Journal of Ankara Medical School

ISSN 1300-5464

Vol. 24 No. 4 2002

Journal Of Ankara Medical School

Cilt: 24, Sayı: 4, 2002
Vol: 24, No: 4, 2002

THE JOURNAL OF THE FACULTY OF MEDICINE
UNIVERSITY OF ANKARA

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JOURNAL OF ANKARA MEDICAL SCHOOL Vol 24, No 4, 2002 159-164

THE EFFECT OF CONTINUOUS RELEASE OF METHYLENE BLUE FROM A DRUG DELIVERY SYSTEM ON THE INTESTINES: AN EXPERIMENTAL STUDY IN CHICK EMBRYO GASTROSCHISIS MODEL✣

Meral Barlas* ✣ Özgür Denli* ✣ Nurşin Gönül** ✣ Canan Hasçiçek**
Şükrü Özdamar*** ✣ Fatin Cedden**** ✣ Attila Elhan*****

SUMMARY

Prolonged exposure to amniotic fluid causes the intestinal changes such as serosal edema, thickening,fibrous coating,and adhesions in gastroschisis. The effect of continuous delivery of methylene blue (CDMB) from a drug delivery system and daily injection of methylen blue (DIMB) on the intestines was evaluated using a chick embryo gastroschisis model.Fifty fertile eggs were divided into5 groups: preliminary study (PSn=10), amnio-allontoic fluid (AAF) control (AAC, n=10), just gastroschisis (JG, n=10), gastroschisis pretreated with daily injection of methylene blue (GP-DIMB, n=10) and gastroschisis pretreated with continuous delivery of methylene blue (GP-CDMB, n=10). The PS group was also divided into 2 subgroups to determine the biochemical differences between the amniotic and allontoic fluid. Gastroschisis was created surgically at the 14th day of incubation. In GPMB group daily methylene blue injection (5 mg/g) was administered into AAF for 5 days. In GP-CDMB, 25 µg/g methylene blue loaded polymer (Hydroxy-Propyl Methyl Cellulosa-Ethyl Cellulosa "HPMC-EC") was placed into AAF.

A significant decrease in mortality and intestinal damage and higher ganglion number were observed both macroscopically and microscopically in the group GP-CDMB compared to JG (p<0.001). Because of multiple intervention of embryos, higher mortality rate were observed in the group GP-DIMB (75.61%). Pretreatment with continuous delivery of methylene blue from a drug delivery system, prevented intestinal damage, and the complication of multiple interventions of fetuses because of gastroschisis.

Key Words: *Fetal Experiment, Gastroschisis, Methylene Blue, Polymers*

ÖZET

İlaç Taşıyıcı Sistemden Devamlı Salınan Metilen Mavisinin Barsaklara Etkisi: Civciv Embriyosu Gastroşizis Modelinde Deneysel Araştırma

Gastroşizis'de, uzun süreli amniyotik sıvıyla temas, barsaklarda serozal ödem, kalınlaşma, fibröz doku ve yapışıklık gibi değişikliklere sebep olur. İlaç taşıyıcı sistemlerden devamlı salınan metilen mavisi ve günlük uygulanan Metilen Mavisi (MM)'nin etkileri, civciv embryosu gastroşizis modelinde değerlendirildi.Elli adet döllenmiş yumurta 5 gruba ayrıldı: Ön çalışma (PS, n=10), Amniyo-allantoik sıvı kontrol (AAC, n=10), yalın gastroşizis (JG, n=10), günlük metilen mavisi injekte edilerek tedavi edilmiş gastroşizis,(GP-DIMB, n=10) ve ilaç taşıyıcı sistemlerden devamlı salınan metilen mavisiyle tedavi edilen gastroşizis (GP-CDMB).Ön çalışma grubunda amniyotik ve allantoik sıvı biyoşimik farklılıkları iki alt grupta araştırıldı. İnkübasyonun 14. gününde cerrahi gastroşizis yapıldı. GP-DIMB grubunda, AAF içine günlük MM enjeksiyonu (5 µg/g) 5 gün boyunca uygulandı. GP-CDMB grubunda, 25mg/g MM yüklenmiş polimer (Hydroxy-propyl methyl cellulose-Ethyl cellulose) AAF içine yerleştirildi.Mortalite ve barsak hasarlanmasının, polimer ile tedavi edilen GP-CDMB grubunda yalın gastroşizis grubuna kıyasla önemli derecede azaldığı (p<001), ayrıca Auerebach gangliyon hücre sayısının da daha fazla olduğu saptandı.Embryona çoğul girişim nedeniyle GP-DIMB grubunda mortalite daha yüksek oranlarda idi.Polimerlere yüklenen ilaçların, gastroşiziste çoğul grişim komplikasyonlarını ve fetüsda mortalite oranlarını düşürerek postoperatif erken cerrahi girişimleri kolaylaştıran yaralı bir tedavi yöntemi olduğu düşünülmektedir.

Anahtar Kelimeler: *Fetal Cerrahi, Gastroşizis, Metilen Mavisi, Polimer*

* University of Ankara, Department of Pediatric Surgery
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**** University of Ankara, Department of Agriculture Faculty - Animal Science
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✣ Ethical Committee consent was obtained from the Medical School of Ankara University.
✣ Presented at the Congress of Innovation-Surgery in Children, 4/November/2002 Münster/Germany

Received: *Dec 31, 2002* Accepted: *Dec 31, 2002*

Materials and Methods

One hundert two fertile chick eggs (Gallus domesticus), weighing 54-70 g, were incubated at 37.5 °C in 80% humidity. The eggs were divided into five groups of 10 eggs each. The eggshell windows were opened by electric hand separator which is used from Dentist.

1. Preliminary study group (PS, n=10): Intestines, amniotic and allantoic fluid obtained from chicken embryos on the 19^{th} day of incubation, without any intervention.
2. AAF Control group (AAC, n=10): Through an eggshell windows the allantoic and amniotic membranes were opened without creating gastroschisis on the 14^{th} day of incubation that resembles the amniotic cavity in humans. Intestines and AAF were harvested on the 19^{th} day of incubation.
3. Just gastroschisis group (JG, n=10): Gastroschisis was created by surgical intervention on the 14^{th} day of incubation. AAF and intestines were harvested on the 19^{th} day of incubation without any treatment.
4. Gastroschisis pretreated with daily injection of methylene blue group (GP-DIMB, n=10) Gastroschisis was created on the 14^{th} day of incubation and methylene blue (5 mg / g) which dissolved in 0.75% NaCl solution (Physiologic serum saline for birds) was instilled (0.1 mL / 50 g) into the AAF on the 15^{th}, 16^{th}, 17^{th} and 18^{th} days of incubation. AAF and intestines were harvested on the 19^{th} day of incubation.
5. Gastroschisis pretreted with Continuous delivery of methylene blue group

(GP-CDMB,n=10): Gastroschisis was created on the 14^{th} day of incubation and methylene blue (25 mg / g) loaded polymer (HPMC -EC) was placed into the AAF. AAF and intestines were harvested on the 19^{th} day of incubation.

By wide distruption of amnio-allantoic membrane, an amnio-allantoic cavity was created surgically to resemble the human amniotic cavity in all groups except PS group. Surgical intervention for creation of gastroschisis was performed by previously described method on the 14^{th} day of incubation(6,7). In a short describtion ,after disruption of amnio - allantoic membranes, a defect was created at the base of the umblical stalk, and intestines were gently pushed out of the abdominal cavity.

The solution of methylene blue used for pretreatment were daily instilled into AAF using a sterile 26 –gauge needle and an injector.

