endoscopic ultrasonography has recently been introduced into clinical practice [6]. Success rates of up to 85% have been reported with endobronchial ultrasonography and up to 78% with endoesophageal ultrasonography [11]. In patients who remain undiagnosed with these methods, apart from cervical mediastinoscopy, anterior mediastinostomy developed by McNeil and Chamberlain [15] in 1966, extended mediastinoscopy developed by Ginsberg *et al.* [16] in 1987, video assisted thoracic surgery (VATS), and, as a last resort, thoracotomy can also be used for sampling mediastinal lymph nodes. Cervical mediastinoscopy allows an easy access to superior and inferior paratracheal lymph node stations on both sides as well as the anterosuperior extension of the subcarinal lymph node station (Nos 2, 4, and 7) [1, 6, 9, 17-19]. However, extended mediastinoscopy or anterior mediastinostomy can be used for sampling subaortic and paraaortic lymph node stations in the aorticopulmonary window which are inaccessible by cervical mediastinoscopy (Nos 5 and 6) [19]. While extended mediastinoscopy can be performed during cervical mediastinoscopy, there is a need for a separate incision and costal cartilage resection for anterior mediastinostomy. In addition, anterior mediastinostomy is associated with potential complications such as internal mammarian artery injury, pneumothorax, and postoperative pain. However, these complications are reportedly rare, and some researchers recommend using anterior mediastinostomy to explore this region [15].

Neither cervical mediastinoscopy nor anterior mediastinostomy can access the inferiorly located parts of the seventh lymph node station except for its anterosuperiorly located part [18]. VATS can be used for this region. VATS can also be useful for sampling aorticopulmonary, paraesophageal [8], and pulmonary ligament [9] lymph nodes. Compared to cervical mediastinoscopy, however, it is associated with a greater morbidity. VATS is indicated when lymph nodes are inaccessible with cervical mediastinoscopy or when multiple tissue sampling is needed [20].

All diagnostic interventions including cervical mediastinoscopy are complementary for each other rather than substitutes. The main point here is to select the most appropriate diagnostic method to reach a final tissue diagnosis in a patient with mediastinal lymphadenopathy. The most important factor to consider in this task is the distribution of the pathologically enlarged lymph nodes. There are insufficient number of studies investigating the rate of the involvement of different lymph node stations by different diseases. Large-scale studies employing diagnostic biopsy sampling reported that a great proportion (76%) of tissue sampling procedures involved the right paratracheal lymph nodes [3, 5]. We also had similar results.

The sensitivity of cervical mediastinoscopy has been reported between 95% and 100%. Porte *et al.* [3] reported a cervical mediastinoscopy sensitivity of 95%. The sensitivity and specificity of cervical mediastinoscopy have been reported to range between 75% and 97% in other large-scale studies [4, 12, 21]. Our study revealed a sensitivity of 94.5%.

The mortality rate with cervical mediastinoscopy ranges between 0% and 0.5% and morbidity 1% and 4.5%. Although low mortality and morbidity rates have been reported so far, some fatal complications have also been reported. These may include pulmonary artery, innominate artery, aortic arch, superior vena cava, and azygos vein injuries. Main minor complications include hemorrhage, pneumothorax, wound infection, tracheal injury, and recurrent nerve injury [1, 4, 9, 17]. We observed no mortality in our study, although there were 19 minor complications, of which 8 were postoperative pain episodes, followed by, in the order of frequency, minor hemorrhage, wound infection, and temporary hoarseness.

In the literature, sarcoidosis and lymphoma are the two main disease categories diagnosed after diagnostic cervical mediastinoscopy [3, 12, 21]. In line with this information, sarcoidosis (40.8%) was the most common diagnosis made in our study. The rate of tuberculous adenitis diagnosed by cervical mediastinoscopy as the final diagnosis was 11-24% in the domestic studies. The corresponding rate in our study was 19.7%, which was in agreement with previous reports [22, 23].

## Conclusions

Our experience indicates that cervical mediastinoscopy with its high diagnostic efficacy should be used for cases with mediastinal lymphadenopathy that remain undiagnosed by other noninvasive and invasive diagnostic methods.

*Conflict of interest*

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# Clinical prognostic factors in patients with idiopathic peripheral facial nerve paralysis (Bell's palsy)

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## ABSTRACT

***Objectives.*** The aim of this study was to analyse the clinical prognostic factors that are associated with treatment outcomes in patients with idiopathic peripheral facial nerve paralysis (PFNP). ***Methods.*** The study was performed retrospectively with 80 patients in a tertiary medical center. All of the patients were treated and followed for idiopathic PFNP (Bell's palsy). The patient charts were reviewed for patient demographics and characteristics. The comorbidities (hypertension, diabetes mellitus), the side, grade and duration of palsy, and the acoustic stapedius reflex were analyzed. ***Results.*** Forty-three male and 37 female patients were diagnosed with idiopathic PFNP. Sixty-seven of patients had complete, 13 patients had partial recovery. Of the partial recoveries, 12 patients had Grade 2 and one patient Grade 3 PFNP at the end of primary treatment. Patients tended to have incomplete recovery if they have a diagnosis of diabetes mellitus, but the difference between groups was not statistically significant ($p$=0.326). A positive stapedius reflex was associated with complete recovery ($p$=0.023). Patients had much more risk of incomplete recovery if age is more than 40 years ($p$=0.006). ***Conclusion.*** A detailed history and complete physical examination are very important in peripheral facial palsy. Co-morbid diseases and demographic features such as high blood pressure, diabetes mellitus and advanced age might influence the treatment outcomes.

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***Keywords:*** Facial paralysis, Bell's palsy, seventh nerve paralysis, risk factors, prognosis

## Introduction

Peripheral facial nerve paralysis (PFNP) is a common health problem with the estimated incidence from 20-30 of every 100,000 individuals [1, 2]. Bell's palsy is the most common cause of PFNP. Taverner [3] described the diagnostic criteria of Bell's palsy that consist of sudden onset of palsy without central neural injury or otologic causes. There are many hypotheses to identify the pathogenesis of Bell's palsy. Herpes simplex virus (HSV-1) reactivation is one of the most widely accepted hypotheses [4, 5]. Also, mumps, rubella and varicella zoster virus infections are the other suspected viral agents in Bell's palsy [6, 7].

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Bell's palsy usually completely recovers even if we don't use medical agents. Unfortunately, almost 15% of Bell's palsy has poor prognosis and may cause severe functional problems such as synchinesia, facial spasm and contracture [8]. Some clinical factors are associated with poor treatment outcome despite this excellent prognosis. These are accepted as poor prognostic factors: advanced age, facial pain, hyperacusis, decreasing of eye tear and associated comorbid diseases such as hypertension or diabetes mellitus [9, 10]. The advanced grade of PFNP at the time of primary treatment is strongly associated with poor prognosis. Marsk *et al.* [11] used Sunnybrook grading system and found a strong correlation of poor prognosis with advanced grade. In addition, House-Brackman scoring system and Yanagihara grading system are the other grading systems [12].

Electroneurography is another prognostic tool of PFNP, especially to predict poor prognosis. But it has many limitations; special equipments are needed to perform electoneurography and it is useful after 2 weeks [13].

We analyzed the factors that associated with treatment outcomes in patients with PFNP.

## Methods

We conducted a retrospective data analysis in a tertiary referral center from May 2008 to December 2010. We include Bell's palsy patients who visited the hospital within a week after onset. If patients had less than 6 months follow up, they were excluded from the study.

All patients were graded according to House-Brackman facial nerve grading system. Patients were categorized depending on etiologic factors and grade of paralysis. All patients were examined addressing to etiology of the paralysis. Patients were accepted as Bell's palsy if they don't have any sign of neurologic or otologic diseases that associated with PFNP. Patients were ordered for audiogram, tympanogram and stapedius reflex tests.

We used the following treatment strategy for Bell's palsy. Intravenous 250 mg prednisolone administration for the first day and then 1 mg/kg/day oral prednisolone administration that tapered 10 mg every 3 days. If patient is less than 18 years old, we did not use 250 mg intravenous prednisolone administration and started 1 mg/kg/day oral treatment. We added an antiviral agent (valacyclovir) to this protocol if patient admitted within 3 days of onset. We hospitalized the patients if they have diabetes mellitus for close follow-up during the treatment. Facial nerve functions were assessed before and after the treatments. A monthly follow up was performed after the first treatment protocol until complete recovery.

### *Statistical Analysis*

SPSS 16 was used for statistical analysis. Chi-square test and Student's t test were utilized to evaluate the correlation between patient characteristics and poor prognosis. A $p<0.05$ was accepted statistically significant.

**Table 1.** Distribution of clinical features of cases according to exact recovery and partial recovery groups

| Variables | | Group 1 (Recovered) (n=67) | Group 2 (Unrecovered) (n=13) | *p* | Odds ratio (95% CI) |
|---|---|---|---|---|---|
| **Age** | **Mean** | 41.4±18.1 | 53.1±17.7 | 0.036 | 1.037 (1.001-1.075) |
| | **Decades** | 5 (1-9) | 5 (3-9) | 0.052 | 1.404 (0.997-1.977) |
| **Gender** | ***Male*** | 37 (55.2%) | 6 (46.2%) | - | 1.000 |
| | ***Female*** | 30 (44.8%) | 7 (53.8%) | 0.548 | 1.439 (0.437-4.739) |
| **Diabetes Mellitus** | | 19 (28.4%) | 6 (46.2%) | 0.326 | 2.165 (0.644-7.283) |
| **Grade of palsy** | | 3 (2-6) | 4 (3-6) | <0.001 | 3.063 (1.711-5.486) |
| **Side** | ***Right*** | 35 (52.2%) | 5 (38.5%) | - | 1.000 |
| | ***Left*** | 32 (47.8%) | 8 (61.5%) | 0.363 | 1.750 (0.519-5.903) |
| **Duration of palsy** | ***First 24 hours*** | 42 (62.7%) | 9 (69.2%) | - | 1.000 |
| | ***1-3 days*** | 17 (25.4%) | 2 (15.4%) | 0.472 | 0.549 (0.107-2.809) |
| | ***≥4 days*** | 8 (11.9%) | 2 (15.4%) | 0.860 | 1.167 (0.211-6.441) |
| **Stapedius reflex** | ***Yes*** | 16 (57.1%) | 1 (11.1%) | - | 1.000 |
| | ***No*** | 12 (42.9%) | 8 (88.9) | 0.023 | 10.667 (1.171-97.185) |

## Results

A total of 80 patients including 43 males and 37 females were diagnosed with idiopathic peripheral facial nerve palsy (Bell' palsy). The mean age at the time of diagnosis was 43.3±18.4 years. Sixty-seven out of those patients had complete recovery but 13 patients had partial recovery (Table 1). Group 1 was conducted by patients with complete recovery while Group 2 was including patients with partial recovery.

The mean ages were 41.4±18.1 years and 53.1±17.1 years in Group 1 and Group 2, respectively. Table 1 summarizes the demographics of subgroups. The age at the diagnosis was significantly associated with treatment outcomes ($p<0.05$). Patients had much more risk of incomplete recovery if age is more than 40 years ($p=0.006$). Sex was not significant between the subgroups. Forty patients had left-side PFNP and forty patients had right-side PFNP. The complete recovery rates were 87.5% and 80% in patients with right- and left-side PFNP, respectively. The difference was not statistical significant according to side of paralysis. Thirty-five patients had hypertension and 25 patients had diabetes mellitus at the time of diagnosis. The rates of diabetes mellitus were 28.4% and 46.2% in Group 1 and Group 2, respectively. Patients tend to incomplete recovery if they have diagnosed diabetes mellitus, but the difference was not statistically significant ($p=0.326$). In Group 2, 69.2% of the patients was received the treatment within 24 hours of onset. There was no significant association between rate of recovery and time of diagnosis (Table 1).

