The importance of CD10 and h-Caldesmon in the distinction of smooth muscle tumors of the uterus and endometrial stromal sarcoma

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Mesenchymal tumors of the uterus are considered rare, while smooth muscle tumors are the most common type within this group. Endometrial stromal tumors is another commonly seen group. Uterine cellular leiomyoma (UCL), uterine leiomyosarcoma (LMS), and endometrial stromal sarcoma (ESS) are tumors, which express common antigens, and are mainly made of spindle-shaped mesenchymal cells.[1] It is difficult to distinguish ESS from uterine smooth muscle tumors through clinical, histological structure, or routine immunohistochemical techniques.[1,2] Although the histological appearance of ESS is thought to facilitate diagnosis, classical morphology may not always be present. Also, uterus smooth muscle tumors (particularly cellular leiomyomas) may undergo fibrosis or myxoid changes, which may lead to misdiagnosis.[3] The immunohistochemical profile of all 3 tumors is similar, and positive results were obtained with the use of common markers such as smooth muscle actin (SMA), muscle specific actin (MSA), or desmin.[1] Such difficulties in the distinction of these tumors lead to different solutions. Recently, several studies have focused on the expression of CD10 and h-Caldesmon on the mesenchymal tumors of the uterus. Immunoreactivity was detected by using the CD10 antigen on endometrial stromal cells and ESS, while h-Caldesmon expression was shown from uterine smooth muscle cells.[1,4] This study researched the importance of immunohistochemical determinants in the distinction of ESS from smooth muscle tumors, along with the expression rate of CD10 and h-Caldesmon in these tumors.

In this study, the archives of Uludag University Medical School, Pathology Department, Bursa, Turkey between 1996 and 2004 were scanned, and those cases diagnosed with ESS, LMS, and UCL stemming from the uterus were identified. Accordingly, 10 cases of ESS, 10 cases of LMS, and 27 cases of UCL were admitted to the study. The hematoxilen-eosine stained preparations of these cases were reviewed, and the immunohistochemical dyes known as h-Caldesmon (prediluted form, Clone h-CALD, Neomarkers, Fremont, USA), and CD10/CALLA (Neutral endopeptidaz-1/20, Ab-2, Clone 56C6, Neomarkers, Fremont, USA) were applied using the streptavidin-biotin technique to cross-sections made of blocks of the best representative preparation for tumored tissue. Positive staining was evaluated subjectively as weak, moderate, and strong. Additionally, cross-sections were grouped into 2: focal, and diffusive positive staining. Staining less than 50% was considered to be focal, whereas, staining more than 50% was considered to be diffusive.[3] Ethical approval was provided from Uludag University Medical School, Bursa, Turkey.

Diffuse-strong positive staining was seen with CD10 immunohistochemical staining in all ESS (100%). In 10 cases of LMS, focal scattered strong positive staining was seen in only 2 (20%). The remaining 8 cases (80%) did not indicate any staining. In the UCL cases, on the other hand, focal scattered weak staining was seen in 6 (22.2%) whereas no staining was seen in the remaining 21 cases (77.8%). With the use of h-Caldesmon immunohistochemical staining, positive staining was not seen in any ESS cases. While diffuse-strong stained was seen in 5 LMS cases (50%), no stain occurred in the remaining 5 (50%), (**Figure 1**). Among the UCL cases, positive staining was observed in 20 (74%). One case (3.7%) displayed diffuse-moderate staining, while another one (3.7%) displayed diffuse-weak staining. In 2 cases (7.3%), focal moderate staining was seen. While no positive staining was found in the tumor itself in 3 cases (11.1%), it was found in the vein walls. Endometrial stromal tumors, and smooth muscle tumors of the uterus are the 2 major types of mesenchymal tumors seen in the uterus.[1]

CD10 is a surface neutral endopeptidase expressed by lymphoid precursor cells (germinal center origin). CD10 expression may also happen through renal tubules, glomerules, mammaries, glands, myoepithelial cells, prostate epithelium, and pulmonary alveolar epithelium.[3] Caldesmon, on the other hand, is a commonly expressed protein however, its high molecule weight isoform (h-Caldesmon) is expressed only to a limited extent in vascular and visceral smooth muscles. Due to the combination and interaction of Caldesmon with calmodulin, tropomyozin, and actin, it is believe to be related to smooth muscle contraction. It is specifically expressed from mature smooth muscle cells.[4] Nucci et al[5] identified positivity with h-Caldesmon in all benign and malign smooth muscle cells in the uterus, and conversely, they found negativity in all endometrial stromal tumors. These findings indicate the importance of the expression of h-Caldesmon in smooth muscle cells. It is a reliable marker in distinguishing endometrial stromal tumors from smooth muscle tumors. It is more specific than desmin in uterus smooth muscle as desmin