After surgical intervention, the eggshells were sealed with sterile plastic dressing and incubated. By daily inspection the survival of the embryos was checked and 52 dead embryos were discharged. Ten chickens from each group were evaluated during the study. The eggshells were opened on the19^{th} day of incubation and embrionic fluid samples were harvested to investigate the glucose, pH, urea, creatinine, uric acid, electrolytes (Na, K, Cl), total bilirubine, indirect bilirubine, direct bilirubine and amylase. After macroscopic evaluation, the intestines were harvested and kept in 10% formalin solution.

A microscopic ruler (ocular micrometer) was attached to the objective, the same pathologist measured intestinal wall thicknessand ganglion cells in a blind manner (M.Trichrom and H&E).

The intestines were graded between 0 to 4 microscopically according the degree of the damage such as normal intestine (=0, no changes), minimal changes (=1, serosal edema), mild (=2, fibrous coating), moderate (=3, fibrous coating and intestinal thickening), severe (=4, fibrous coating and intestinal thickening and adhesions).

The results were presented as the means $\pm$ SEM. Statistical analysis was performed by analysis of variance followed by Kruskal–Wallis 1-way Anova (8). Differences were considered significant when p values were less than 0.05.

Results

The survival rates were decreased in the chick embryos with creation of gastroschisis compared with PS and AAC groups. The survival rates were greater in the chick embryos with GP-CDMB

(76.92%) group compared with the GP-DIMB(24.39%) and JG (41%) group (p<0.001). Because of multiple intervention of embryos in the GP-DIMB groups mortality was highest(75.61%), (Table.1).

Table 1: The Experimental Groups Mortality and Survival Rate of the Chick Embryos

Group	Mortality	Alive (%)	Total
PS	0	10 (100)	10
CG	4	10 (71.42)	14
JG	14	10 (41.66)	24
GP-DIMB	31	10 (24.39)	41
GP-CDMB	3	10 (76.92)	13
Total	52	50 (49.01)	102

Abbreviations:PS; Preliminary study group, CG; Control group, JG; Just gastroschisis group, GP-DIMB: Gastroschisis pretreated with methylene blue group,GP-CDMB: Continuous delivery of methylene blue group

The biochemical parameters, histopathologic changes of the intestines, intestinal wall thickness and Auerbach ganglions of the chick embryos are summarized in Table. 2

Urea, creatinine, uric acid, total bilirubine, indirect bilirubine and glucose concentrations were higher in the allantoic fluid of PS group whereas lower in the GP-DIMB and GP-CDMB groups compared with the AAC group (p<0.05).

There were significant signs of intestinal damage in the JG group while intestinal damage was partially prevented in the GP-DIMB and GP-CDMB groups The intestines were thickened and covered by fibrous peel in the JG group. In the GP-CDMB and GP DIMB groups the intestinal thickness was nearly normal and there was no fibrine deposition on serosal surface (Figs1-4). There was no adhesion in the GP- CDMB and GP-DIMB groups compared with the JG group (p<0.001), (Fig.3). There were no significant signs of intestinal damage between GPMB and GP-CDMB groups (p>0.05). The total number of the ganglion cells was higher in the GP-CDMB group than the JG group (p<0.05).

Table 2: The Biochemical Parameters, Macroscopic and Histopathological Changes of the intestines, Intestinal Wall Thickness and Number of Auerbach Ganglion Cells of the Chick Embryos

Group	Amn - PS	All - PS	CG	JG	GP-DIMB	GP-CDMB
Glucose (mg/dL)	8.6 ± 0.2	44.8 ± 1.0	6.6 ± 0.3	12.1 ± 0.6	8.6 ± 0.3	6.2 ± 0.1
Ürea (mg/dL)	7.6 ± 0.1	93.5 ± 2.1	81.4 ± 1.7	34.8 ± 0.9	47.60 ± 0.9	46.90 ± 0.8
Creatinine (mg/dL)	0.4 ± 0.1	3.6 ± 0.4	2.43 ±0.4	0.8 ± 0.1	0.77 ± 0.1	0.91 ± 0.1
Ü.acid (mg/dL)	31 ± 0.5	298.2 ± 9.1	204 ± 8.7	80.9± 4.3	83.60 ± 3.3	76.78 ± 5.7
T.bilirubine(mg/dL)	0.6 ± 0.1	2.4 ± 0.4	1.71 ± 0.6	0.4 ± 0.1	0.53 ± 0.1	0.53 ± 0.3
D.bilirubine(mg/dL)	0.4 ± 0.1	0.3 ± 0.1	0.17 ± 0.1	0.1 ± 0.1	0.13 ± 0.1	0.03 ± 0.0
Ind. Bil (mg/dL)	0.2 ± 0.1	2.08 ± 0.4	1.53 ± 0.2	0.3 ± 0.1	0.40 ± 0.1	0.5 ± 0.1
Amylase (mg/dL)	61.4 ± 3.4	31 ± 0.5	108.8 ± 7.5	248.1 ± 10.3	177.4 ± 8.3	282.6 ± 9.3
Na (mEq/L)	122 ± 6.5	45.16 ± 2.1	83.1 ± 3.1	87.04 ± 3.3	90.6 ± 3.3	88.6 ± 3.9
K (mEq/L)	21.1 ± 0.9	20.6 ± 0.7	20.9 ± 0.8	29.39 ± 0.9	33.11 ± 0.8	33.85 ± 0.3
Cl (mEq/L)	18.08 ± 0.6	33.9 ± 1.2	57.8 ± 1.1	72.05 ± 1.1	73.90 ± 0.7	69.9 ± 0.7
PH	7.6 ± 0.4	6.3 ± 0.5	6.67 ± 0.4	7.26 ± 0.4	7.35 ± 0.1	7.22 ± 0.3
Int. Macroscopic-Grade	0 ± 0.0	0 ± 0.0	0 ± 0.0	2.50 ± 0.1	1.00 ± 0.1	1.00 ± 0.1
Int. Microscopic-Grade	0 ± 0.0	0 ± 0.0	0 ± 0.0	3.0 ± 0.1	2.0 ± 0.1	1.0 ± 0.1
Intestinal Thickness (hm)	288.61 ± 17.5	280.24 ± 18.6	305.34 ± 24.4	818.59 ± 121.4	401.30 ± 66.5	402.03 ± 18.7
Auerbach Ganglion (number)	19.0 ± 3.7	18.4 ± 3.6	17.3 ± 4.4	11.5 ± 2.8	12.2 ± 5.9	14.6 ± 3.3

Abbreviations: PS; Preliminary study group (Amn – amnion control, All – allantois control), CG; Control group, JG; Just gastroschisis group, GP-DIMB: Gastroschisis pretreated with methylene blue group,GP-CDMB: Continuous delivery of methylene blue group

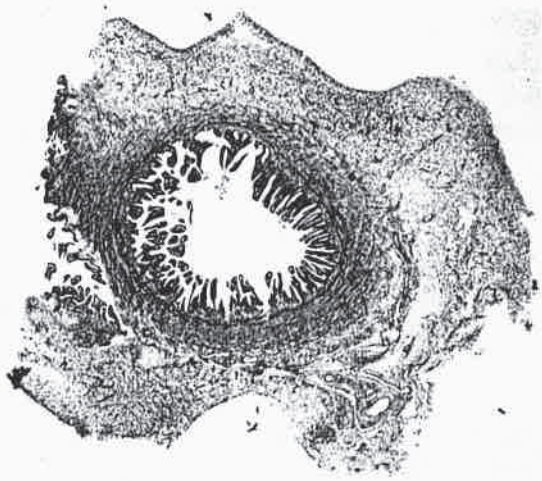

Figure 1. Intestinal wall thickening in the just gastroschisis group(No treatment) (Masson's Trichrome x 28)