We observed complete recovery of those patients with Grade 2 paralysis. One patient had partial recovery of Grade 3 paralysis. Table 2 summarizes the recovery rates addressing to the Grade of paralysis. The grade of paralysis was strongly associated with prognosis ($p<0.001$). One patient underwent facial nerve decompression despite all medical treatments that had complete facial nerve paralysis. Thirty-seven patients were underwent audiologic examination, none of those had hearing loss associated with facial palsy. The rates of stapedius reflex were 57% and 11% in Group 1 and Group 2, respectively. The positive stapedius reflex was significantly associated with complete recovery ($p=0.023$).

## Discussion

Anatomy and function of the seventh cranial nerve was described in the early 1800s by Sir Charles Bell [1]. Many prognostic factors have been evaluated in patients with peripheral facial nerve palsy [14-16]. These are the most common analysed factors: Age, sex, side of palsy, hypertension, diabetes mellitus, grade of facial palsy, stapedius reflexes and timing of treatment. Advanced age at the time of diagnosis was found as a poor prognostic factor [17]. Smith and Cull [18] reported the association of healing process with regeneration and central adaptation. Central adaptation decreases with age that explains the poor prognosis in elder patients. Additional, co-morbid systemic diseases (diabetes mellitus, hypertension, etc.) are mostly seen in the advanced age. These co-morbid diseases may play an important role in elder patients. Diabetes mellitus might influence the neuro-degenerative process in patients with Bell's palsy [19].

This association plays an important role in recovery process. The rate of diabetes mellitus was much more in incomplete recovery group in the current study, unfortunately difference was not statistical significant. Further studies should address to analyse type of medication and control status of diabetes mellitus. Wasano *et al.* [20] reported the better outcomes in women patients. They advocated the neuro-regenerative effects of progesterone in these patients. Abraham *et al*. [10] have supported the

**Table 2.** Distribution of peripheral nerve palsy grades before and after the treatment

| | | | After Treatment | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Grade 1** | **Grade 2** | **Grade 3** | **Grade 4** | **Grade 5** | **Grade 6** |
| **Pre-Treatment** | **Grade 2** | 25 | 25 | - | - | - | - | - |
| | **Grade 3** | 29 | 28 | 1 | - | - | - | - |
| | **Grade 4** | 13 | 7 | 6 | - | - | - | - |
| | **Grade 5** | 7 | 4 | 3 | - | - | - | - |
| | **Grade 6** | 6 | 3 | 2 | 1 | - | - | - |
| | **Total** | **80** | **67** | **12** | **1** | - | - | - |

increased mean arterial pressure as a poor prognostic factor. In our study, neither hypertension nor sex had any effect on the prognosis. We concluded that sex, side of palsy, hypertension and timing of the treatment did not have any effect on the prognosis.

Absence of stapedius reflexes are one of the most known bad prognostic factor in PFNP [21]. Our results are supporting the poor treatment outcomes with absence of stapedius reflexes. The grade of palsy is another important prognostic factor. Patients have much more risk of incomplete recovery, if patients have Grade 4 or more palsy. Also, duration of recovery process is extended in these patients [11, 12]. We found that all grade 2 palsies had complete recovery. However, complete recovery rates were 97%, 58%, 58% and 50% in Grade 3, 4, 5 and 6, respectively.

Pietersen [22] published 1,011 patients with Bell's palsy that did not receive any kind of medication. According to this study, complete recovery rate was in 71% of the patient, incomplete recovery with mild sequel in 13%, and experienced residual weakness, synkinesis and/or contracture in 16% of those. The complete recovery rate was 94% in patients with partial paralysis whilst complete recovery rate was 60% in patients with complete paralysis. Jabor and Gianoli [23] reported that the improved rate of complete recovery if patient has any sign of recovery at third week of onset. On the basis of this finding, electromyograhic study at second week of onset supports us valuable findings addressing to the prognosis.

Systemic glucocorticoids are the main treatment agent of Bell's palsy [24]. Antiviral agents might be the other pharmacologic agent with suspect of Herpes Simplex virus activation as a cause of Bell's palsy. Engstrom *et al*. [25] compared the treatment outcomes of 829 patients with Bell's palsy [25]. They compared the treatment outcomes among the 4 subgroups; prednisolone, valacyclovir, valacyclovir+prednisolone and placebo arm. They reported the extended recovery time if patients do not receive prednisolone treatment. But, there was no significant difference between prednisolone alone and prednisolone plus valacyclovir groups according to recovery time [25]. Hato *et al.* [26] reported improved treatment outcomes of valacyclovir plus glucocorticoid treatment in comparison with glucocorticoid alone. We use glucocorticoids with antiviral agents as a treatment protocol depending on the literature.

## Conclusions

A detailed history and complete physical examination are very important in peripheral facial palsy. Co-morbid diseases and demographic features such as high blood pressure, diabetes mellitus and advanced age, pre-treatment grade of palsy might influence the treatment outcomes. More satisfactory information can be given to the patients about their treatment and outcome expectations with the light of current study.

*Conflict of interest*

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# The evaluation of epilepsy and other contributing disorders in patients with cerebral palsy using the Gross Motor Function Classification System

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## ABSTRACT

***Objectives.*** Morbidity and mortality in patients with cerebral palsy are related to motor function disability, as well as other contributing disorders. The aim of this study was to evaluate the contribution of epilepsy and other disorders in cerebral palsy patients using the Gross Motor Function Classification System (GMFCS), and to determine their relationship. ***Methods.*** This study was performed in Eskişehir Osmangazi University School of Medicine, Department of Pediatric Neurology between May 2011-January 2012, with a total of 154 patients diagnosed with cerebral palsy. Epilepsy and other contributing disorders were evaluated in the patients. GMFCS was used to measure the motor function. ***Results.*** Mental retardation (89.6%), ophthalmological problems (68.2%) and epilepsy (61.0%) were the leading disorders, followed by oromotor dysfunction (48.7%), malnutrition (40.9%), orthopedic problems (38.3%), dental problems (18.8%), sleep disorders (17.5%) and hearing loss (9.1%) in cerebral palsy patients. Epilepsy, mental retardation, oromotor dysfunction and malnutrition were mostly observed in GMFCS level 5 ($p<0.001$). Sleep disorder and dental problems were mostly observed at level 5 ($p<0.05$). Epilepsy was seen more frequently in patients who had neonatal seizure history, microcephaly and mental retardation ($p<0.05$). ***Conclusions.*** There are correlations between the occurrence of disorders such as mental retardation, epilepsy, oromotor dysfunction, malnutrition, sleep disorders, dental problems and gross motor function levels. GMFCS levels were thought to be instructive for possible additional disorders.

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***Keywords:*** Cerebral palsy, Gross Motor Function Classification System, disorders, epilepsy

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## Introduction

Cerebral palsy was first described in 1862 by the Orthopedics surgeon William James Little [1]. Cerebral palsy is a non-progressive disorder caused by damage of the brain in the intrauterine period and at the first months of life; however, the disorder changes in characteristics with aging, with permanent motor function loss and limited movement, postural and movement disorders [2]. Cerebral palsy may progress due to many causes in the prenatal, natal or the early postnatal period [3]. The essential finding of the disease is loss of motor function. Auditory, visual, cognitive, perceptional and behavioral disorders, malnutrition and epilepsy mostly contribute to the disease [2, 4]. Morbidity and mortality are due to motor function disorder, as well as other contributing disorders [5].

The determination of the prognosis of cerebral palsy in the early period is difficult. The most prominent fear of the family is whether their child would be able to resume a normal life in the future and be able to walk or not. Initially, Palisiano et al. [6] developed a scale to determine the motor prognosis. The Gross Motor Function Classification System (GMFCS) consists of measuring the sitting and walking ability in children with cerebral palsy. To determine the motor prognosis, it can be a guide for possible additional disorders and for planning of the treatment [7, 8]. The aim of this study was to evaluate the contributing epilepsy and other disorders and to determine their relationship in patients with CP, using GMFCS.

## Methods

This study was performed in Eskişehir Osmangazi University School of Medicine, Department of Pediatric Neurology between May 2011-January 2012, with a total of 154 patients with a diagnosis of cerebral palsy. Epilepsy and other contributing disorders were evaluated in patients. GMFCS was used to measure the motor function. The age-dependant GMFCS groups children into 1 of 5 levels based on their ability to mobilize and reflects overall gross motor skills and severity of motor impairment. Level 1 (walks and climbs stairs, without limitation) represents the highest level of gross motor function and level 5 (unable to walk, severely limited self-mobility) the lowest [6]. Patients under the age of 3 were not included in the study. Cerebral palsy was classified clinically according to the recommendations of the workshop held in Bethesda and the European Cerebral Palsy Surveillance Group [9].

The nutritional and defecation habits and the sleep disorders of the patients were evaluated. Each patient was ophthalmologically evaluated and Visual Evoked Potentials were performed. All patients were examined by the ear, nose and throat specialist and Otoacoustic Emission (OAE) was performed. Patients who failed the OAE were evaluated with electrophysiological audiometry. The seizure history was evaluated in detail. All patients underwent electroencephalography (EEG). The electroencephalography records were performed using the Nihon Kohden Neurofax 7310 F EEG device. The patients who had 2 or more seizures in the absence of any stimulating factor and with no repeated seizures in the same day were accepted as epileptic. A decrease in the seizure's frequency of more than 50% was accepted as partial response, a less than 50% frequency was accepted as refractory seizure, and absence of seizure was accepted as total response.

For children over 6 years of age, the WISC-R intelligence test, and for children under 6, the Denver developmental screening test, were performed. All patients routinely underwent urine-blood amino acid analysis and cranial MRI. MRI examinations were performed on 1.5 T MR scanner (Siemens, VisionPlus, Germany) equipped with the head coil. The motor functions were measured with GMFCS.

*Statistical Analysis*

SPSS (Statistical Package for Social Sciences) for Windows 16.0 (SPSS Inc., Chicago, IL) statistical pocket program was used to evaluate the data. The frequency distribution was expressed as %, and the age was expressed as “months”, and given as the average values ± standard deviation. The Student t test was used for comparison of 2 average values, and the chi-square test used for comparison of the percentages. The Spearman correlation analysis was used for the correlation analysis. A $p$ value of $<0.05$ was accepted as the statistically significant level.

## Results

The mean age of the total of 154 patients was

8.07±4.15 (3-18 years) and the male/female ratio was 1.3. One hundred and forty-one (91.6%) of the patients were spastic, 8 (5.2%) were dyskinetic, 3 (1.9%) were ataxic, and 2 (1.3%) had mixed type cerebral palsy. 36.4% of (56 patients) the spastic cerebral palsy patients were quadriparetic, 28.6% (44 patients) were diplegic, and 26.6% (41 patients) were hemiparetic cerebral palsy.