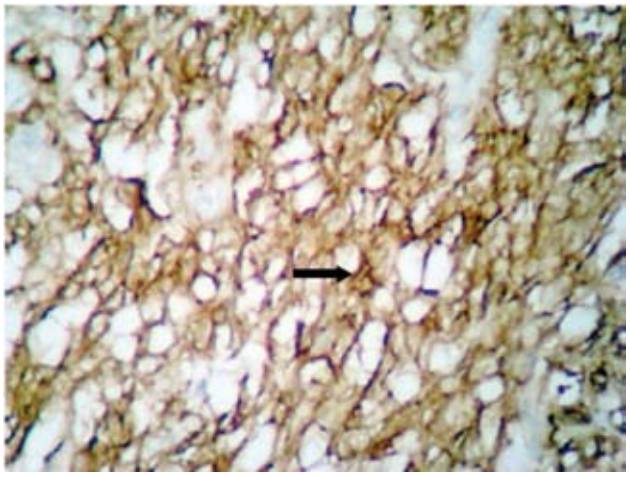

Figure 1 - Diffuse-strong h-Caldesmon positivity in LMS (h-Caldesmon, x400).

may lead to positive staining in non neoplastic and neoplastic endometrial stroma as well. h-Caldesmon is a highly sensitive and specific marker that shows smooth muscle differentiation, and helps the identification of difficult lesions.[5] In an earlier study, CD10 was used in ESS, UCL and LMS cases, and 100% of ESS, and 20% of UCL yielded positive results. No staining was found in LMS cases.[1] In a study conducted by McCluggage et al,[3] CD10 led to diffuse-strong staining in 13 low grade ESS cases, and one endometrial stromal nodule case. Focal weak staining was seen in 3 of the 10 UCL cases, and in 3 of the 5 LMS cases. The positivities in UCL and LMS cases were explained by the existence of cells differentiated to stroma. The present study reached similar conclusions about the use of CD10. Diffuse-strong positive staining was seen in all ESS cases, and weak scattered staining was seen in 6 UCL and 2 LMS cases. Our conclusions on h-Caldesmon in UCL are parallel to others in the literature,[4] and we found staining in all cases except in 3. We identified diffuse-strong staining in 50% of the LMS cases. In half of the LMS cases, diffuse-strong staining was identified.

In conclusion, CD10 and h-Caldesmon were useful markers in distinguishing endometrial stromal tumors from smooth muscle tumors to their diffuse-strong positivity in these respective tumors.

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References

1. Chu PG, Arber DA, Weiss LM, Chang KL. Utility of CD10 distinguishing between endometrial stromal sarcoma and uterine smooth muscle tumors: an immunohistochemical comparison of 34 cases. ***Mod Pathol*** 2001; 14: 465-471.
2. Baker P, Oliva E. Endometrial stromal tumors of the uterus: a practical approach using conventional morphology and ancillary techniques. ***J Clin Pathol*** 2007; 60: 235-243.
3. McCluggage WG, Sumathi VP, Maxwell P. CD 10 is a sensitive and diagnostically useful immunohistochemical marker of normal endometrial stroma and endometrial stromal neoplasms. ***Histopathol 2001***; 39: 273-278.
4. Rush DS, Tan J, Baergen RN, Soslow RA. h-Caldesmon, a novel smooth muscle-specific antibody, distinguishes between cellular leiomyoma and endometrial stromal sarcoma. ***Am J Surg Pathol*** 2001; 25: 253-258.
5. Nucci MR, O'Connell JT, Huettner PC, Cviko A, Sun D, Queda BJ. h-Caldesmon expression effectively distinguishes endometrial stromal tumors from uterine smooth muscle tumors. ***Am J Surg Pathol*** 2001; 25: 455-463.