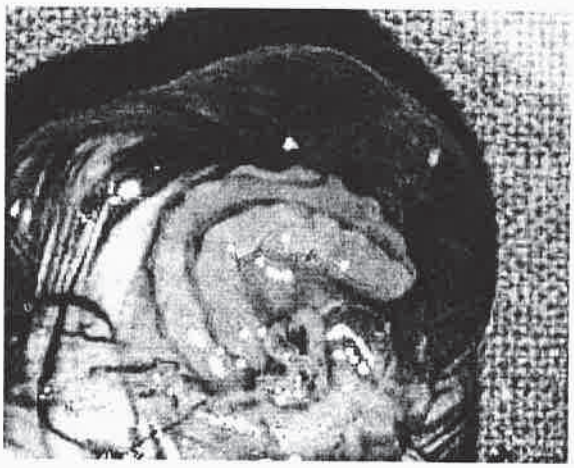

Figure 3. Macroscopic appearance of intestinal wall thickening, edema and adhesion was seen in the just gastroschisis group(No treatment)

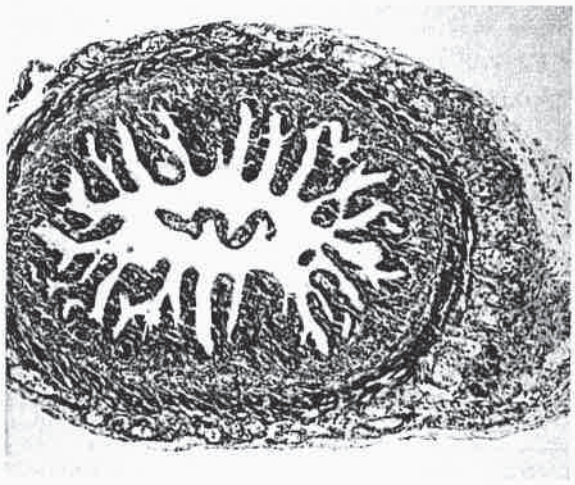

Figure 2. Nearly normal appearance of intestinal wall which was treated with polymer (Continuous Delivery of Methylene Blue Group) (H&E x 115)

Figure 4. Macroscopic appearance of nearly normal intestinal wall which was treated with polymer (Continuous Delivery of Methylene Blue Group)

Discussion

Evaluation of embryonic fluids from chicken embryo gastroschisis model shows remarkable similarities with human amniotic fluid on composition. Chicken embryo discharged urinary and intestinal waste products to allantoic cavity, however, human fetus discharged to the amniotic cavity. Common amnio-allantoic cavity of chicken embryo resembles human amniotic cavity (9). The etiology of intestinal damage in gastroschisis is still uncertain and attributed to either direct exposure to the amniotic fluid or ischemia and lymphatic congestion caused by abdominal wall compression of the eviscerated mesentery (9 -11).

Kanmaz et al found a correlation between intestinal damage and pH of amnioallantoic fluid (AAF) in a chick gastroschisis model and they concluded that acidity of AAF is responsible for this damage (1).

Dilsiz et al showed that nitric oxide is an important mediator of the ID in gastroschisis and they concluded that the inhibition of NOS with L-NAME could prevent ID in gastroschisis (2). Tibboel et al have suggested that intestinal damage was the result of venous and lymphatic obstruction at the site of narrow abdominal defect. They showed that the normal human fetus defecates routinely during fetal life and the intestinal damage in gastroschisis is a result of prolonged contact with gastrointestinal waste products in amnio-allantoic fluid (10,12). Klück et al found a correlation between ID and exposure to amnioallantoic fluid (AAF) and concluded that urinary products are responsible for this damage (3). There was no intestinal damage when the protruded bowels were not related with allantoic fluid in their study.According to Langer et al intestinal dysfunction in gastroschisis must have been caused by both amniotic fluid exposure and chronic vascular compromise. They have suggested that amniotic fluid was responsible for intestinal adhesions, vascular compromise for intestinal damage, and both together for malabsorption and dysmotility (9,10).

In the present study drug delivery system was investigated first time to treat the ID in the chick gastroschisis model. The aim was to determine whether the ID in gastroschisis could be prevented by controlled release of methylene blue from a drug delivery system (HPMC – EC). Methylene blue (methylthionine chloride; MB) is a thiazine dye used in the treatment of methemoglobinemia (6,7). It may represent a new class of anti-oxidant drugs, which competitively inhibits the reduction of molecular oxygen to superoxide by acting as an alternative electron acceptor for tissue oxidases . It is a widely accepted pharmacological tool in the analysis of the nitric oxide – pathway.

MB is known to be an inhibitor of soluble guanylate cyclase and have shown that it is a direct inhibitor of NOS and other iron containing enzymes (5-7). Increased NOS-2 gene expression has been shown in the intestinal mucosa of patients with inflammatory bowel diseases (ulcerative colitis and Crohn's disease) and necrotizing enterocolitis (4). In the current study, because of NOS inhibition of MB, intestinal mucosa, submucosa, muscular, serosal thickening and ID were significantly decreased in GP-DI MB and GP-CDMB groups. These findings show us that NO is an important mediator of the ID in gastroschisis. However, the mortality rate was higher in the GP-DIMB group because of multiple interventions to the embryo. The survival rates were greater in the GP- CDMB (76.92%) group than the GP-DIMB (24.39%) group. The adverse effects of AF on the intestines may be preventable by altering the compositions of AF by either exchange or injecting appropriate solution into the amniotic cavity. This could be achieved by use of an amniotic cavity catheter or repeated amniocentesis, however, in these groups the mortality rate were high (1,2,11,13,14). Controlled drug release system provides localized delivery, lowers the risk of systemic side effects and prevents multiple intervention.

As a first preliminary study, we believe that drug delivery system could be a new simple technique and alternative for human fetuses with gastroschisis. We hope the use of drug delivery system of an appropriate drug for treatment, will prevent ID of gastroschisis totally in the future by eliminating the effect of amniotic cavity catheter and multiple amniocentesis.

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SURVEY ON THE LENGTH OF STAY FOR THE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE: AN APPLICATION ON ATATURK CHEST DISEASE HOSPITAL

Afsun Ezel Esatoğlu* ✣ Songül Bozat**

SUMMARY

This study has been made on the patients with Chronic Obstructive Pulmonary Disease (COPD) in Atatürk Chest Disease Hospital in order to put forth the factors prolonging the length of stay, decreasing the discharge time, preventing the nonessential staying and contributing to servicing more patients with the existing available conditions of the hospital. During the research, the files, service protocol books and outlet summaries of 113 patients staying in Non Tuberculosis (TB) clinics between 1-31 January 2001 were examined. It was researched that how the variables such as age, staying service, sex, domicile, staying status, educational level, number of COPD diagnosis had before, additional diseases, COPD age, complication and reasons for complication, effected the length of stay. According to the results of the research, there are meaningful relations between the average length of stay and educational level, number of COPD diagnosis, additional diseases, COPD age, existence of complication and reasons for complication. On the other hand, staying service, age of the patient, sex, domicile, staying status are not related meaningfully with the average length of stay statistically.