According to the GMFCS, 12 patients (12%) were at Level 1, 49 patients (31.8%) were at Level 2, 13 patients (8.4%) were at Level 3, 22 patients (14.3%) were at Level 4, and 58 patients (37.7%) were at Level 5. 47.5% of the patients at GMFCS Levels 4 and 5 had a birth weight of >2500 and their relationship was statistically significant ($p$<0.05). There was no statistical significance between the gestational age, perinatal asphyxia and MRI findings and the GMFCS levels ($p$>0.05). Level 5 was detected mostly in quadriplegic type cerebral palsy ($p$<0.001) (Table 1).

The other contributing disorders were, in particular, mental retardation (89.6%), ophthalmological problems (68.2%) and epilepsy (61%), followed by oromotor dysfunction (48.7%), malnutrition (40.9%), orthopedic problems (38.3%), dental problems (18.8%), sleep disorders (17.5%) and hearing loss (9.1%). The most common ophthalmological problems were strabismus (48%), refractive disorders (12.9%) and nystagmus (12.3%). Pes equinovarus deformity (11.7%) and scoliosis (9%) were the most common orthopedic problems. 38.9% of the patients had sialorrhea and 73 (47.4%) of them had constipation. Two (1.3%) of the patients had gastrostomy. Mental retardation, epilepsy, oromotor dysfunction and malnutrition were most commonly observed at GMFCS Level 5 ($p$<0.001). Sleep disorder and dental problems were most commonly observed at Level 5 ($p$<0.05) (Table 2).

**Table 1.** The distribution of patient characteristics according to the GMFCS levels

| GMFCS | Level 1 n (%) | Level 2 n (%) | Level 3 n (%) | Level 4 n (%) | Level 5 n (%) |
|---|---|---|---|---|---|
| **Birth weight** | | | | | |
| <1500 gr | 1 (8.3) | 9 (18.4) | 1 (7.7) | 2 (9.1) | 9 (15.5) |
| 1500-2500 gr | 1 (8.3) | 11 (22.4) | 9 (69.2) | 8 (36.4) | 23 (39.7) |
| ≥2500 gr | 10 (83.4) | 29 (59.2) | 3 (23.1) | 12 (54.5) | 26 (44.8) |
| **Gestational age** | | | | | |
| <32 weeks | 0 (0) | 16 (32.7) | 6 (46.1) | 3 (13.6) | 14 (24.1) |
| 32-36 weeks | 2 (16.7) | 7 (14.3) | 3 (23.1) | 6 (27.3) | 12 (20.7) |
| ≥37 weeks | 10 (83.3) | 26 (53) | 4 (30.8) | 13 (59.1) | 32 (55.2) |
| **Perinatal asphyxia** | | | | | |
| Present | 3 (25) | 14 (28.6) | 6 (46.2) | 9 (40.9) | 19 (32.8) |
| absent | 9 (75) | 35 (71.4) | 7 (53.8) | 13 (59.1) | 39 (67.2) |
| **Cerebral palsy type** | | | | | |
| Spastic quadriparesis | 0 (0) | 0 (0) | 3 (23) | 6 (27.3) | 47 (81) |
| Spastic diplegia | 1 (8.3) | 24 (49) | 8 (61.5) | 9 (40.9) | 2 (3.5) |
| Spastic hemiparesis | 11 (91.7) | 25 (51) | 1 (7.7) | 3 (13.6) | 1 (1.7) |
| Other | 0 (0) | 0 (0) | 1 (7.7) | 4 (18.2) | 8 (13.7) |
| **MRI finding** | | | | | |
| Present | 12 (100) | 47 (95.9) | 12 (92.3) | 22 (100) | 55 (94.8) |
| Absent | 0 | 2 (4.1) | 1 (7.7) | 0 | 3 (5.2) |

MRI: Magnetic resonance imaging

Ninety-four (61.0%) of the patients had epilepsy. The average starting age of the seizures in the patients was 29.31±32.21 (range; 1-180) months. The seizures had begun before 1 year of age in 44.7% of the patients. Gender, birth weight and perinatal asphyxia had no statistically significant relationship with epilepsy ($p$>0.05). Epilepsy was seen more frequently in patients who had a history of seizure and microcephaly at the neonatal period ($p$<0.05, $p$<0.05, respectively). Mental retardation was more common in patients who had epilepsy, compared with patients without epilepsy ($p$<0.05) (Table 3). Thirty-five (37.2%) of the patients with epilepsy, and 3 (5%) of the patients who did not have epilepsy demonstrated epileptiform discharges on EEG.

**Table 2.** Distribution of the contributing disorders according to their GMFCS levels

| GMFCS | Level 1 n=12 n (%) | Level 2 n=49 n (%) | Level 3 n=13 n (%) | Level 4 n=22 n (%) | Level 5 n=58 n (%) |
|---|---|---|---|---|---|
| **Mental retardation** | | | | | |
| Present | 9 (75) | 38 (77.5) | 12 (92.3) | 22 (100) | 57 (98.3) |
| Absent | 3 (25) | 11 (22.5) | 1 (7.7) | - | 1 (1.7) |
| **Ophthalmological problems** | | | | | |
| Present | 5 (41.7) | 31 (63.3) | 10 (76.9) | 16 (72.7) | 43 (74.1) |
| Absent | 7 (58.3) | 18 (36.7) | 3 (23.1) | 6 (27.3) | 15 (24.9) |
| **Epilepsy** | | | | | |
| Present | 8 (66.7) | 24 (49) | 2 (15.4) | 16 (72.7) | 44 (75.9) |
| Absent | 4 (33.3) | 25 (51) | 11 (84.6) | 6 (27.3) | 14(24.1) |
| **Oromotor dysfunction** | | | | | |
| Present | 1 (8.3) | 11 (22.5) | 5 (38.5) | 11 (50) | 47 (81) |
| Absent | 11 (91.7) | 38 (77.5) | 8 (61.5) | 11 (50) | 11 (19) |
| **Malnutrition** | | | | | |
| Present | 3 (25) | 7 (14.3) | 3 (23.1) | 9 (40.9) | 41 (70.7) |
| Absent | 9 (75) | 42 (85.7) | 10 (76.9) | 13 (59.1) | 17 (29.3) |
| **Orthopedic problems** | | | | | |
| Present | 5 (41.7) | 24 (49) | 5 (38.5) | 7 (31.8) | 18 (31) |
| Absent | 7 (58.3) | 25 (51) | 8 (61.5) | 15 (68.2) | 50 (69) |
| **Dental problems** | | | | | |
| Present | 0 (0) | 4 (8.2) | 1 (7.7) | 8 (36.4) | 21 (36.2) |
| Absent | 12 (100) | 45 (91.8) | 12 (92.3) | 14 (63.6) | 37 (63.8) |
| **Sleep disorders** | | | | | |
| Present | 1 (8.3) | 2 (4.1) | 2 (15.4) | 3 (13.6) | 19 (32.8) |
| Absent | 11 (91.7) | 47 (95.9) | 11 (84.6) | 19 (86.4) | 39 (67.2) |
| **Hearing loss** | | | | | |
| Present | 1 (8.3) | 4 (8.2) | 2 (15.4) | 1 (4.5) | 6 (10.3) |
| Absent | 11 (91.7) | 45 (91.8) | 11 (84.6) | 21 (95.5) | 52 (89.7) |

Eighty (85.1%) patients had one type of seizure, while 14 (14.9%) had more than one type of seizure. The most common seizure types were generalized tonic-clonic (GTC) and tonic seizures. The most common type of seizure in spastic quadriparesis was GTC, generalized tonic in spastic diplegia, and complex partial in spastic hemiparesis. Thirty-two (34%) of the patients had status epilepticus history. Thirty-eight (40%) epileptic patients were using monotherapy, and 56 (60%) were using polytherapy. Eighteen (19%) patients had refractory seizures. Sixteen (17%) patients had a history of infantile spasm.

The epileptic patients demonstrated more GMFCS Levels 4 and 5 compared to the non-epileptic patients ($p<0.001$). There was no statistically significant relationship between the response to the seizure treatment and the GMFCS levels ($p>0.05$). Status epilepticus was most commonly observed in Level 5 ($p<0.05$). Cognitive and mental retardation were most common in GMFCS Level 4 and 5. A statistically significant relationship was detected between the mental retardation and GMFCS levels ($p<0.001$). The patients with mental retardation and epilepsy were most common in Level 5. This difference was statistically significant ($p<0.001$). No relationship was detected between the response to seizure treatment and cerebral palsy type ($p>0.05$).

Fifty-six (70%) of 80 patients who could sit without support before age 3, could walk before age 6, while 3 (23.1%) of 13 patients who could sit without support after age 3, could walk. The potential to walk was higher in patients who could sit without support before age 3, and this relationship was statistically significant ($p<0.001$).

## Discussion

In spite of the fact that intensive care conditions have improved, the frequency of cerebral palsy has not

**Table 3.** The characteristics of the patients with or without epilepsy

| Variable | Epilepsy | | | |
|---|---|---|---|---|
| | Present | | Absent | |
| | n=94 | % | n=60 | % |
| **Gender** | | | | |
| Male | 55 | 58.5 | 32 | 53.3 |
| Female | 39 | 41.5 | 28 | 46.7 |
| **Birth weight** | | | | |
| < 2500 gr | 40 | 42.5 | 34 | 56.7 |
| ≥2500 gr | 54 | 57.5 | 26 | 43.3 |
| **Gestational age** | | | | |
| <37 weeks | 36 | 38.3 | 33 | 55 |
| ≥37 weeks | 58 | 61.7 | 27 | 45 |
| **Neonatal seizure** | | | | |
| Present | 37 | 39.4 | 12 | 20 |
| Absent | 57 | 60.6 | 48 | 80 |
| **Perinatal asphyxia** | | | | |
| Present | 30 | 31.9 | 21 | 35 |
| Absent | 64 | 68.1 | 39 | 65 |
| **Microcephaly** | | | | |
| Present | 51 | 54.3 | 17 | 28.3 |
| Absent | 43 | 45.7 | 43 | 71.7 |
| **Cerebral palsy type** | | | | |
| Spastic quadriparesis | 45 | 47.8 | 11 | 18.4 |
| Spastic diplegia | 14 | 14.9 | 30 | 50 |
| Spastic hemiparesis | 26 | 27.7 | 15 | 25 |
| Other | 9 | 9.6 | 4 | 6.6 |
| **Mental retardation** | | | | |
| Present | 88 | 91.2 | 50 | 68.7 |
| Absent | 6 | 8.8 | 10 | 31.3 |

changed due to the effort to keep babies with very low birth weight and prematurity alive [10, 11]. With a difference due to the socioeconomic level, the frequency of cerebral palsy has been determined as 1.5-2.5 in 1000 live births [12, 13]. In this study, a total of 154 patients with a cerebral palsy diagnosis between 3-18 years of age were evaluated. Comparable with the literature, the male/female ratio was 1.3. The most common clinical cerebral palsy form is the spastic type. Dyskinetic and mixed type cerebral palsy are seen rarely [14,15]. In this study, 91.6% of the patients had spastic cerebral palsy, quadriplegic type being the most common.

There are no specific criteria for the severity of the movement disorder in patients with cerebral palsy. In previous classification systems, it had been graded generally as mild, moderate and high according to the walking ability [16]. The GMFCS, which was created by Palisano *et al.* [6] in 1997, has been stated as an available and reliable method to detect the future motor prognosis. The frequency of the contributing problems varies according to the patients' GMFCS levels. As it may be a predictive issue for the possible problems in patients, it may also lead the clinician in planning the treatment [7, 8]. In our study, a GMFCS Level of 5 was detected most commonly in spastic quadriparetic patients. Mental retardation, speech disorders, oromotor dysfunction, malnutrition, sleep disorders and dental problems were determined to be most frequent in Level 5. Although epilepsy and EEG findings were most commonly observed in Level 5, there was no relationship between the response of seizures to therapy and GMFCS levels.