Effects of walnut leaf aqueous extract on blood sugar and lipids in male diabetic rats

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Diabetes mellitus is a metabolic disease that cause suffering for about 3 million people in Iran. Coronary heart disease (CHD) is a common factor of death throughout the world. In Iran, CHD is the cause of 38% of death. The rate of CHD has been reported to be 2-4 times higher in diabetic patients when compared with non-diabetic, and therefore approximately 75% of these patients die from CHD, or myocardial infarction. Hence, the mortality rate of CHD is higher in diabetics.[1] Cardiac ischemia caused by partial or complete blockade of coronary vessels, and hyperlipidemia is the most important factor of arteriosclerosis. Lipid disorders are a risk factor for cardiac ischemic diseases. Epidemiologic and experimental studies have shown that increase in blood cholesterol is the most important risk factor related to CHD, so that one percent increase in blood cholesterol causes 2% increase in the rate of CHD. The frequency of mortality due to cardiovascular diseases has been reported to be 28.7% in diabetic patients while 16.3% among non-diabetic subjects. Although, the administration of insulin is a routine treatment for diabetes, however the nutritional approaches to treatment of diabetes have many advantages in developing countries. Before the

discovery of insulin and current anti-diabetic drugs, diabetic patients were treated with therapeutic plants, or traditional medicines.[2] At this time positive effects of approximately 1200 types of therapeutic plants was believed to reduce glucose level in diabetics.[2] The use of therapeutic plants in traditional medicine for treatment of diseases has been practiced. Administrations of these plants in Asia especially in Iran, India, China, and some African countries such as Morocco is common. It has been reported that 14 types of therapeutic plants including olive and walnut leaves have been used in Morocco for the treatment of diabetes.[3] Another report from Morocco indicated that from 320 diabetics, and 380 hypertensive and cardiovascular patients, 80% used therapeutic plants for their disease treatment. These people believed that herbal medicine is better than chemical drugs, as they are cheaper and more effective.[4] It seems that the usage of therapeutic plants has fewer side effects, when compared with chemical drugs. Some studies have shown that administration of stewed walnut leaves decreased glucose level in diabetic patients.[5]

The present research was designed to study the effects of walnut leaf extract on the levels of glucose and lipids such as cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), in type I diabetic rats as a model of human diabetes.

Twenty four male adult Wistar albino rats within weighing ranges of 200-230 g were kept in single cages for 5 days. They were kept in 12 hour in a light-dark on a cycle at 22±3°C temperatures with free access to food and water for 2 weeks before the beginning of the experiment. All animal tests were carried out in accordance with the guidelines of Zahedan University of Medical Sciences for care and use of laboratory animals. This study was carried out in 2007 in the research laboratory of Physiology Department, Faculty of Medicine in Zahedan, Iran.

Aqueous Extract Assay. Proper walnut leaves were collected and washed with tap water, then dried at 37°C in the incubator. They were ground up mechanically, dissolved in water, and remained untouched for 24 hours. The resulting fluid was filtered with paper and dried in laboratory conditions to yield the powder. During the experiment, some of this extract in appropriate doses was dissolved in water each day, and fed to the rats using was a mouth catheter.

Diabetes induction and experiments protocol. The animals were divided randomly into 3 groups, each including 8 rats. The first group received normal saline, the second group received single dose of streptozotocine as much as 60 mg/kg, intra-peritoneally, and the third group one week after the diabetes induction similar, to the second group, received 400 mg/kg aqueous extract of walnut leaves orally for 4 weeks. The intake of food and water were measured daily throughout the study period. One week after the injection of streptozotocine, to confirm the induction of diabetes, blood samples were taken and glucose, cholesterol, triglyceride, LDL-C and HDL-C were measured. At the end of the study, animals were given ether anesthesia and the blood sample were taken, and then glucose, cholesterol, triglyceride, LDL-C and HDL-C in samples of serum were measured.

Blood sampling and measurements. Blood samples were kept in laboratory conditions for 20 minutes, and then were centrifuged in 3000 rpm for 15 minutes to extract the serum. In order to measure the level of glucose and lipids such as cholesterol, triglyceride and HDL-C in serum, the peroxide, method and biochemical kits (produced by Ziest Chem Diagnostics, Tehran, Iran) were used routinely as auto analyzer. Low density lipoprotein cholesterol value was calculated using Freed Walled formula.

Statistical analysis. Data including mean±SD were collected from each group. Groups were compared using ANOVA test, followed by Tukey, Kramer post-hoc test for multiple comparisons. The level of significance was $p<0.05$. Data were analyzed using SPSS version 11.