Key Words: *Chronic Obstructive Pulmonary Disease (COPD), Length Of Stay, Average Length Of Stay.*

ÖZET

Kronik Obstrüktif Akciğer Hastalıkları'nın Yatış Süreleri Yönünden İncelenmesi: Atatürk Göğüs Hastalıkları Hastanesi'nde Bir Uygulama

Tanımlayıcı nitelikte bir çalışma olan araştırma, Atatürk Göğüs Hastalıkları Hastanesi'nde yatan Kronik Obstrüktif Akciğer Hastalığı (KOAH) tanılı hastaların, yatış sürelerini uzatan faktörleri ortaya koyarak, hasta devir hızının artırılması, dolayısıyla varolan yatak sayısı ile daha fazla sayıda hastaya hizmet verilmesini olanaklı kılmak ve hasta bekleme sürelerini en aza indirerek hasta yataklarını daha verimli kullanıp gereksiz yatışları önlemeye katkıda bulunmak amacıyla gerçekleştirilmiştir. Araştırmada, Atatürk Göğüs Hastalıkları Hastanesi Non TB kliniklerinde 1-31 Ocak 2001 tarihleri arasında KOAH tanısı ile yatan 113 vakanın hasta dosyaları, servis protokol defterleri ve hasta çıkış özetleri incelenmiştir. Araştırmada hastalara ait yaş, yatılan servis, cinsiyet, ikamet yeri, yatış statüsü, eğitim durumu, KOAH tanısıyla hastaneye yatış sayısı, ek hastalık durumu, KOAH yaşı, komplikasyon ve komplikasyon nedenleri gibi değişkenlerin yatış sürelerini nasıl etkilediği incelenmiştir. Araştırma sonuçlarına göre öğrenim düzeyi, KOAH tanısıyla hastaneye yatış sayısı, ek hastalık bulunma durumu, KOAH yaşı, komplikasyon bulunma durumu ve komplikasyon nedenleri ile ortalama yatış süresi arasında istatiksel açıdan anlamlı ilişki bulunmuştur. Hastanın yattığı servis, yaş, cinsiyet, ikamet yeri, yatış statüsü ile yatış süresi arasında istatiksel açıdan anlamlı ilişki bulunmamıştır.

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Received: *Jan 08, 2003* Accepted: *Feb 03, 2003*

In the assessment of hospital performance, several measurements related to the hospital facilities have been employed. One of these is the length of hospital stay (LOS), that is the number of the days of a patient's stay in hospital to get treatment in a certain period (1, 2). The term average length of stay (ALOS) has been used to express hospital stay related assessments more meaningfully. ALOS is calculated by dividing the total number of the days of the discharged patients' stay (including the ones who died) by the number of the patients discharged (2, 3).

LOS is an indicator showing the quality and effectiveness of the medical care in hospital. When the type of illness is taken into consideration LOS can give us a clear idea about inappropriate or unnecessary hospitalisation; appropriate care and cost (4, 5).

LOS varies according to which social security institution the hospital belongs to and the countries. These differences are based on the differences in medical facilities, the procedure used to offer medical care, different ways of organising and medical care organisations. In U.S.A. shorter than 30 days stay is classified as short term, while longer than 30 days is called long term. It has been mentioned that in U.S.A ALOS stay in 7 days throughout the county(6). However the duration varies from hospital to hospital. Unnecessary hospitalisation, days and procedures have been maintained because of applied management programmes (7). In Turkey the study carried out by Çelik et al (8), mentioned that 49 out of 221 patient day is unnecessary. In state hospitals in Turkey LOS sloped backwards from 5.7 to 5.5 (9).

It is obvious that reducing unnecessary hospitalisation by controlling the variables of LOS has numerous beneficial aspects in terms of hospital management and national economy. As a result of decrease in the cost of LOS, the increase in the number of in patients and the increase in the circulation positively affect the efficiency of the hospital. There fore hospital sources have been used more effectively and efficiently (10, 11). As a result of reducing unnecessary hospital stay; wasting money, labour and time can be prevented. Instead of building new hospitals it can be said that the number of the patients to be hospitalised can be increased using available beds. Several studies has been made to determine the factors affecting variables of LOS and to control them. As Morgan and Beech mention (12), reducing LOS depends on several factors enabling available beds to be used more effectively. Some of these are increasing the quality of treatment, improving the service given to the patients as well as increasing the number and availability of operating rooms and using the beds more effectively. In the study by WHO the factors influencing ALOS have been mentioned as follows: rapid increase in population, prolonged life expectancy, excessive number of bed in the area, prolonged diagnosis, increase in the hospital acquired infections, inappropriate clinical services, prolonged decisions on admissions and discharges, staff lacking in services training, lack of outside care opportunities, doctors paid in accordance with the ALOS (13).

Mawajdeh et al (11), have stated four categories –patient, physician, hospital and source and type of payment- determine the LOS. Çelik et al (8), mention that age, sex, residence, institution at which the patient admitted and insurance status determine unnecessary stay. In the study on factors affecting diagnosis and treatment in acute rheumatic fever and controllable variableities of these procedures carried out by Turkish Armed Forces in different power forces it has been found that the physicians attitude and behaviour have significant influence on ALOS (14). The type, severity, structure of illnesses were also found to be significant predictors of ALOS in the study of Mawajdeh (11).

In different departments of the same hospital, different LOS for the same illness has been observed. This leads to an increase in the cost of the illness. Differences in the treatment of the same illness is considered an important factor in terms of foresight of the result management and hospital planning and it is claimed that this causes considerable differences in the effective use of hospital beds (10, 15). There fore establishing the LOS according to the illnesses is one of the subjects to be studied in hospital management (4, 5). That LOS should be analysed on the basis of physicians and diagnostic groups seems another approach on which authors agree (10, 11),

Length of Stay in Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a slow but progressive disease which is characterised by obstructive air flow due to chronic bronchitis and emphysema. It is one of the common respiratory diseases in the world; it has become a favourite research topic because of its relatively increasing morbidity and mortality and expenditure on health. In U.S.A among the causes of death COPD appears in the fifth place (16, 17).

There has been 58 % increase in the number of COPD patients between 1975-1994 in Turkey. When examined ALOS owing to COPD in the last 20 years, it was observed that the mean was 8.8. It has also been observed that ALS has had stability in years and has never showed any decrease (18). In Table 1, the ALOS of COPD patients in 1995-2000 according to years in ACDH and generalised in Turkey is given. LOS for ACDH in USA is 5.7 days (19). The difference between these figures should be studied considering the loss in both country-scale and hospital-scale.

Atatürk Chest Disease Hospital (ACDH) is one of the 24 specialised hospitals offering services of chest diseases, run by Ministry of Health. There are 5 more chest disease hospitals run by other institutions in Turkey. According to Ministry of Health Data (9), in ACDH, average length of stay is 22.0 days. In Table 1 the figures of Ministry of Health and ACDH do not correlate with each other, which is due to the problems of recording system. In table 1, ALOS shows slight fluctuation from 1995 to 2000 but it tends to decrease in general. It is emphasising that ALOS due to COPD is twice as long as ALOS throughout Turkey.

The aim of this study is to identify the factors affecting hospital LOS for the patients with COPD in ACDH. It is believed that is would provide managerial control in patient care and planning. The result of this study would also provide the health staff with the data to reduce and/or eliminate the factors affecting length of hospital stay for patients with COPD. Thus, hospital management would be able to determine estimated length of hospital stay due to certain illnesses and to plan bed need, manpower equipment expanses, laboratory and blood bank services.