Although the main problem in cerebral palsy is the motor disorder, the damage is not limited to the motor area. Epilepsy contributes with a frequency of 15-90% [17, 18]. The seizures start at 1 year of age in approximately half of the patients and a relationship has been detected with the neonatal seizures [19]. In a study held in 17 centers with 9654 cerebral palsy patients, it was reported that epilepsy was present in 35% of the patients, and this was related to neonatal seizures and microcephaly, and it was most commonly seen in dyskinetic and bilateral spastic cerebral palsy [20]. In this study, epilepsy was present in 61.0% of the patients. In the neonatal period, epilepsy was more

common in patients with a seizure history and microcephaly. Mental retardation was more common in epileptic patients. In approximately half of the patients, the seizures had begun before 1 year of age. The most common seizure type in spastic quadriparesis was GTC, generalized tonic in spastic diplegia, but complex partial in spastic hemiparesis. The most common EEG finding was spike-wave activity. In approximately one fifth of the patients, the seizures were refractory. No relationship was determined between the response to the seizure treatment and the cerebral palsy type.

Mental retardation was determined in 30-50% of cerebral palsy patients and it was related to the cerebral palsy type. In spastic diplegia, while the cognitive functions were affected mildly due to the sparing of the cortical gray matter, in spastic quadriparesis, mental retardation was observed more frequently and in a more severe form [21]. It has been reported that approximately half of the hemiparetic cerebral palsy patients have an average intelligence [22]. The frequency of mental retardation increases in epileptic patients [23]. In this study, 89.6% of the patients had mental retardation at variable degrees. Mental retardation was most commonly detected in the spastic quadriparetic cerebral palsy type. In children with cerebral palsy, sleep disorders may be observed due to the mental state levels, the treatment and the epilepsy [24]. In this study, sleep disorder was observed in 17.5%, and it was most frequent at GMFCS Level 5.

Ophthalmological problems are observed in approximately half of the patients with cerebral palsy. The most common types are refractive disorders and strabismus [25]. In this study, ophthalmological problems such as strabismus, refractive disorders and nystagmus were detected in 68.2% of the patients. No relationship was detected between the ophthalmological problems and the GMFCS levels. Hearing loss is seen in 7-15% of the cerebral palsy patients [26, 27]. In this study, this rate was 9.1%. It is crucial to perform a complete opthalmological examination and to detect and treat the ocular problems in cerebral palsy patients. With early diagnosis and treatment of opthalmological problems and hearing loss, the negative influence of the quality of life in children with cerebral palsy should be prevented and contribution to their mental, social and spiritual improvement should be promoted.

Alimentation problems and malnutrition are more frequent in children with cerebral palsy compared to the normal population. These children develop malnutrition due to oral motor disability, low calorie intake, stagnation, spasticity and difficulty in feeding. In their study, Del Giudice *et al.* [28] reported gastrointestinal system symptoms in 92% of the patients. In another study performed in Turkey with 120 cerebral palsy patients, sialorrhea, constipation, dysphagia and alimentation disorder were observed in 30.6%, 25%, 19.2%, and 21.7%, respectively. GIS problems and alimentation problems were more common in patients with severe GMFCS levels. The time spent for a meal was longer in patients with alimentation disorders [29]. In this study, oromotor dysfunction was seen in 48.7%, malnutrition in 40.9%, dental problems in 22.1%, and they were most common at GMFCS Level 5. Because the required time for a meal is longer in these children compared to normal children, it is one of the most difficult problems for the family. Furthermore, dental problems are observed in CP patients in relationship with feeding disability and excessive sialorrhea [28, 29].

The stretching of the muscles is inadequate in cerebral palsy, and contracture, muscle weakness, spasticity, and as the child grows, due to contractures, bone deformities such as equinus deformity, increased lordosis, kyphosis and scoliosis may develop [30]. In this study, orthopedic problems were detected in 38.3% of the patients. The most common types were pes equinovarus and scoliosis. The treatment of the orthopedic problems in patients with a walking potential is crucial to develop functional ambulation, and in non-ambulatory patients, to render sitting easy, to develop hygiene and to prevent pain.

The prognosis of cerebral palsy differs with the cerebral palsy type, severity, contributing mental retardation, epilepsy, presence or absence of malnutrition, and the opportunity of the patient to benefit from the rehabilitation resources. The most prominent fear of the family when their child is first diagnosed with cerebral palsy is whether their child would be able to walk or not. The patients who can sit without support by 2 years of age are most likely to be able to have the walking potential, and most of the hemiparetic cerebral palsy patients can walk independently [31]. In this study, it was detected that children who could sit without support before 3 years of age had an increased chance of walking. Beside the physical therapy and rehabilitation, detection of epilepsy and other contributing disorders and their treatment are crucial for the patient's life quality and prognosis.

## Conclusions

As a result, although the main problem is the motor disability in patients with cerebral palsy, other contributing problems such as mental retardation, epilepsy, alimentation problems, audio- and visual problems also exist. There are correlations between the occurrence of disorders such as mental retardation, epilepsy, oromotor dysfunction, malnutrition, sleep disorders, dental problems, and the gross motor function levels. GMFCS, as a scale used to detect the motor prognosis, is thought to be an instructive instrument in possible additional problems. It is crucial to detect and treat the contributing problems for the quality of life and prognosis in patients with cerebral palsy.

*Conflict of interest*

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# Endolaryngeal diode laser surgery for early glottic carcinomas involving anterior commissure

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## ABSTRACT

***Objectives.*** In this study, we present the results of endolaryngeal diode laser surgery in patients with an early stage laryngeal carcinoma involving the anterior commissure (AC). ***Methods.*** A total of 108 patients (103 males, five females; mean age: 52.4 years; range; 31 to 75 years) who were treated between January 2010 and January 2015 were included in this study. All patients with glottic cancer had anterior commissure lesions. Complications, recurrence rates, and overall survival associated with diode laser surgery were recorded. ***Results.*** Based on the AC classification, the initial local control rate was 89.4% for the AC0 tumors, 81.3% for the AC1 tumors, and 90% for the AC2 tumors. Five-year disease-free and overall survival rates were 93.1% and 98.3, respectively. ***Conclusion.*** This study provides evidence that microscopic endolaryngeal diode laser surgery is a safe and effective option for the treatment of early glottic cancer involving the AC.

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***Keywords:*** Glottic carcinoma, anterior commissure, diode laser surgey, survival

## Introduction

The advances in laser technology have produced refined techniques and devices, resulting in a major change from open surgical procedures to endolaryngeal laser surgery in the treatment of early laryngeal cancer. In 1970s, $CO_2$ laser-complemented cold instrumentation began to use in the endoscopic laryngeal surgery [1]. The $CO_2$ laser provides a bloodless surgery with minimum charring and without postoperative edema and need for tracheostomy. Despite these advantages, there are certain disadvantages of $CO_2$ laser surgery. In particular, the equipment requires a large capital outlay and some extensions of the pathology, such as the anterior commissure (AC) and subglottis, and is unable to be approached transorally with a free beam $CO_2$ laser. In addition, microlaryngeal tangential dissection and office-based laryngeal procedures are limited by the lack of a fiber-based delivery system [2]. To address these limitations, a fiber transmissible wavelength, such as potassium titanyl phosphate (KTP) and diode laser, was introduced to endolaryngeal surgery. These lasers function similarly to a $CO_2$ laser with the benefit

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of being delivered through a small glass fiber.

One laser which has gained utilization during the past decade is the 980-nm wavelength diode laser system for the microscopic endolaryngeal surgery. Many studies have demonstrated that diode laser is easily used, effective, and safe for the management of different pathologies in the larynx [3, 4].

In the present study, we aimed to assess the oncological results using microscopic endolaryngeal diode laser surgery (MDLS) in early glottic carcinomas involving the AC.

## Methods

The study was approved by the Institutional Review Board of Ankara Oncology Training and Research Hospital and was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki. A written informed consent was obtained from each patient.

Between January 2010 and January 2015, 108 patients with a T1-T2 glottic carcinoma involving the AC were treated with MDLS. Inclusion criteria were the lack of a previous history of potentially metastatic adenopathy or distant secondary neoplasms, and being treatment-naïve. All the patients had squamous cell carcinoma of the glottic region with normal or impaired vocal cord mobility.

The tumors were defined according to the 2012 American Joint Committee on Cancer/International Union against Cancer Tumor - node - metastasis (TNM) system and the involvement ofthe AC. AC is the area where both vocal ligaments form anterior.According to the AC classification, AC0 describes tumors which do not involve any subsite of the AC, AC1 describes tumors with the involvement of the AC on only one side of the midline, AC2 describes tumors with the involvement of the subsite which crosses the midline on only part of the longitudinal extension of the subside, and AC3 describes tumors with the involvement of the whole subsite on both sides of the midline [5].

A gallium-aluminum-arsenide diode laser (Wuhan Gigaa Optronics Technology, Wuhan, China) with the following specifications was used to excise the tumor in all cases: power 3 to 9 W and wavelength 980 nm with a 400-mm fiber and continuous wave mode.

*Technique*

A gallium-aluminum-arsenide diode laser (Wuhan Gigaa Optronics Technology, Wuhan, China) with the following specifications was used to excise the tumor in all cases: power 3 to 9 W and wavelength 980 nm, with a 400-mm fiber and continuous wave mode. MDLS is performed through a laryngoscope with the benefit of an operating microscope. The cutting is performed with a hand held fiber delivery systems using straight or 30º angled instruments. The instruments are specifically designed for laryngeal surgery with different lengths. The laser works in a contact manner to cut the tissue but can also be used in a spread form for coagulation. The tip of the fiber needs to protrude from the instrument 3-4 mm so that the fiber can touch the tissue.

The patients were intubated with a protected endotracheal tube, and the tube was inflated using saline with methylene blue dye. The patient was placed in a supine position with the head fully extended. The dentition was protected using a plastic tooth guard. Using a rigid laryngoscope, the larynx was visualized through the laryngoscope. After adequate exposure was obtained, the laryngoscope was fixed using a suspension arm. Then the microscope was brought into position. At first, the surgical margins were marked by making small shots around the tumor. En bloc resection was preferred by cutting around the tumor and removing it in 1 piece. After removal, all specimens were analyzed under the operative microscope and frozen sections were sent only if the surgeon was suspicious for unclear surgical margins. Repeated frozen sections were performed until the section was free of tumor.

*Statistical Analysis*

The cumulative probability of surviving was analyzed according to the Kaplan-Meier method. Using this method, we studied the disease-free interval according to local control results.The statistical significance of the differences in the cumulative survival tables was tested with the log-rank test with an error of 5% (i.e., significant at $p \leq 0.05$). The data were collected using an Excel (Microsoft, Redmond, Washington, USA) spreadsheet. All analyseswere performed using the SPSS for Windows version 15.0 software (SPSS Inc., Chicago, IL, USA).