Table 1 indicates the glucose and lipids values in all groups. Analysis for the level of glucose showed a significant decrease in the treatment group and not in diabetic control group. Consuming the walnut extract appears, therefore, to decrease the level of cholesterol in the treatment group, while it has no effect in diabetic control group ($p=0.041$). The mean triglyceride level also decreased significantly after treatment with the extract however, the level of HDL-C increased significantly in the treatment group ($p=0.037$). Low density lipoprotein cholesterol level in diabetic control group increased significantly when compared with the control group, and the administration of walnut leaf extract decreased

Table 1 - Glucose, lipids and lipoproteins variation in all animal groups.

Groups	Control	Diabetic (control)	Diabetic + treatment
Glucose (mg/dl)	130.2 ± 24	524.7 ± 55	434.7 ± 65.2
Triglyceride (mg/dl)	82.5 ± 15	141.5 ± 25.2	121.5 ± 18.4
Cholesterol (mg/dl)	70 ± 12	101.9 ± 7.5	85.4 ± 8
LDL-C (mg/dl)	17.4 ± 3.8	42.3 ± 5.7	24.6 ± 4.1
HDL-C (mg/dl)	37.4 ± 3.5	28.7 ± 4.9	35.1 ± 5.4
LDL/HDL ratio	0.47	1.5	0.67†
Cholesterol/HDL ratio	1.87	3.52	2.36†

Results are Mean ± SD, N = 8 in each group,
†$p=0.04$ in compared with diabetic control group, LDL-C - low density lipoprotein cholesterol, HDL-C - high density lipoprotein cholesterol, LDL - low density lipoprotein, HDL - high density lipoprotein.

significantly the level of LDL-C in the experimental group. According to the findings, LDL/HDL and total cholesterol/HDL ratios decreased significantly in treated animals in comparison with the control diabetic group (p=0.042).

The results showed that chronic administration of aqueous extract of walnut leaves in male diabetic rats can decrease glucose level significantly (**Table 1**). Administration of aqueous extract of walnut leaves in this study significantly decreased cholesterol, LDL-C and triglyceride and increased HDL-C values in diabetic animal (**Table 1**). Zavvarreza et al[5] also reported that the administration of Iranian walnut oil extract caused dose-dependent decrease in triglyceride, cholesterol and LDL-C level in rats that received hypercholestrolemic diet. Koohsoltani et al[1] has shown that administration of walnut pulp caused a dose-dependent decrease in triglyceride, cholesterol and LDL-C values in normo lipidemic and hyper lipidemic people. Also, they reported that the administration of 35g of walnut for 4 weeks in diet, caused a significant decrease in triglyceride and LDL-C and increase in HDL-C amount in blood. Furthermore, administration of walnut leaf extract in this study caused significant decrease in LDL/HDL and total cholesterol/HDL ratios, similar to previous reports.[1] Therefore, it is possible that the apparent hypoglycemic effect of aqueous extract of walnut leaves in diabetic animals and the apparent decrease in cholesterol, LDL-C, and triglyceride and the elevation of HDL-C seen after treatment with walnut leaf extract in this study, and pervious reports need further studies in order to discover their mechanism of action so as to increase possibility of future treatment in humans.

As a limitation to this study, we failed to analyze and determine the effective constituents of the extract, and we have not measured its effect on liver function. Further studies is appropriate to prove its effectivity.

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References

1. Koohsoltani Y, Amir Esfahani M, Shayesteh M, Dastgiri S. Effects of pulp walnut administration on blood lipids in Normo and hyper lipidemic men's, ***J Tabriz Univ Med Sci*** 2004; 62: 55-60.
2. Fallah HH, Fakhrzadeh H, Larigani B, Sheikh SAH. Review on therapeutic plant used in diabetes, ***J Med Plants*** 2006; 5: S1-S8.
3. Eddouks M, Maghrani M, Lemhadri A, Ouahidi ML, Jouad H. Ethnopharmacological survey of medicinal plants used for the treatment of diabetes. ***J Ethnopharmacol*** 2002; 82: 97-103.
4. Bnouham M, Mekhfi H, Legsseyer A. Ethnopharmacology forum Medicinal plants used in treatment of diabetes in Morocco, ***J Diab Metab*** 2002; 10: 33-50.
5. Zavvarreza J, Doosti M, Soleimani S, Asadi F, Farzamfar B, Ariyabarzan S, et al. Effects of oil extract of Iranian walnut on lipids and lipoproteins changes in animal model (hyper cholestrolemic male rat). ***Iranian J Lipids Diabetes*** 2006; 5: 319-324.