Method

The coverage of the research consists of 114 patients who have been treated with the diagnosis of COPD (the disease which is listed with code A-93 in international diseases list with the title 150, and which coded 490-496 in the list number 999), in Non Tuberculosis B Clinics of Atatürk Chest Diseases Hospital (ACDH) from January 1 to January 30, 2001 and who were contacted by hospital registrations. In the research, exemplifying wasn't chosen and all of 114 patients were included in the research. Data of 113 patients were evaluated because one of the patients' file hasn't been found in archives. January 2001 has been chosen deliberately as the month of operation because of the increase in patients in winter months. Patient's files, hospital registrations, and summary of patients' discharge have been studied with a data collecting direction developed by the researchers with the retrospective record screening method.

Data were evaluated by using SPSS 9.05 package programme. In statistical analysis: correlation, the importance control of the difference between two averages (t test) and one sided variance analysis were used and meaningful differences between groups were explained by Least Significant Difference (LSD) test.

Table 1. ALOS of COPD patients according to years

Year	1995	1996	1997	1998	1999	2000
Türkiye ALOS (Day) *	7.7	7.9	7.7	7.6	7.3	7.2
ACDH ALOS (Day) **	19.1	18.0	18.9	18.3	18.3	17.9

Reference: * MOH, Disease Statistics , 1996b, 1997, 1998, 1999, 2000, 2001, **ACDH Annual Health Statistics.

Finding and Discussion

Table 2. Distribution of patients with COPD in ACDH according to their sociodemographic characteristics

Sociodemographic characteristics	N	(%)	Sociodemographic characteristics	N	(%)
Sex			**Residence**		
Male	80	70.8	From Ankara	64	56.6
Female	33	29.2	Out of Ankara	49	43.4
Age Groups			**Stay Statutes**		
30-39	3	2.7	Civil Servant	9	8.0
40-49	12	10.6	Private payment	2	1.8
50-59	21	18.6	SIO (SSK)*	17	15.0
60-69	52	46.0	SIAMASE (Bağkur)**	41	36.3
70-79	25	22.1	GERF (Emekli S.) ***	17	15.0
Educational Status			Green Card ****	18	15.9
Illiterate	33	29.2	Non Payment *****	9	8.0
Literate	10	8.8			
Primary School	34	30.1			
Junior High School	21	18.6			
High School and above	15	13.3			
Total	113	100.0	**Total**	113	100.0

* Social Insurance Organisation (SSK)
** Social Insurance Agency of Merchants Artisans and Self -Employed (Bağkur)
*** Government Employees Retirement Fund (Emekli Sandığı)
**** Green Card (The system that pays the treatment payments of patients who are not able to afford).
***** Non Payment (Social insurance that is given to people who need care at the age of 65 and above, with in the law 2022)

Distribution of sociodemographic status of the patients included in the research has been shown in Table 2. 70.8 % of patients were men, 46 % were between the age of 60-69, 30.1 % were primary school graduated, 56.6 % were living in Ankara, and 36.3 % were insured by Bağkur (The Social Insurance Agency of Merchants Artisans and Self-Employed)

Table 3. Distribution of patients with COPD in ACDH according to patients' characteristics

Patients' characteristics	N	(%)	Patients' characteristics	N	(%)
Clinic in Stay			**Additional Illness Situation**		
1 Non TB	43	38.1	Present	59	52.2
2 Non TB	45	39.8	Absent	54	47.8
3 Non TB	4	3.5	**Age of COPD**		
4 Non TB	8	7.1	1-11 month	13	11.5
5 Non TB	13	11.5	1-2 year	20	17.7
Number of Stay (Day)			3-4 year	23	20.4
1-7	13	11.5	5 year and above	57	50.4
8-15	41	36.3	**Complication**		
16-23	41	36.3	Present	48	42.5
24-31	11	9.7	Absent	65	57.5
32 and over	7	6.2	**Reason of Complication**		
Number of stay From COPD			Patient	35	72.9
1 time	51	45.1	Hospital	13	27.1
2 time	25	22.1			
3 time	23	20.4			
4 time and above	14	12.4			
Total	113	100.0	**Total**	113	100.0

Distribution of various characteristics of their disease of COPD patients included in the research in table 3. It has been established that 39.8 % of patients were those staying in Non TB Clinics, 36.3 % were those who were staying 8-15 days and 16-23 days, and 45.1 % were staying for the first time with diagnosis of COPD. In addition to this information, it has been found that 52.2% of patients had another contributing disease, 50.4 % had this disease over 5 years (COPD age). It has been also been found that 42.5 % of patients had complications, and 72.9 % of whom had the complication from patients, 27.1 % of whom had from the hospital. In the research knowledge about the existence of hospital infections evaluated as the course of complication have been gathered by studying the information in patient's files. The complications originated from the disease itself were systemic diseases developed with COPD.

Table 4. ALOS according to sex in patients of COPD in ACDH

Sex	N	ALOS (X)	Standard Deviation	Standard Error
Mail	80	17.6	9.16	1.02
Female	33	15.8	6.34	1.10

(t = 1.044; p>0.05)

When the distribution of ALOS of the patients according to their to their sex examined (Table 4), it has been observed that the ALOS of male patients (17.6), were higher than female patients (15.8). The difference in ALOS according to sex hasn't been found meaningful in the statistical aspect. In the studies carried by Varankesh (5), in patients with inguinal hernia in literature, by Özgen (20) in patients with diabetes, by Ersoy (21) in patients with appendicitis, by Şeref (22) in patients with ischemic hearth disease, by Mawajdeh and colleagues (11) in patients with appendectomy, bronchial asthma and caesarean sex of the patients haven't been found meaningful in the statistical aspect. However, there has been meaningful connection with sex and LOS in researches carried by Dinçer and colleagues in different groups of patients (10), by Çelik and colleagues (8) on inappropriate use of beds, and Dowd and colleagues (23) in researches dealing with LOS.

The relation between patient's age and ALOS has been examined by correlation analysis, and a negative relation has been found (r = –0.115). Although ALOS shows a decline trend in correlation with increasing age, the relation is not meaningful in statistical aspect (t = 0.224, p>0.05). The findings of research carried by Özgen (20), Farren (24), Toraman (13), Şeref (22), and Çelik and colleagues (8), are very similar to

Table 5. The ALOS of patients with COPD in ACDH according to their education level

Educational Level	N	ALOS (X)	Standard Deviation	Standard Error	ALOS at 95 % reliance limit		LSD				
					Down Level	High Level	1	2	3	4	5
Illiterate	33	18.7	7.58	1.32	16.0	21.4	1		*		*	*
Literate	10	31.0	11.45	3.62	22.8	39.1	2	*		*	*	*
Primary School	34	17.0	6.39	1.09	14.7	19.2	3		*		*	*
Junior High School	21	12.9	4.47	0.97	10.9	14.9	4	*	*	*		
High School and above	15	11.5	6.12	1.58	8.1	14.9	5	*	*	*		
Total	**113**	**17.2**	**8.52**	**0.80**	**15.6**	**18.8**						

(F=14.490; p< 0.001)

this research. However, it has been found that there is a positive relation between age and LOS (5, 10, 11, 25, 26,).

When ALOS according to the patients' education level studied, it has been found that the illiterates stayed 18.7 day, literate people stayed 31.0 days, primary school graduates 17.0 days, junior high school graduates were 12.9 days; and high school and above were 11.5 days (Table 5). As can be seen in the results of research ALOS tended to decline as the patients education level gradually went up. The difference between ALOS according to the patients' education level has been meaningful in the statistical aspect ($p<0.001$). In the advanced statistical analysis, the groups of illiterate people and primary school level and junior high school group were not different from another meaningfully from the statistical point, but the average of all other groups were meaningfully different from another in the statistical aspect.