## Results

Of the patients, 103 were males and five were females with a mean age of 52.4 (range; 31 to 75)

years. The mean follow-up was 51.5 (range; 25 to 60) months. There was no perioperative mortality. None of the patients were lost to follow-up. Web developed in 6 (5.5%) patients. The lesions of the patients developing web were AC1-2. The web was involving more than one-third of the vocal cords only in one patient and it was resected with a diode laser. Perichondritis developed in 2 (1.8%) patients. These complications were spontaneous in all patients.

Intraoperative frozen-sections were sent for 28 patients. The results showed that the margins were clear. Pathological examination of the specimens revealed that the surgical margins were positive for the tumor in 22 (20.4%) patients. Finally, free surgical margins were achieved in 86 (79.6%) patients.

The initial five-year local control rate was 93% for the tumors with negative surgical margins and 63.6% for the tumors with positive surgical margins. This positivity was not related to the tumor localization and TNM stage, but it may be associated with tumor volume The initial five-year disease-free survival was 92.7% for the tumors with negative surgical margins and 47.7% for the tumors with positive surgical margins. These findings indicated a significant difference ($p$=0.001).

Details on tumor classes and recurrences are given in Table 1. The initial five-year local control and laryngeal preservation rates for all patients were 87% and 99.1%, respectively. Regarding the AC classification, the initial local control rate was 89.4% for the AC0 tumors, 81.3% for the AC1 tumors, and 90% for the AC2 tumors. However, there was no significant difference between the initial local control rates of AC0 and AC1-2 tumors ($p$=0.456).

At the time of this analysis, 106 of 108 patients (98.1%) were alive with no evidence of disease, two (1.8%) patients died from a second primary lung tumor. Twelve (11.1%) patients had local and two (1.9%) patients had locoregional failure within 25 to 38 months after treatment. Among 22 patients whose surgical margins were reported as positive, eight (36.4%) had recurrence.

According to the Kaplan-Meier method, the estimated five-year disease-free and overall survival

**Table 1.** Patient characteristics

| | T1 (n=64) | T2 (n=44) | AC0 (n=66) | AC1-2 (n=42) |
|---|---|---|---|---|
| Locoregional control | 60 (93.8) | 34 (77.3) | 59 (89.4) | 35 (83.3) |
| Local recurrence | 4 (6.2) | 8 (18.2) | 7 (10.6) | 5 (11.9) |
| Locoregional recurrence | - | 2 (4.5) | - | 2 (4.8) |
| **Treatment of local recurrence** | | | | |
| MDLS | 4 (6.2) | 5 (11.4) | 5 (7.6) | 4 (9.5) |
| RT | - | 2 (4.5) | 2 (3) | - |
| Partial laryngectomy | - | 2 (4.5) | - | 2 (4.8) |
| Total laryngectomy | - | 1 (2.3) | - | 1 (2.4) |
| Second recurrence | 1 (1.5) | 2 (4.5) | 1 (1.5) | 2 (4.8) |
| Ultimate larynx preservation rate, % | 100 | 97.7 | 100 | 97.7 |

*Values are presented as number (percentage) unless otherwise indicated, MDLS=microscopic endolaryngeal diode laser surgery, RT=radiotherapy; AC=anterior commissure, - =no patients

**Table 2.** Oncological Results

| | DFS at 5 years | *p* value | OS at 5 years | *p* value |
|---|---|---|---|---|
| **T1** | 93.1% | 0.019 | 98.3% | 0.783 |
| **T2** | 76.3% | | 97.4% | |
| **AC0** | 88.7% | 0.603 | 98.4% | 0.117 |
| **AC1** | 79.6% | | 100% | |
| **AC2** | 90% | | 90% | |

AC=anterior commissure, DFS=disease free survival, OS=overall survival

for MDLS were 85.9% and 98.1%, respectively. The estimated five-year disease-free survival after any type of salvage treatment was 100%. Regarding T stage, the estimated five-year disease-free rate was 93.8% for T1 tumors and 77.3% for T2 tumors. The difference between the initial local control rates of T1 and T2 tumors was significant ($p$=0.019).The estimated five-year disease-free and overall survival rates according to the AC classification are shown in Table 2. There was no statistically significant difference in terms of prognosis according to AC and TNM classifications The five-year disease-free survival curves according to the AC classification are presented in Figure 1.

## Discussion

A diode laser is a portable laser which is delivered down a fine glass fiber and used with straight or angled handheld probes [3]. The use of handheld probes allows the surgeon resecting deep andcurved parts of the larynx easier and safer than with a standard $CO_2$ laser. The 980-nm diode laser is a new technology, and it has been demonstrated that the 980-nm diode laser accelerates the wound-healing process by changing the expression of PDGF and bFGF genes responsible for the stimulation of cell proliferation and fibroblast growth [6]. This special feature of the 980-nm diode laser allows it to become a strong alternative to the $CO_2$ laser [7]. In a review, Arroyo *et al.* [7] reported that diode laser can be a useful tool for the treatment of different laryngeal pathologies.

Diode laser has recently been used for pediatric airway diseases [3, 4]. In 2008, Ferri *et al.* [8] were the first who reported the early oncological results of endolaryngealendoscopic diode laser surgery for Tis and T1 glottic laryngeal cancer. In 2010, diode laser was introduced in Turkey and, in the light of this study, we began to use MDLS for early glottic cancer. In addition to the oncological and functional success of diode laser surgery, this is predominantly due to the cost of $CO_2$ laser per patient being more than 2.5 fold of diode laser treatment in Turkey and the lack of reimbursement policies from the current health insurance systems.

On the other hand, although almost 40% of the patients in our study underwent AC1-2 resection, the rate of anterior glottic web was lower in MDLS than in standard $CO_2$ laser surgery (5.5% vs 10.6%).

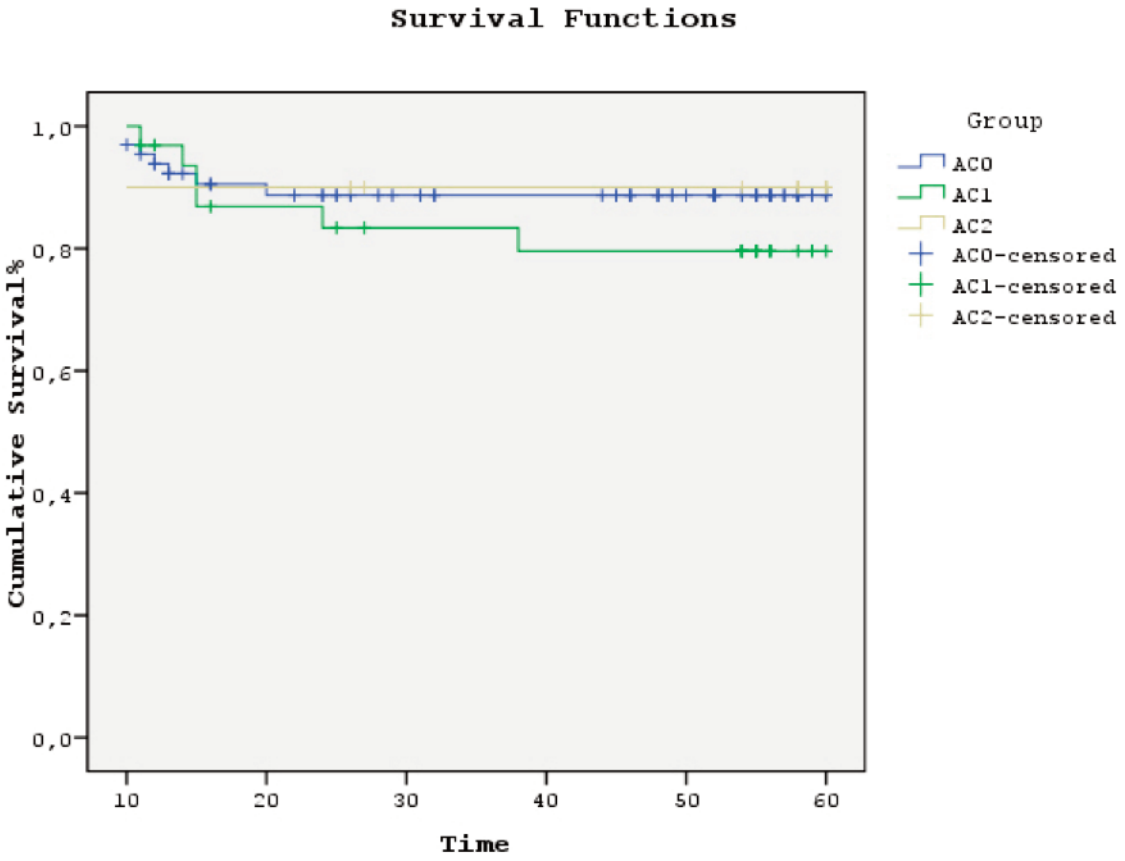


**Figure 1.** Disease-free survival for AC0, AC1, and AC2 tumors at five-year after primary surgery

In our practice, the use of angled probes during AC resection allows the surgeon resecting deeply enough to reach and feel the thyroid cartilage. The deep resection results in a defect in the AC region which prevents the contact of the vocal folds and heals with a fibrotic tissue attached to the AC, rather than a bulky tissue obliterating the AC. Furthermore, the complication rate of perichondritiscaused by the dissection of the inner surface of thethyroid cartilage which we experienced is problematic. This likely relates to the necrosis of the inner perichondrium. In our experience, it is much more difficult to preserve the inner perichondriumof the thyroid cartilage in MDLS than in $CO_2$ laser. The fact that high-power densities used is the additional factor relating to our complication rate. We, therefore, recommend using the MDLS with a laser power setting of 3 to 5 W.

Furthermore, for $CO_2$ laser surgery, there is a controversy about the impact of AC involvement on the recurrence rates in the literature. Some authors found an association between AC involvement and increased rate of recurrence,whereas some authors suggested that AC involvement was not significant for local control [13, 14]. It is critical to remind that resection of the tumors involving AC is very difficult and has a very steep learning curve using standard $CO_2$ laser [14]. Our results demonstrated that the rates of local recurrence were similar in both AC0 and AC 1-2 tumors with MDLS. In our practice, no special experience is needed for the resection of AC in MDLS, as the handheld probe is so much like a cutting instrument used in routine microlaryngeal surgery.

In addition, there is a controversy about the impact of positive surgical margins on the recurrence rates in the literature for $CO_2$ laser surgery. The main reason of this controversy is the collateral thermal effect of laser on the margins of the incision which results in an additional control margin. Our study demonstrated that although the collateral thermal effect of diode laser is much higher than $CO_2$ laser, the five-year disease-free survival of the patients who had positive surgical margins was significantly shorter, compared to the patients who had clear surgical margins. Lesions of patients with positive surgical margins may have progressed submucosally. Lesions of patients with positive surgical margins may have progressed submucosally. These lesions are macroscopically safe when removed by surgically. The patients were not treated with adjuvant treatment. Follow-up was performed at close intervals and re-resection was performed on suspicious lesions.