Association between tryptophan hydroxylase gene polymorphism and painful non-osseous temporomandibular disorders

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Temporomandibular disorder (TMD) is the term that embraces many painful and/or dysfunctional conditions of temporomandibular joints and muscles of mastication. Temporomandibular disorder, which has also been identified as the non-dental cause of pain within orofacial region has been audited as the most prevalent painful condition among population.[1] However, the underlying mechanism of this widespread disorder remains unclear. Although TMD is known as a local event, systemic changes such as pain perception alterations,[2] and genetic variations of serotonin system components[3] have been reported. Serotonin is a neurotransmitter, which plays a crucial role in daily activities such as sleep, appetite, sexual behavior, and pain.[3] The role of serotonin in pathogenesis of TMD has been implicated. Serotonin is synthesized from tryptophan by the enzyme tryptophan hydroxylase (TPH). Tryptophan hydroxylase is involved in the first and rate limiting step of serotonin synthesis. Thus functional and proportional alterations of TPH may directly affect serotonin synthesis. Tryptophan hydroxylase enzyme is encoded by TPH gene which is located on chromosome 11, and alterations of genetic structure in the position of A218C on intron 7 have been linked with many disorders associated with serotonin metabolism.[4] Although several polymorphisms of TPH gene have been identified, however, the relationship between A218C polymorphism of TPH gene and TMD has not been previously investigated. The aim of

this study was to analyze whether an association exists between TPH gene polymorphism and TMD.

Fifty patients with TMD who applied to the Department of Oral and Maxillofacial Surgery, Selcuk University Faculty of Dentistry, between June 2005 and January 2006, and 45 age and gender matched pain-free healthy volunteers were involved in this study (**Table 1**). The patient group was examined clinically and radiographically with panoramic radiographs and magnetic resonance imaging. All patients were diagnosed according to Research Diagnostic Criteria for TMD[1] for group I disorders, with or without a disc displacement. Inclusion criteria for TMD patients was complaint of pain within the temporomandibular region and masticatory muscles for at least 6 months duration. Patients with TMD without pain complaints and TMD patients with degenerative osseous changes like osteoarthritis were excluded. A visual analog scale (VAS) of 0-100 mm, which was designed as 0 being no pain, and 100 being the worst pain ever experienced, was given to the subjects to quantify the subjective pain at maximum mouth opening. This study was approved by the local ethic's committee, and the patients and controls were informed on the procedure, and written consent was obtained.

Seven ml venous blood were drawn from the subjects and kept into the tubes that contained one ml EDTA (ethylenediaminetetraacetic acid). The DNA was extracted from leucocytes by using standard procedures as previously described.[3] The polymorphic region at position 218 on intron 7 of the TPH gene was determined with polymerase chain reaction (PCR) technique by using the primers (forward: 5' TTC CAT CCG TCC TGT GGC TGG TTA 3', reverse: 5' TTT GAA CAG CCT CCT CTG AAG CGC 3'). The PCR products were classified according to the length during cutting period. The "C" allele was cut while the "A" allele was not. Finally, 3 fragments with 3 different extents were observed (AA genotype for a single band at 1024 bp level, AC genotype for 3 bands at 1024 bp, 660 bp and 364 bp, CC genotype for a single band at 364 bp level). The χ^2 test was used in order to compare the genotype distribution of the patients and controls (SPSS version 10.0 for Windows, SPSS Inc, Chicago, IL). A $p<0.05$ was considered as significant. The patients and the control subjects were similar to each other in terms of age, gender, and ethnic origin. The CC genotype was over represented among the patient group ($\chi^2=7.00$, $p=0.03$), while the number of the individuals with AA and AC genotypes between groups were similar. In the patient group the frequency of C allele was significantly higher than the controls. However, there was no significant difference between the A allele frequency of the patients and controls. In the patient group, 13 patients had mild pain (VAS score <35 mm), 19 had moderate pain (VAS score between 35-65 mm) and 18 had severe pain (VAS score is higher than >65 mm). Genotype frequency of the patients according to the subjective pain intensity was similar ($p>0.05$)

Table 1- Characteristics of the patients and control subjects.