Table 6. ALOS of COPD patients in ACDH according to their residence

Residence	N	ALOS (X)	Standard Deviation	Standard Error
From Ankara	64	17.7	9.21	1.15
Out of Ankara	69	16.2	7.34	1.04

($t = 0.963$; $p > 0.05$)

ALOS of patients according to their residence is shown in table 6. Patients living in Ankara stay 17.7 days, and who live anywhere out of Ankara stay 16.2 days. The difference wasn't meaningful statistically. The related research findings of Ersoy (21), Özgen (20), Varankesh (5), and Mawajdeh et al (11), have all been well-adjusted to this result of research. In some other researches, LOS of people living in the city has increased (8, 23).

When the patients' ALOS compared in accordance with their staying departments, it has been found that ALOS was the longest in 1 Non TB department (18.6), and the ALOS was the shortest in no 4 Non TB department (14.6) (Table 7). It has been established that the other ALOS in departments were 15.9 days in 2 Non TB department, 16.7 days in 3 Non Tb department, 17.6 days in 5 Non Tb department. In the variability analysis there hasn't been found any meaningful difference statistically between ALOS in accordance with departments ($p>0.05$).

Although there has been meaningful difference in LOS of different departments in Turkey (8, 10), it can be mentioned that the cause of in existence of difference between departments in aspect of ALOS in this research is that all departments are composed of Non TB departments. It can be considered that patients with different diseases stay in Non TB departments affect the results.

Table 7. ALOS of COPD patients in ACDH according to departments where they were hospitalised

Clinical Name	N	ALOS (X)	Standard Deviation	Standard Error	ALOS at 95 % reliance limit Down level	Upper level
1 Non TB	43	18.6	8.58	1.30	16.0	21.3
2 Non TB	45	15.9	7.54	1.12	13.6	18.2
3 Non TB	4	16.7	14.54	7.27	-6.4	39.9
4 Non TB	8	14.6	4.65	1.64	10.7	18.5
5 Non TB	13	17.6	10.77	2.98	11.1	24.1
Total	**113**	**17.2**	**8.52**	**0.80**	**15.6**	**18.8**

($F = 0.772$; $p>0.05$)

Table 8. ALOS of COPD patients in ACDH according to their staying status

Stay Status	N	ALOS (X)	Standard Deviation	Standard Error	ALOS at 95 % reliance limit Down level	Upper level
Civil Servant	9	19.5	12.12	4.04	10.2	28.9
Private payment	17	18.2	9.24	2.24	13.4	22.9
SIO (SSK)	41	16.0	7.14	1.11	13.7	18.2
SIAMASE (Bağkur)	17	18.6	9.49	2.30	13.7	23.5
GERF (Emekli S.)	18	16.3	7.80	1.83	12.4	20.1
Green Card	2	11.5	7.77	5.50	-58.4	81.4
Non Payment	9	19.5	9.50	3.16	12.2	26.8
Total	**113**	**17.2**	**8.52**	**0.80**	**15.6**	**18.8**

(F = 0.594 ; p>0.05)

In Table 8, ALOS is given in accordance with patients' payment status of hospital expenditure. It has been found that ALOS of patients staying as civil servants is 19.5 days, patients from Social Insurance Organisation (SSK), 18.3 days, from Social Insurance Agency of Merchants Artisans and Self -Employed (Bağkur) 16.0, members of Government Employees Retirement Fund (Emekli Sandığı) 17.3, those who has Green Card 16.3, those who pay expenditure themselves 11.5, those staying with free status 19.5. As shown in Table 8, ALOS of patients who pay their expenditure themselves is shorter than staying status of other patients. Nevertheless, ALOS of patients staying as civil servants and those who are staying with free status has been found longer, but there hasn't been found a meaningful difference statistically in the variability analysis (p> 0.05).

In the researches carried by Ersoy (21), Özgen (20), and Varankesh (5) in Turkey there hasn't been a meaningful relation between the patient's staying status and LOS, as hasn't been found this research. Çelik et al, (8), have found that there has been a meaningful relation between staying status and LOS and the inappropriate staying rate of patients who has insurance is longer than the others. In other researches carried on this subject, it has been stated that LOS of patients with health insurance is longer than those who don't have any insurance (4, 11, 27).

Table 9: ALOS of COPD Patients who were hospitalised with the diagnosis of COPD in ACDH according to their number

Number of stay with COPD diagnosis	N	ALOS (X)	Standard Deviation	Standard Error	ALOS at 95 % reliance limit		LSD			
					Down Level	High Level	1	2	3	4
1 time	51	15.1	8.69	1.21	12.6	17.5	1			*
2 time	25	16.7	6.12	1.22	14.2	19.2	2			*
3 time	23	18.7	7.78	1.62	15.4	22.1	3			
4 time and above	14	23.4	9.99	2.67	17.6	29.2	4	*	*	
Total	**113**	**17.2**	**8.52**	**0.80**	**15.6**	**18.8**				

(F= 4.082; p <0.01)

In table 9, we examined the relation between ALOS and staying frequency of patients with COPD diagnosis. ALOS of patients whose staying frequency is only one stay 15.1 days, those who were hospitalised, twice stayed 16.7 days, 3 times stayed 18.7, 4 times and above 23.4 days. ALOS of patients who were hospitalised with COPD diagnosis for the first time is shorter. The ALOS that is in relation with staying frequency of patients with COPD diagnosis is also meaningfully different in the statistical aspect ($p<0.01$). The difference between ALOS of patients who were hospitalised 4 times and above with COLD diagnosis and those who were hospitalised once and twice has statistically been found meaningful, the difference in relation with each other in other groups has been found meaningless.

The distribution of patients' ALOS is shown in Table 10 in accordance with coexistence of additional diseases together with COPD. ALOS of patients with additional diseases is 19.1 days, ALOS of those who don't have any additional disease is 14.8 days. It has also been statistically found meaningful that ALOS of patients with an additional disease is longer.

Table 10. ALOS of patients with COPD in ACDH according to coexistence of additional disease.

Additional Disease	N	ALOS (X)	Standard Deviation	Standard Error
Present	59	19.1	8.11	1.05
Absent	54	14.8	8.33	1.13

($t = 2.734$; $p<0.01$)

Distribution of ALOS of patients in accordance with COPD age is shown in Table 11. We have established that ALOS of patients whose COPD age is between 1 and 11 months is 9.9, those whose COPD age is 1-2 years is 15.3, 3-4 years is 19.6, 5 years and above is 18.6 days ($p<0.001$). According to advanced statistical analysis, we have discovered that the difference originated from patients whose COPD age is between 1 and 11 months.

Status of the disease, its severity and age are important factors that affect the patients' LOS and support the result of the research (11, 28, 29). Özgen (20) has mentioned that there isn't any meaningful relation between their diabetes age and LOS.

Table 12. ALOS of COPD patients in ACDH according to existing complications

Complication	N	ALOS (X)	Standard Deviation	Standard Error
Present	48	20.1	8.16	1.17
Absent	65	14.9	8.03	0.99

($t = 3.369$; $p<0.01$)

In Table 12, ALOS has been studied in accordance with existing complication in patients with COPD. Of the patients who were included in the research, it has been established that ALOS of those with complication is 20.1 days, those without complication is 14.9 days. ALOS of patients with complication is longer than the others. There

Table 11. ALOS of COPD patients in ACDH according to their COPD age

Number of stay with COPD diagnosis	N	ALOS (X)	Standard Deviation	Standard Error	ALOS at 95 % reliance limit		LSD			
					Down Level	High Level	1	2	3	4
1-11 month	13	9.9	5.58	1.55	6.5	13.3	1		*	*
1-2 year	20	15.3	4.77	1.06	13.1	17.5	2			
3-4 year	23	19.6	9.28	1.93	15.6	23.7	3 *			
5 year and over	57	18.6	8.88	1.17	16.2	20.9	4 *			
Total	**113**	**17.2**	**8.52**	**0.80**	**15.6**	**18.8**				

($F = 5.184$; $p < 0.01$)

has been a statistically meaningful difference in ALOS of COPD patients in accordance with coexisting complications ($p<0.001$). Also, it has been established that there was meaningful difference between complications and ALOS.