## Conclusions

This study provides evidence that MDLS is a safe and effective option for the treatment of early glottic cancer involving the AC. Similar rates of local recurrence in both AC0 and AC1-2 tumors suggest that MDLS can be a strong alternative of $CO_2$ laser, particularly for the tumors involving the AC.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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# Silent cerebral embolism after carotid endarterectomy: a two-center experience

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## ABSTRACT

***Objectives.*** Carotid endarterectomy (CEA) is considered the most effective treatment for stroke prevention in patients with critical carotid stenosis. The incidence of new ischemic lesions ranges from 0% to 33% at diffusion-weighted magnetic resonance imaging (DW-MRI) after CEA in previous studies. We determined the rate of silent cerebral embolism in CEA patients by DW-MRI in this study. ***Methods.*** This study was conducted between January 2016 and April 2016 in two centers. Thirty-five consecutive patients (three with bilateral) with 38 CEAs were included in the study. There were no new postoperative symptoms in all patients. Preoperative and postoperative brain DW-MRIs were performed within one day preoperatively and second day postoperatively. Two DW-MRIs were screened and compared in terms of newly occurring lesions. Thus, we attempted to find the rate of silent cerebral embolism. ***Results.*** New brain lesions were detected in six (6/38 CEAs; 15.8%) cases with unilateral CEAs. All of these lesions were ischemic. In five cases, new lesions were located within the operated carotid artery territory (ipsilateral parietal lobe). However, in one case, a new lesion was located outside of the operated carotid artery territory (ipsilateral occipital lobe). Thirty-day morbidity and mortality rates were 0% and 2.85% (1/35), respectively. ***Conclusion.*** Silent cerebral embolisms may frequently occur during postoperative period in CEA patients. Even if these lesions are asymptomatic, we have to be rigorous to avoid microembolism during all stages during surgery.

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***Keywords:*** Cerebral embolism, carotid endarterectomy, magnetic resonance imaging

## Introduction

More than 150 known causes play role in the etiopathogenesis of stroke. The most common causes of them are cardiac and small vessel diseases. The other causes are exta- or intra-cranial atherosclerosis, dissection, coagulopathy, vasculitis, metabolic disease with arteriopathy and unknown.

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Almost 20% of all strokes are related to carotid stenosis [1]. Carotid endarterectomy (CEA) is the most effective treatment for stroke prevention in patients with critical carotid stenosis [2-4]. Recent studies have demonstrated that surgical morbidity is approximately 6% for symptomatic stenosis and 3% for asymptomatic stenosis in CEA patients [5, 6].

Most of perioperative ischemic neurological complications are commonly caused by an embolism released from the fragile plaque in the course of arterial dissection, shunt insertion and cross-clamping, or uncommonly by hemodynamic hypoperfusion [7-9]. Intraoperative hypoperfusion should rarely be an issue because brain perfusion can be maintained by collateral circulation or selective shunting. Conversely, a small embolism arising from a fragile plaque during arterial dissection, shunting and cross-clamping constitutes a risk of perioperative ischemic complications. In cardiovascular diseases, plaque size, luminal narrowing, and plaque structures are also considered causally related to the development of cardiovascular cases [10-12].

Diffusion-weighted magnetic resonance imaging (DW-MRI) is a highly sensitive tool for detecting cerebral ischemia [13]. Ischemic areas smaller than 3 mm in diameter can be indicated only on DW-MRI sequences. It is a suitable new method for improving quality control in cerebrovascular interventions.

The main objective was to evaluate the incidence of silent ischemic brain lesions in CEA patients by using DW-MRI.

## Methods

*Patients*

This study consisted of 40 consecutive patients with high grade carotid artery stenosis who were treated between January 2016 and April 2016 in two cardiovascular surgery centers. Two of 40 patients with new postoperative ischemic symptoms were excluded from the study. Color doppler-assisted duplex investigations and computerized tomography (CT) with contrast was applied for the diagnoses of carotid stenosis. There were bilateral critical carotid stenoses in three patients. Therefore, CAE was separately performed bilaterally in three of 38 patients. The contralateral internal carotid artery (ICA) was occluded in two patients. A critical carotid stenosis was defined as a stenosis of ≥70% for symptomatic patients and a stenosis of ≥80% for asymptomatic patients. Preoperative and postoperative brain DW-MRIs were performed within 1 day before the operation and again 2 days after the operation in patients. Three patients whose unilateral carotid stenosis had contraindications (one with a cardiac pacemaker, one with a mechanical mitral valve prosthesis and one with claustrophobia) for DW-MRI and were excluded from the study. Thus, 38 CEA (35 patients) outcomes were examined by DW-MRI.

All patients were administered with acetylsalicylic acid (100 mg) and statins (40 mg) before the operation. Hypertension was defined as a blood pressure of 140/90 mmHg or higher. Diabetes mellitus was defined as a fasting blood glucose of >126 mg/dl on two measurements, or if the patient was being treated with insulin or oral antidiabetic medication. Chronic obstructive pulmonary disease (COPD) was defined as an FEV1/FVC less than 70%, or if they were undergoing bronchodilator medication.

This study was approved by the ethics committee of the university hospital. We also obtained the patients' written informed consents to be included in the study.

*Carotid Endarterectomy*

The CEA operations were performed under general anesthesia. Near-infrared spectroscopy were implement to all patients for assessment of neurological status during surgery. The ICA, ECA (external carotid artery), and CCA (common carotid artery) were adequately exposed. Intravenous heparin (100 U/kg) was administered; then the ECA, CCA, ICA, and superior thyroid artery were cross clamped. An arteriotomy was performed from the CCA to ICA and fully extended high above the upper extent of the lesion to be removed. The lumen was rinsed with heparinized saline for removing crumbs. A shunt was first placed into the CCA and the balloon of the proximal tube was inflated adequately with saline. Then, the distal tube of the shunt was inserted into the ICA and the balloon was inflated very gently to avoid intimal damage and debris embolization. The distal and proximal tubes of the shunt were fixed by using silicon vessel tape. Arterial flow in the shunt was tested. Thus, cerebral perfusion was restored through the shunt. All of the atherosclerotic plaques were rigorously removed in the subintimal plane and the lumen was washed with saline. The endarterectomized area was thoroughly inspected. If there was any intimal flep, it was skinned or fixed with 7-0 prolene suture. The arteriotomy was closed with a running 6-

0 prolene suture with a patch. A few loops before completion, the shunt device was removed and the ICA and CCA were clamped. Before tying the suture, the lumen was washed with heparinized saline. After tying, the ICA was unclamped for 5 seconds, then clamped again. The ECA and CCA were then declamped and then the ICA was finally declamped. Thus, any possible particulates were prompted into the ECA. Aspirin (100 mg/day) and statins (40 mg/day) were routinely administered after surgery. The excised plaques were examined macroscopically for classification after surgery. Near infrared spectroscopy (NIRS) were used for hemodynamic changes of brain function in all patients during the surgery.

*Diffusion-Weighted Magnetic Resonance Imaging*

DW-MRIs were performed on the brain within the 1 day before and again 2 days after the operation with a MAGNETOM Avanto 1.5-T scanner (Siemens AG, Erlangen, Germany). Diffusion gradients were applied in each of the x, y, and z directions with three b values. The imaging protocol was the same in all patients. Conventional T1- and T2-weighted spin-echo imaging with a fluid-attenuated inversion recovery (FLAIR) sequence was also performed at each examination. On the DW-MRI, any signal-intensity abnormalities were recorded. For all diffusion-weighted abnormalities, we identified the size, vascular distribution, lobe, and area of the brain in which the lesion was situated. All diffusion-weighted abnormalities were correlated with the findings of the T2-weighted and FLAIR images. The DW-MRI images were then evaluated by two neuroradiologists blinded to the clinical status of the patients. The presence of any new hyperintensities in the brain was interpreted as a sign of new ischemic lesions after CEA.

*Statistical Analysis*

Statistical analyses were performed by using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package. In the evaluation of the data, descriptive statistical methods (mean, standard deviation, frequency and percentage distributions) as well as repeated measures of variance were used in repeated measures of the groups, Newman Keuls multiple comparison test in subgroup comparisons and Mc Nemar's test in repeated measures of qualitative data. Values of $p<0.05$ were significantly evaluated.

## Results

*Clinical outcomes*

There were ischemic symptoms in two (5%) patients of 40 CEA procedures postoperatively (hemiplegia and transient ischemic attacks [TIA]). These patients were excluded from the study. The mean age of the 35 participants was 66 years (range; 42-80 years). There were 11 (31.4%) females and 24 (68.6%) males. Fifteen (42.9%) patients had no symptoms preoperatively. Additionally, there were 6 (17.1%) patients with stroke and 14 (40%) patients with TIAs preoperatively. The contralateral ICA was totally occluded in two patients and three patients had critical stenoses in the contralateral internal carotid artery. So, CAE was separately performed bilaterally in these patients. The mean percent of stenosis in the operated carotid artery was 86.85% (range: 70%-98%). Saphenous vein patch were used in 15 (15/38; 39.5%) cases, synthetic patch were used in 14 (14/38; 36.8%) cases and external jugular vein patch were used in 9 (9/38; 23.7%) cases to close the arteriotomy. None of the arteriotomies were closed primarily. The mean cross clamp time was 1.86 minutes before shunting and 2.6 minutes after removing the shunt. Mean arterial pressure was between 80 mmHg and 115 mmHg in all patients and hypotensive period was not observed in all patients during the surgery. When the excised plaques were identified macroscopically, 15 (15/38; 39.5%) plaques had ulcerations, 25 (25/38; 65.8%) plaques had calcifications, 12 (12/38; 31.6%) plaques had thromboses, and 20 (20/38; 52.6%) plaques had signs of degeneration. During the postoperative period, there was no case revealed postoperative hyperperfusion syndrome. Control carotid doppler ultrasound were performed in all patients postoperatively and results were satisfactory. The 30-day morbidity and mortality rates were 0% and 2.85% (1/35), respectively. Cause of mortality was myocardial infarction. The other clinical and laboratory parameters are demonstrated in Tables 1 and 2.

*DW-MRI outcomes*

There were relatively bigger ischemic lesion (9 mm and 13 mm) in ipsilateral parietal lobe in two patients that excluded from the study due to postoperative ischemic symptoms. Therefore, rate of postoperative ischemic symptoms were 5% in the study. Six (6/38; 15.7%) cases whose no new symptoms postoperatively had new brain lesions in

**Table 1.** The clinical and laboratory parameters of patients (n=35)

| Parameters | Data |
|---|---|
| Age (year) | 66.02±10.37 (42-80) |
| Gender | |
| Male | 24 (68.6) |
| Female | 11 (31.4) |
| PAD | 7 (20) |
| DM | 11 (31.4) |
| HT | 24 (68.6) |
| COPD | 8 (22.9) |
| Smoking | 18 (51.4) |
| Symptom | |
| Asymptomatic | 15 (42.9) |
| Stroke | 6 (17.1) |
| TIA | 14 (40) |
| LDL (mg/dL) | 128.68±35.9 (48-189) |
| HDL (mg/dL) | 23±10.66 (12-68) |
| Cholesterol (mg/dL) | 200±50.01 (108-302) |
| Trigliserid (mg/dL) | 134.4±40.96 (56-229) |
| Urea (mg/dL) | 20.54±8.38 (8-46) |
| Creatinin (mg/dL) | 1± 0,95 (0.5-6.3) |
| Hemoglobin (g/dL) | 12.76±1.62 (9.1-16) |
| Hematocrit (%) | 38.76±4.42 (27.9-48) |
| Platelet ($x10^9$/L) | 231.4± 59.76 (151-388) |
| Ipsilateral Stenosis (%) | 86.57±6.96 (70-98) |
| Contralateral Stenosis (%) | 32.82±24.12 (0-90) |

Data are shown as mean±standart deviation (range; mininumum-maximum) or number (percent). COPD=chronic obstructive pulmonary disease, DM=diabetes mellitus, HDL=high-density lipoprotein, HT=hypertension, LDL=low-density lipoprotein, PAD=peripheric arterial disease

postoperative DW-MRIs. All lesions were ischemic. In five cases, new ischemic lesions were located within the operated carotid artery territory (ipsilateral parietal lobe). In one case, new lesion was located outside of the operated carotid artery territory (ipsilateral occipital lobe). The range of these lesion sizes were from 3 mm to 6 mm (Table 3, Figure 1). The other 29 patients (32 CEA cases) were normal according to the DW-MRI results.