Characteristics	n	Male	Female	Mean age ± SD
		n (%)		
Control	45	8 (17.8)	37 (82.2)	22 ± 3.3
TMD	50	9 (18)	41 (82)	23 ± 6.6
Total	**95**	**17 (17.9)**	**78 (42.1)**	**22 ± 5.3**

TMD - temporomandibular disorder, n - number

Tryptophan hydroxylase gene is located at the chromosome 11, which is mapped as 11p15.3-p14.[4] This location does not exist at the promoter region of the gene since the amount of TPH enzyme and serotonin is not directly changed by polymorphism of this gene. However, it has been reported that this polymorphism may alter pre-mRNA splicing of the intron and strong linkage disequilibrium within TPH gene, or a closer gene may affect serotonin biosynthesis.[5] Temporomandibular disorder patients have been considered as different in terms of pain perception and psychological situations.[2] Hence, possible role of serotonergic system on the cause of TMD needs to be investigated. Previous reports have attempted to exhibit potential genetic alterations in TMD pathogenesis. Most of the mentioned studies have focused on the candidate genes, which affects serotonin metabolism and function. The possible role of serotonin transporter gene polymorphism and serotonin 2A receptor gene polymorphism in Turkish community were previously studied, and the importance of these genetic alterations on development of TMD was implicated.[3] On the other hand, genetic alterations of other important elements of nervous system and some inflammatory cytokines have been previously investigated, and genotype frequencies of catechol-O-methyltransferase enzyme on TMD development and pain sensitivity have been implicated.[6] We did not found any relation between genotype frequency and subjective pain expression of individuals. This finding may be due to relatively insufficient amount of the subjects, pain assessment method and lack of association between TPH gene polymorphism and pain perception. More recently, Erdal et al[5] proposed that absence of AA variant of A218C polymorphism of TPH gene in Turkish population might be related with migraine, which has been known as a common painful disorder. In this study, no association was found between the presence of A allele and AA variant and

TMD. However, the frequency of A allele was less prevalent among TMD patients that was in accordance with the findings of Erdal et al.[5]

Despite some limitations such as small subject quantity of the subjects with specific TMD diagnosis, the present study may be considered as a preliminary report, which was the first to investigate a possible relationship between TPH gene polymorphism and TMD. According to the results of this study, CC genotype and C allele may be risk factors for TMD development. However, the effect of TPH gene polymorphism on TMD development, and whether AA genotype or A allele are preventive factors for TMD remains unclear. The possible role of TPH gene polymorphism on serotonin synthesis and function has been related with linkage disequilibrium with functional alterations within TPH or nearby gene.[5] Further studies in which enhanced number of subjects that mimic almost whole of certain community with specific TMD diagnosis should be developed to understand the role of TPH gene polymorphism on pathogenesis of TMD. The results of this study may also imply possible role of antidepressant medication therapy, which targets deficiency of TPH enzyme on improvement of TMD.

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References

1. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria examinations and specifications, critique. ***J Craniomandib Disord*** 1992; 6: 301-355.
2. Etoz O, Ataoglu H. Evaluation of pain perception in patients with temporomandibular disorders. ***J Oral Maxillofac Surg*** 2007; 65: 2475-2478.
3. Herken H, Erdal E, Mutlu N, Barlas O, Cataloluk O, Oz F, et al. Possible association of temporomandibular joint pain and dysfunction with a polymorphism in the serotonin transporter gene. ***Am J Orthod Dentofacial Orthop*** 2001; 120; 308-313.
4. Shen P, Buchholz M, Sung R, Roxas A, Franco C, Yang WH, et al. Population genetic implications from DNA polymorphism in random human genomic sequences. ***Human Mutat*** 2002; 20: 209-217.
5. Erdal N, Herken H, Yilmaz M, Erdal E, Bayazit YA. The A218C polymorphism of tryptophan hydroxylase gene and migraine. ***J Clin Neurosci*** 2007; 14: 249-251.
6. Etoz OA, Erdal ME, Herken H, Bayazit YA. Lack of association between the 308GA polymorphism of the tumor necrosis factor alpha gene and temporomandibular dysfunction. ***The Pain Clinic*** 2006; 18: 175-180.