Suggestions

1. An effective infection protection chain should be set to provide a sterilised environment during the patients stay at hospitals, since hospital originated infection is an important factor affecting ALOS. This kind of infection protection chain can be provided and checked by means of such committee.
2. An inspecting committee should be formed to check the quality and convenience of the treatment give of the patients and the rate of unnecessary and/or under hospitalisation.
3. The length of the in patients hospital stay related to the type of the disease should be standardises throughout the country. Therefore the length of could be taken under control and bed capacity, which is on important income of a hospital, could be used at more effectively and efficiently. In addition, such standardisation would make the bed planning based on scientific principles possible.
4. Standard methods of treatment based on the type of the disease should be developed to make the length of hospital stay controllable and to prevent unnecessary patient care.
5. In order to assess convenience of services the patients who are insured by the government use, it can be mentioned that forming an inspecting mechanism of usage of services at the insurance programmes would decrease the unnecessary staying period of such patients.
6. The length of hospital stay of the patients with another contributing disease is longer-Hence, before the patient is hospitalised, these contributing diseases should be examined and required treatment should be determined and taken under control.
7. In this study physicians are not taken in to consideration as on effecting factor since patients with COPD are not followed by the same physician from their admission to their discharge . Different physician's attitude is considered to be on influence on the length of hospital stay of the patients. Physicians should be trained on the importance of using the hospital sources effectively and efficiently.
8. According to A list of Turkish Health Statistics and Annual Hospitalised Care Institutions, the ALOS of the patients in all hospitals throughout Turkey can be calculated considering their diagnosis. However average length of stay ca not be obtained according to the level where the hospital stay, which results in not being able to make comparison among hospitals. It is recommended that a data base should be provided to make comparisons among hospitals.
9. Hospital management should examine the factors affecting the length of hospital stay and take measurements. This is only possible with the staff qualified with scientific health institution management skills and techniques. Each management post should be occupied by the ones who have such management skills and training.
10. No or inefficient system of data to provide objective researches on the length hospital stay is a curical problem which influences the kind and the, applicability of the studies. Hospital management should develop a reliable, accurate and current hospital information system to evaluate and inspect the length of hospital stay.

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JOURNAL OF ANKARA MEDICAL SCHOOL Vol 24, No 4, 2002 177-182

LATERAL ORBITAL APPROACH TO INTRAORBITAL LESIONS

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SUMMARY

In this study, we present operative results of lateral orbital approach of eight patients with intraorbital tumors such as cavernous hemangioma, schwannoma, epidermoid and other tumors. Lesions were removed completely in all patients with this route. Operative complications were seen in three patients including transient impairment of eye movements in two patients and visual impairment with atonic pupil in one patient. Lateral approach to the orbit is less invasive technique than transcranial approach and can be safely used for tumors that located in the inferior, superior, and especially lateral compartment of the orbit.

Key Words: *Lateral, Orbit, Surgery, Tumor.*

ÖZET

Intraorbital Lezyonlara Lateral Orbital Yaklaşım

Bu çalışmada, kavernoz hemanjiom, Schwannoma, epidermoid tümör gibi intraorbital tümörü olan, lateral yaklaşım ile tedavi edilmiş sekiz hastanın operasyon sonuçları sunulmuştur. Hastaların tümünde lezyonlar bu yolla total çıkarılmıştır. Üç hastada operasyon komplikasyonları görülmüş olup; iki hastada göz hareketlerinde geçici kısıtlılık, bir hastada görme keskinliğinde azalma ve atonik pupil olmuştur. Orbitaya lateral yaklaşım, transkranial yaklaşıma göre daha az invaziv bir tekniktir ve orbitanın süperior, inferior ve de özellikle lateral kompartmanında yerleşmiş tümörlerin çıkarılması için güvenli bir şekilde kullanılabilir.

Anahtar Kelimeler: *Lateral, Orbita, Cerrahi, Tümör.*

Orbital lesions may arise from the contents of the orbit, from surrounding structures, or from a metastatic source. Various surgical approaches have been used for exposure of these lesions (1-4). By the development of modern neuroradiological techniques, it is possible to determine the exact location and extend of the intraorbital lesion, and its relationship with blood vessels, nerves and muscles in this region (5). Beside of these neuroradiologic advances, surgical approaches have also been modified and have become less invasive by the developments in microsugical techniques.

The lateral approach to the orbit first proposed in 1889 by Krönlein (6) and modified by Berke in 1953 (7), and by Maroon and Kennerdell in 1976 (8). This approach has been selected for tumors that located in the lateral, superior or inferior compartment of the orbit (1, 8, 9). This approach has also been used for orbital decompression in patients with Graves' ophthalmopathy (10).

In this study, we present our surgical experience in eigth patients with intraorbital lesions that treated with lateral orbital approach.

Patients and Methods

Between 1997-2002, eigth cases with intraorbital lesions were treated surgically with lateral orbital approach in our institution. There were 4 male (50%) and 4 female (50%), ranging in age from 4 to 63 years, with a mean of 27.1 years. Characteristics of patients are shown in Table 1. Plain X-ray films, computed tomography (CT) and/or magnetic resonance imaging (MRI)

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Received: *Nov 07, 2002* Accepted: *Feb 28, 2003*

TABLE 1. Characteristics of eigth patients

Patient No/ Sex/Age (years)	Symptoms/ Duration (months)	Location of tumor (Compartment)	Type of tumor	Complication	Follow-up (months)	Prognosis
1 / F / 35	Proptosis 12 mo.	Superior	Cavernous Hemangioma	TIEM	36	Good
2 / M / 8	Proptosis, pain 2 mo.	Lateral	Epidermoid cyst	-	48	Good
3 / M / 49	Proptosis, visual impairment, pain 9 mo.	Superior, lateral, inferior, posterior	Schwannoma	TP, VI	38	Good
4 / M / 41	Proptosis, visual impairment 6 mo.	Lateral, superiror	Schwannoma	-	41	Good
5 / F / 63	Proptosis, pain 6 mo.	Superior, lateral	B-cell lymphoma	-	2	Good
6 / F / 12	Proptosis, visual impairment 3 mo.	Lateral, inferior	Epidermoid	TIEM	13	Good
7 / F / 5	Proptosis 4 mo.	Lateral, superior	Hemangioma	-	16	Good
8 / M / 4	Proptosis 1 mo.	Lateral	Ectopic lacrimal gland	-	14	Good

TIEM; Transient impairment of eye movement, TP; Tonic pupil, VI; visual impairment.

were used for diagnosis and surgical planing. Ultrasonography was also used in some cases, especially for differential diagnosis.