## Discussion

In this consecutive series of 35 patients undergoing elective CEA, the stroke rate was 0% and the mortality rate (from any cause) was 2.85% in first

**Table 2.** The operative parameters (n=38)

| Parameters | Data |
|---|---|
| Patch | |
| Saphenous | 15 (39.5) |
| Synthetic | 14 (36.8) |
| EJV | 9 (23.7) |
| First Clamp Time (min) | 1.86±0.77 (1-4) |
| Second Clamp Time (min) | 5±1.12 (1-2.6) |
| Shunt Time (min) | 32.63±9.84 (15-62) |
| Ulcer | 15 (39.5) |
| Calcification | 25 (65.8) |
| Thrombosis | 12 (31.6) |
| Degeneration | 20 (52.6) |

Data are shown as mean±standart deviation (range; mininumum-maximum) or number (percent). EJV=external jugular vein

30 postoperative days. The incidence of postoperative silent brain ischemia that demonstrated by DW-MRI was six (6/38 CAEs; 15.8%) cases.

CEAs reduce the stroke risk in symptomatic or asymptomatic patients with high grade carotid stenosis [2, 4, 14]. In the European Carotid Surgery Trial, among the 1,745 patients who underwent CEA, there were 122 (7.0%) major strokes or death. The death rate was 1.0%, the disabling stroke rate was 2.5%, and the non-disabling stroke rate was 3.5% in 30 days postoperatively [13]. Rapp *et al.* [15] demonstrated that new DW-MRI lesions were observed over a large range after both carotid artery stenting (9% to 70.3%) and CEA (0% to 27.27%). The incidence of new ischemic lesions after CEA was between 0% and 33% at DW-MRI in different studies [16-19].

DW-MRIs have been shown to be far more sensitive to acute cerebral infarctions than either CTs or conventional MRIs [20, 21]. Therefore, DW-MRIs are commonly used for identifying an ischemic lesion in the brain. Furthermore, this imaging method is a great for quality control in carotid interventions. Most of the lesions are ischemic at postprocedural DW-MRIs in patients with carotid interventions.

Embolization can occur during all phases of the CEA operation (e.g., dissection, shunting, clamp release, and wound closure) [22]. Intra-arterial shunting for the maintenance of cerebral perfusion is often blamed for new ischemic brain lesions in CEA patients. Aksun *et al.* [23] demonstrated that requirement of shunt usage may be clear by applying of cerebral oximetry monitoring during the CEA operation. Schnaudigel *et al.* [24] observed that the general use of intra-arterial shunts during carotid artery clamping was associated with a significantly higher incidence of new ipsilateral DW-MRI lesions. Effect of shunt to the development of cerebral ischemia is obscured in this study. Therefore, the effect of shunt to the cerebral embolism could be more clear if the comparison were performed between the patients with and those without shunt.

On the other hand CEA could performed under local anesthesia. Toktas *et al.* [25] demonstrated that CEA procedure with local anesthesia may provide better assessment of neurological status during operation. Whereas all procedure were performed under general anesthesia in this study. Therefore assessment of neurological status were performed by near-infrared spectroscopy during surgery.

Shunt were used in all study patients during the procedures. The arteriotomy area was rinsed with saline before inserting the shunt and after the endarterectomy procedure. Also, the inside of shunt was bled to the outside thorough a third tube to avoid

**Table 3.** Patients with silent cerebral embolism

| No | Age/ Gender | Preoperative Symptom | Carotid Stenosis (%) | Contraleteral Carotid Stenosis (%) | Patch | Ulcer | Calcification | Thrombosis | Degeneration | Localization of Lesions | Size of Lesion (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 62/M | No | 80 | 26 | EJV | - | + | + | + | İpsilateral Pariatel | 3 |
| 2 | 67/M | No | 85 | 15 | Synthetic | - | + | - | + | İpsilateral Oxipital | 4 |
| 3 | 68/M | TİA | 98 | 55 | Synthetic | - | + | - | - | İpsilateral Pariatel | 6 |
| 4 | 56/M | TİA | 75 | 40 | Saphenous | + | + | - | + | İpsilateral Pariatel | 6 |
| 5 | 67/M | No | 90 | 33 | Saphenous | - | - | - | - | İpsilateral Pariatel | 5 |
| 6 | 69/M | No | 85 | 15 | Synthetic | - | + | - | - | Ipsilateral Pariatel | 4 |

EJM=external jugular vein, TIA=transient ischemic attack

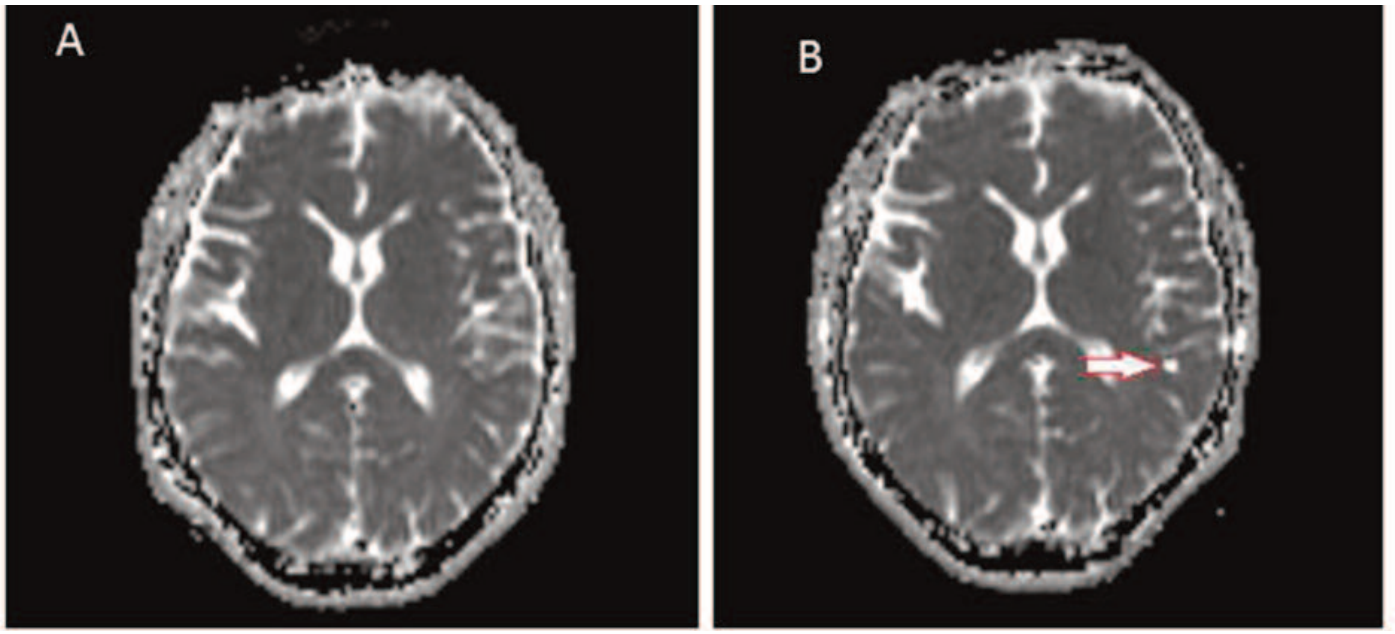


**Figure 1.** (A) Preoperative and (B) postoperative diffusion weight magnetic resonance imaging scans of the brain. Arrow shows the new asymptomatic microischemic lesion that was detected at the ipsilateral parietal lobe.

the formation of microemboli. Therefore, rinsing all possible particulate fragments and carefully inserting the shunt into the ICA and CCA have vital importance. The effect of plaque morphology on the CEA outcome is unclear. However, some studies have reported that microemboli occur during the dissection phase because of fragile carotid plaques [26]. Verhoeven *et al.* [22] divided carotid plaque morphology into three types (fibrous, fibroatheromatous, and atheromatous). According to this study, fibrous plaques were more related to the occurrence of microemboli than atheromatous plaques during the declamping of carotid artery and the closing of the arteriotomy. Atheromatous plaques were more related to occurrence of microemboli during the dissection phase. As a result, this study emphasized that cerebrovascular adverse events occurred more often in patients with atheromatous plaques than in patients with fibrous or fibroatheromatous plaques.

In the present study, six (6/38 CAEs; 15.8%) cases had silent cerebral emboli in the postoperative DW-MRI. In these patients, ulcerated and thrombosed plaque rates were 16.6%, the calcified plaque rate was 83.6%, and the degenerated plaque rate was 50%. This rates demonstrate that the importance of gentle handling of the carotid artery.

All ischemic lesions of brain may not be related to microembolism. Another possible mechanism might be hemodynamic compromise during the procedure. Critical reduction of the cerebral perfusion can cause cerebral ischemia in a certain group of patients [27, 28]. This mechanism may clarify

ischemic lesions outside of the treated carotid artery territory. Therefore, one (16.6%) of the new ischemic brain lesions was located outside of the operated carotid artery territory (ipsilateral occipital lobe) in the DW-MRI in our study.

Additionally, the temporary reduction of carotid blood flow can cause an ischemic event in the ipsilateral brain side in patients with inadequate cerebral collateral circulation (incomplete circle or occlusion of the circle of Willis) [29]. Therefore, systolic arterial pressure has to be at ± 20% from the preoperative baseline value during the cross clamping [30].

*The Limitations of the Study*

Our study has some limitations, including a relatively small sample size and there were no statistical comparisons. Long-term results of patients were also not presented. All operation were performed by using the shunt. So the comparison was not performed between patients with and those without shunt. Additionally effect of local anesthesia to the cerebral microembolism was unknown in this study. Because all operation were made under general anesthesia. Other factors that may be responsible for the silent cerebral embolism (cardiac rhythm disorders, cerebral blood flow, etc.) were not analyzed in this study. Also cerebral collateral circulation was not examined in all patients.

## Conclusions

Silent cerebral ischemia may occur frequently postoperatively in CEA patients. Even if these ischemic lesions that detected with DW-MRI are asymptomatic, we have to be rigorous during all stages of the surgery. Each stage of the CEA may be responsible to cerebral embolism. Additionally, a lower than critical level of blood flow may be the cause of cerebral ischemia. Therefore, we have to maintain adequate blood pressure during the surgery to avoid cerebral hypoperfusion.

*Conflict of interest*

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# A case with unscarred uterus rupture during late postpartum period

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## ABSTRACT

An unscarred uterus rupture is uncommon. It has non-specific symptoms and presentation differs according to the site and time of rupture. It is usually diagnosed intrapartum or shortly after delivery. Here we report a spontaneous rupture of unscarred uterus with delayed presentation and without any usual risk factors. A 33-year-old and 38 weeks' pregnant woman presented with regular uterine contractions. The labor was successfully completed vaginally without any augmentation and no intrapartum complications were observed. After 72 hours the patient complained of an abdominal distension and tenderness. She underwent emergency explorative operation. Intraoperative examination revealed a rupture area on the right posterolateral wall of the uterine fundus approximately 5-6 cm in width. The post-operative period was uneventful and the patient was discharged on 5th postoperative day. Spontaneous rupture of unscarred uterus should be kept in mind even though it is rarely seen complication at late postpartum period.