Effect of intraoperative mitomycin C application on recurrence of endoscopic dacryocystorhinostomy

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Epiphora, or abnormal tearing, occurs because of blockage in the lacrimal drainage system and recurrent infection may follow as a result of the stasis. The dacryocystorhinostomy (DCR) operation may alleviate the symptoms and it can be performed through a cutaneous incision, external DCR, or via an endonasal approach under either microscopic or endoscopic guidance. The success rates of different endoscopic approaches were greater than 90% in several series of publications. Mitomycin C (MMC) is a chemotherapeutic antibiotic isolated from the broth of *Streptomyces caespitosus*. The ability of this alkylating agent to modify the normal wound healing pathway by inhibiting fibroblast and endothelial cell growth and replication, has made it an attractive adjunct in glaucoma and pterygium surgery, as well as in DCR surgery. The primary cause of failure in DCR surgery is closure of the surgical osteotomy due to fibrosis, scarring, and granulation tissue. The intraoperative application of the anti-metabolite MMC to the surgical anastomosis can theoretically inhibit such closure. While MMC application varies in different published articles according to duration, manner, and procedures, our study was designed to evaluate the effect of intraoperative mitomycin C application in recurrence rate of nasolacrimal duct obstruction 12 months later of endoscopic DCR.[1]

This prospective double-blinded randomized clinical trial was conducted in Besat Hospital, Hamadan University of Medical Sciences, Hamadan, Iran from 2006-2007 and 92 consecutive cases were included in our study. All patients underwent an ophthalmologic examination along with regurgitation test, irrigation test, and in some instances, dacryoscintigraphy of the nasolacrimal drainage system, followed by otorhinolaryngologic and endoscopic intranasal examinations. This study was approved by the Ethic's Committee of our university, and informed consent was obtained from all patients. All the patients was blinded to the treatment option and randomly allocated in each group. The inclusion criteria was the patients with primary nasolacrimal duct obstruction and with no other lacrimal disease. Patients were excluded if there was a history of severe facial trauma, previous lacrimal surgery, and suspicion of malignancy, lower lid problems involving the canaliculi, secondary obstruction of lacrimal duct or failure of follow-up. All

surgeries were performed under general anesthesia by the same surgeon (first author). Endonasal endoscopic DCR uses sinus surgery instrumentations for the nasal mucosa and bone, with a sickle knife to open the lacrimal sac. After the mucosal elevation, without the usage of intracanalicular light pipe, an approximate 15x15 mm bony rhinostomy was created by protected-powered drilling on the lower and upper parts of the lacrimal bone and frontal process of the maxilla. The lacrimal sac was incised vertically using a sickle knife, and the medial part of lacrimal sac was removed by Weil forceps. Finally, short silicone tubes inserted and knotted in the nose. Our trail group was divided in 2 subgroups (3 and 15 minutes) according to exposure of MMC. In both subgroups 0.2 mg/ml of MMC was applied topically by cotton pledget on the rhinostomy site, and after a desired period of time, the surgical area was washed out by saline to prevent additional exposure to MMC. All patients discharged were the following day, and reevaluated in the 3rd, 7th, and 30th days after surgery by the same surgeon for endoscopic rhinostomy check and removal of crusts, if necessary. Silicone tube was removed 3 months after surgery. The final evaluation and determination of successful outcome was carried out 12 months later by an ophthalmologist that was blinded to the undertaken methods in each group and subgroups. At the final visit, subjective and objective outcomes were assessed. Subjective success was based on the patient's symptoms of epiphora. This was recorded as asymptomatic, significantly improved (mild epiphora), moderate epiphora (intermittent epiphora although patient can endure it), or severe epiphora (persistent epiphora). The successful outcome was defined as asymptomatic or mild epiphora, with a normal irrigation test. All postoperative complications were recorded. Finally, all the data were extracted manually and presented in a descriptive manner. Chi square, Fisher's exact, and t tests were used for non-parametric and parametric variables.

There were 68 (73.9%) females and 24 (26.1%) males in the series. The mean age of patients was 43.00±15.95 years. The age, gender, duration of symptoms, operation duration and follow up period in both control and trial groups compared (Table 1), and showed no statistical differences. In 92 consecutive patients of both groups only 10 (10.8%) endoscopic DCR surgeries were failed. The recurrence was 6 (13.0%) of nasolacrimal duct obstruction , and 4 (8.7%) patients in control and trail groups (P=0.799). Also, according to duration of mitomycin C application, there were 2 (8.69%) failed patients in both 3 and 15 minutes subgroups. Overall postoperative complications in both control and trial groups were 7 (7.6%) patients included 5 (5.4%) patients with periorbital ecchymosis, 2 (2.2%) patients with minor epistaxis and no cases of periorbital emphysema. Also, spontaneous early removal of silicone tubes (before 3 months) occurred in 5 (5.4%) patients apart from any influence on the outcomes.