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***Keywords:*** Uterine rupture, postpartum rupture, postpartum hemorrhage

## Introduction

Uterus rupture is a rare condition that is characterized with tear of uterine wall and its serosa layer. It can be associated with perinatal maternal mortality or morbidity [1]. Major risk factor for uterine rupture is a prior uterine scar and unscarred uterus rupture is uncommon [2]. Advanced maternal age, grand multiparity, macrosomia, multiple gestations, delayed labor, uterine anomalies, abnormal placentation, trauma, obstetric maneuvers (e.g.; internal version and breech extraction, instrumental delivery), vigorous fundal pressure, labor induction and augmentation are other risk factors for uterine rupture [3]. Clinical signs and symptoms are fetal bradycardia, abdominal pain, signs of intra-abdominal hemorrhage, maternal tachycardia and hypotension, uterine tenderness and change in uterine shape; but more often presentation and symptoms are non-specific. Uterine atony, placental abruption, placenta

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previa, uterine inversion, cervical or vaginal tear may be considered in the differential diagnosis of uterine rupture [4]. They are usually diagnosed intrapartum or shortly after delivery. We report a delayed presentation of a spontaneous rupture of unscarred uterus.

## Case Presentation

A 33-year-old and 38 weeks' pregnant woman presented with regular uterine contractions. Her obstetric history revealed two uncomplicated term vaginal deliveries and she had no history of abdominal surgery or curettage. The labor was successfully completed vaginally 7 hours after admission without any augmentation and no intrapartum complications had been seen. The baby was female and had Apgar scores of 7 and 9 at one and five minutes, respectively. The placenta was delivered spontaneously and was intact. Postpartum hemoglobin concentration was revealed no decrease. In the first 24 hours of the postpartum, vital signs were stable with a blood pressure of 120/80 and there was no active vaginal bleeding. After 72 hours the patient complained of abdominal distension and no gas-gaita discharge occurred. She had abdominal tenderness and symptoms of rebound. Laboratory analyses showed 2g/dL decrease in hemoglobin concentration. Transabdominal sonography revealed an intra-abdominal free fluid collection. An abdominal computed tomography (CT) was performed. According to CT there was free fluid in the abdomen, free air bubbles and dilated intestines could be seen behind the posterior uterus. Hypodense parenchymal contusion-laceration areas, starting from uterine serosa and extending into the myometrial muscle group were observed (Figure 1).

The patient consulted to general surgery and decided to be operated. Intraoperative exploration revealed a rupture area on the right posterolateral wall of the uterine fundus approximately 5-6 cm in width (Figure 2). Approximately 1000 mL of blood was evacuated from the abdominal cavity. Bilateral salpinx and ovaries were intact. The operation was terminated after the rupture site was sutured and two closed system drainages were placed in the abdomen. The post-operative period was uneventful and the patient was discharged on the fifth postoperative day.

## Discussion

Spontaneous uterine rupture is a rare complication which can lead maternal and fetal death when the diagnosis is delayed. It occurs in 5.3 per 10000 deliveries [5]. The most common risk factor is previous uterine scars where the risk of rupture is 22-74 in 10000 deliveries [6]. Advanced maternal age,

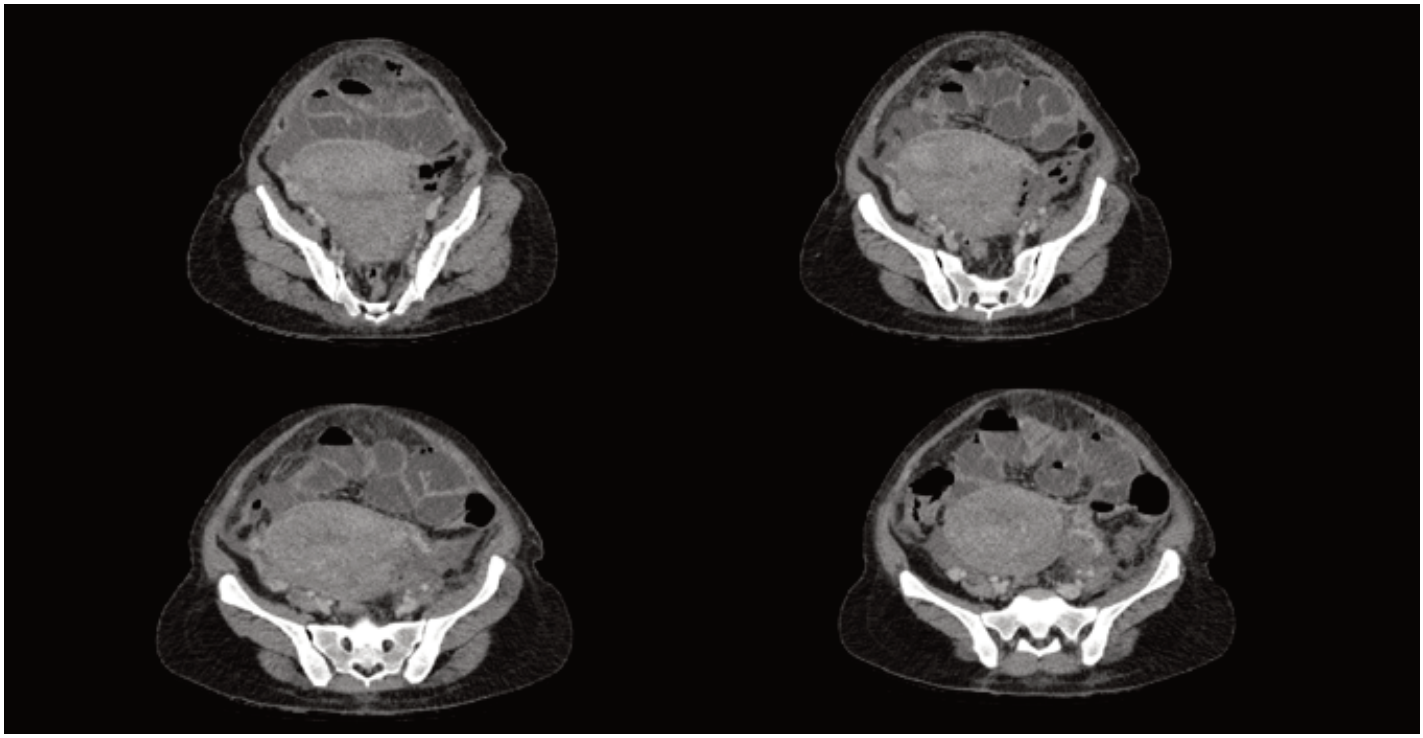

**Figure 1**. Preoperative CT image of the patient.

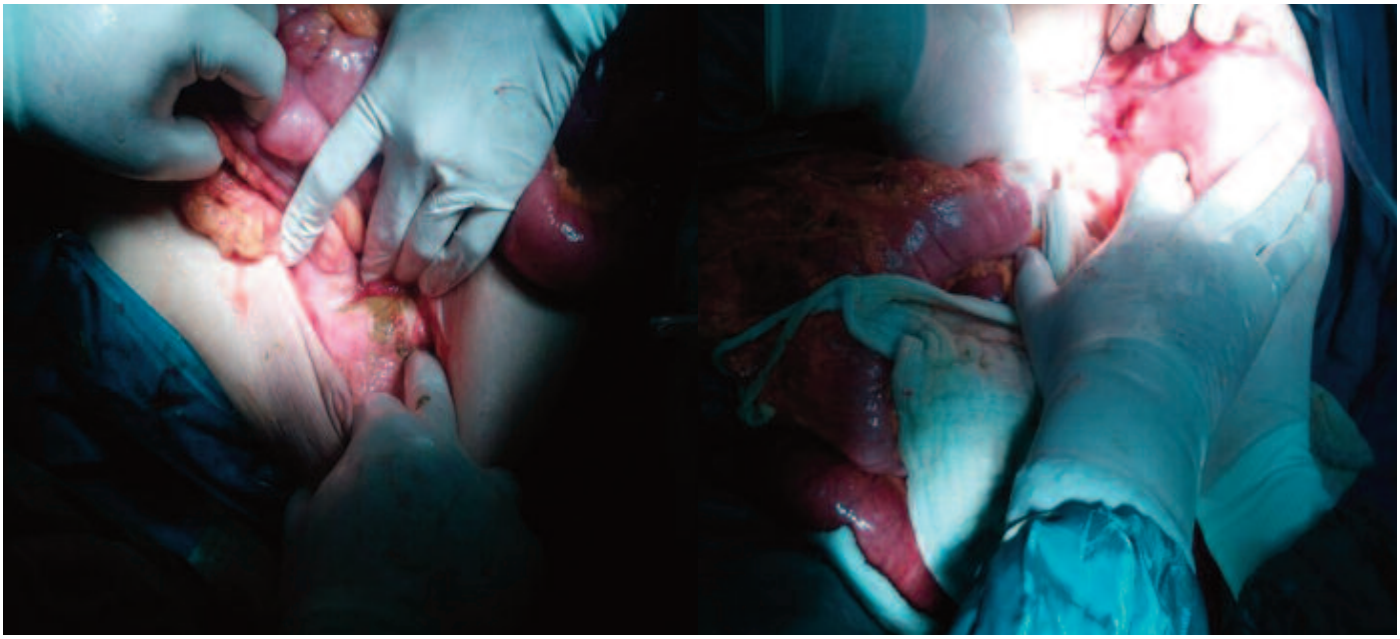

**Figure 2.** Intraoperative exploration and the repair of uterine rupture.

grand multiparity, macrosomia, multiple gestation, delayed labor, uterine anomalies, abnormal placentation, trauma, obstetric maneuvers (e.g. internal version and breech extraction, instrumental delivery), labor induction and augmentation are the other risk factors for uterine rupture [7]. At the same time, there are many cases reported without any risk factors [8]. In this patient the only possible risk factor was multiparity with two previous vaginal deliveries. It was reported that for a patient who had seven previous vaginal deliveries the uterine rupture risk is 20 times higher than a nulliparous [9].

Uterine rupture has non-specific symptoms and presentation differs according to site and time of rupture. The first symptom of rupture that occurs during intrapartum is generally fetal heart rate abnormalities such as bradycardia and usually the changes of the shape of uterus. Then, vaginal bleeding and maternal tachycardia may follow. Ruptures due to previous caesarian occur at the site of previous scar. However, in unscarred uterus it can be on lateral, fundal or posterior region. As in our case posterior defects need higher index of suspicion compared to lower uterine segment due to late onset of symptoms. Making a diagnosis was difficult due to lack of usual risk factors and significant clinical signs and symptoms until postpartum 72$^{th}$ hours in the presented case. It can be supposed that the rupture had been occurred intrapartum or just after the delivery. But intrapartum monitorization had shown no fetal bradycardia and there was no sign of abnormal bleeding. The postpartum period was completely uneventful until postpartum 72$^{th}$ hours.

## Conclusion

In conclusion spontaneous rupture of unscarred uterus is a serious complication that should be kept in mind even though it is rarely seen. It's also a complication that should be considered at late postpartum period. Any situations that cause hemoperitoneum and abdominal pain should be suspected as uterine rupture.

*Informed consent*

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

*Conflict of interest*

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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