Our results showed that 6 (13%) recurrence of nasolacrimal duct obstruction, and 4 (8.7%) patients in control and trail groups. Based on our data, it appeared that patients with nasolacrimal obstruction who underwent endoscopic DCR did not benefit from adjunctive topical application of MMC. Along with our findings, Liu et al[2] mentioned that MMC application during silicone tube insertion did not benefit outcome. Complications from such application were mild and infrequent. Also, Roozitalab[3] concluded that the use of intraoperative mitomycin C in DCR surgery does not change the success rate of this procedure. But, some studies found that the satisfaction and success rates of the mitomycin C group were higher than those of the control group and no deleterious effect was noted with MMC application, however, the differences did not reach statistical significance and concluded that intraoperative MMC application seems to be a safe adjuvant that could help in increasing the success rates of surgery in primary acquired nasolacrimal duct obstruction. As mentioned by Liao, intraoperative MMC application is effective in increasing the success rate of DCR surgery in standard nasolacrimal duct obstruction, and no significant complications resulted from its use.[4] The success rate of our previous study without the usage of MMC was 88.5% on 12 month follow up[5] and in the present study 87% were the success rate of control and 91.3% trail groups. Because of time consumption and increase in operation duration in MMC application and non significant difference in success rate in both control and trail groups, we don't advocate the routine usage of it in endoscopic DCR and precise clinical evaluation, more scrutiny in patients selection and surgeon experience

Table 1 - Patients' characteristics of control and trail groups that underwent DCR (N=46).

Characteristics	Control	Mitomycin C	*P*-value
Age (year)	44.00 ± 15.33	42.06 ± 13.02	NS
Gender (male/female)	13/33	11/35	NS
Duration of symptom (month)	19.26 ± 9.99	22.07 ± 14.08	NS
Operation duration (minute)	23.67 ± 7.50	34.01 ± 3.59	<0.05
Follow up period (month)	12.80 ± 1.52	12.17 ± 1.18	NS

NS - non significant, DCR - dacryocystorhinostomy

may play more important role in the success rate of this procedure. The limitation of this study was our sample size. Therefore, we suggest further evaluation in larger scale and in multicentral setting.

In conclusion, topical intraoperative MMC application could not reduce the recurrence rate of endoscopic DCR in both 3 and 15 minutes application subgroups. Although we suggest further multi-central trails for comparing results in different hospital setting.

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References

1. Deka A, Bhattacharjee K, Bhuyan SK, Barua CK, Bhattacharjee H, Khaund G. Effect of mitomycin C on ostium in dacryocystorhinostomy. ***Clin Experiment Ophthalmol*** 2006; 34: 557-561.
2. Liu D, Bosley TM. Silicone nasolacrimal intubation with mitomycin-C: a prospective, randomized, double-masked study. ***Ophthalmology*** 2003; 110: 306-310.
3. Roozitalab MH, Amirahmadi M, Namazi MR. Results of the application of intraoperative mitomycin. ***Eur J Ophthalmol*** 2004; 14: 461-3.
4. Liao SL, Kao SC, Tseng JH, Chen MS, Hou PK. Results of intraoperative mitomycin C application in dacryocystorhinostomy. ***Br J Ophthalmol*** 2000, 84: 903-906.
5. Farahani F, Hashemian F, Fazlian MM. Endoscopic dacryocystorhinostomy for primary nasolacrimal duct obstruction. ***Saudi Med J*** 2007; 28: 1611-1613.

Illustrations, Figures, Photographs

Four copies of all figures or photographs should be included with the submitted manuscript. Figures submitted electronically should be in JPEG or TIFF format with a 300 dpi minimum resolution and in grayscale or CMYK (not RGB). Printed submissions should be on high-contrast glossy paper, and must be unmounted and untrimmed, with a preferred size between 4 x 5 inches and 5 x 7 inches (10 x 13 cm and 13 x 18 cm). The figure number, name of first author and an arrow indicating "top" should be typed on a gummed label and affixed to the back of each illustration. If arrows are used these should appear in a different color to the background color. Titles and detailed explanations belong in the legends, which should be submitted on a separate sheet, and not on the illustrations themselves. Written informed consent for publication must accompany any photograph in which the subject can be identified. Written copyright permission, from the publishers, must accompany any illustration that has been previously published. Photographs will be accepted at the discretion of the Editorial Board.