Operative technique

Under general anesthesia, S-shaped skin incision was used and curved up to the brow and then posteriorly along the upper margin of the zygomatic arch for approximately 35 to 40 mm from the lateral canthus to avoid to damage the frontal branches of the facial nerve (8). After skin incision, the fascia of the temporal muscle was incised along the skin incision, the temporal muscle was dissected subperiostally and retracted posteriorly to expose the lateral orbital bone. Midas Rex pneumatic tool (Midas Rex Inc, Texas, USA) was used to open a bone window. The periorbita was dissected from the inner surface of the lateral wall of the orbit. After the bone removal was completed, an insicion was made in the periorbital fascia parallel to the lateral rectus muscle. The orbital-self retaining retractor was used for superior and inferior retraction of the orbital fat. Lateral rectus muscle was dissected from surrounding structures and retracted superiorly or inferiorly with silk sutures. Two main microsurgical routes including above and below the lateral rectus muscle were used to visualize the tumor. The neural and vascular structures in the lateral compartment of the orbit were carefully dissected and preserved. After tumor resection was completed, hemostasis was established and the periorbita was reapproximated. The subcutenous tissue and skin were closed with Vicryl (Ethicon Ltd., UK).

Results

The lesions of the orbit were completely removed in all cases. Operative complications were seen in three patients including transient impairment of eye movement in two cases and worsened visual impairment with atonic pupil in another case. In our series, the mean follow-up period was 26 months and recurrence was not seen. One patient with malign B-cell lymphoma (Fig. 1) was referred to the medical oncology and radiation threapy unit to further investigation and treatment.

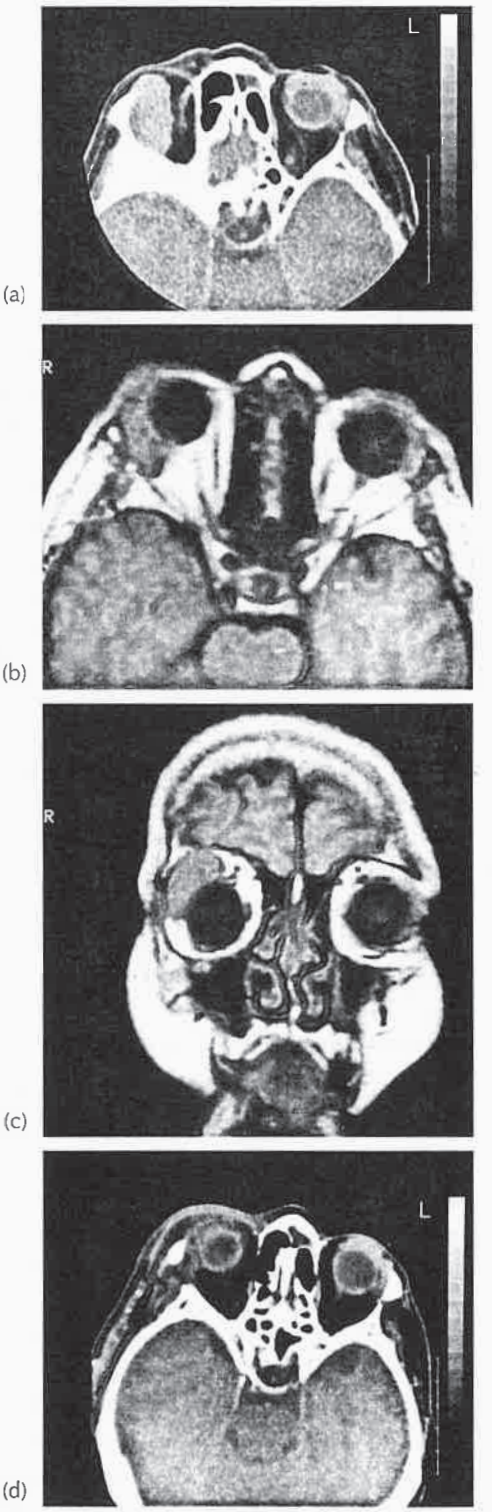

Figure 1. A 63-year-old woman with malign B-cell lymphoma.
a: contrast-enhanced axial computed tomography
b: contrast-enhanced axial magnetic resonance imaging
c: contrast-enhanced coronal magnetic resonance imaging
d: postoperative axial computed tomography.

Discussion

It is crucial that selection of proper approach to intraorbital lesions depends on various criteria including the location of the tumor, the size of the lesion, the vascularity and the ultrasonic characteristics of the tumor and the probable pathology anticipated (1). Modern diagnostic methods including angiography, computed tomography and magnetic resonance imaging have been used to define these criteria. Transcranial approach to the intraorbital lesions generally selected for lesions with intracranial extension, for tumors located in the orbital apex and deep medial orbital compartment and intracranial tumors with extension into the orbit (1). Although transcranial approach provides better exposure to the orbital cavity for superiorly and medially located tumors, it is far more invasive than lateral orbital approach. Intraorbital tumors except located in the middle compartment of orbital fossa or tumors that extend into the optic canal can be safely removed through lateral orbital approach. The optic canal opening is difficult with this approach so this approach is contraindicated for resection of optic nerve gliomas or for tumors that extends to the superior orbital fissure or optic canal (1, 4). In our series, we provided enough exposure to remove the lesions completely without major complication in all cases via lateral orbital approach. Illustrative cases are shown in Figure 1 and Figure 2.

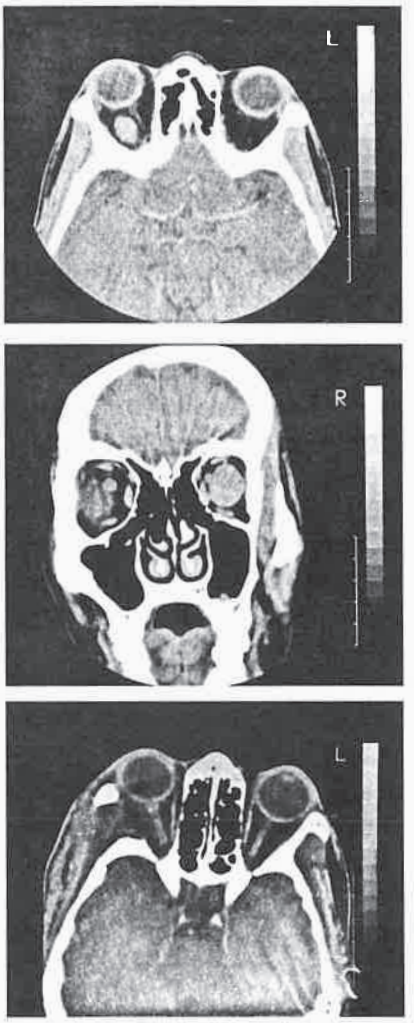

Figure 2. A 35-year-old woman with cavernous hemangioma.
a: contrast-enhanced axial computed tomography
b: contrast-enhanced coronal computed tomography
c: postoperative axial computed tomography

The extend of the bone removal was 2 mm beyond spheno-zygomatic suture posteriorly, 5 mm beyond fronto-zygomatic suture superiorly, zygomatic arc inferiorly and inferior orbital fissure medially (Figure 3). Contrary to description by Arai et al., lateral orbital rim was preserved and we had no difficulties to remove anteriorly placed tumours (4). Wirtschafter et al. also described preservation of the lateral rim in lateral orbitotomy (10). We also did not need to remove the sphenoid bone and expose anterior temporal dura because none of our lesions was placed within the muscle cone.

Visual impairment is one of the most serious postoperative complication and may due to traction on the optic nerve or sacrifice its vascular supply (4). In our series, visual impairment worsened with a tonic pupil in one patient who had a schwannoma that was located on the superior, lateral, posterior and inferior compartment of the orbit. It was possible that either the ciliary ganglion or short ciliary nerve was damaged during the resection of tumor. It was also possible that visual impairment was due to traction of the optic nerve during tumor

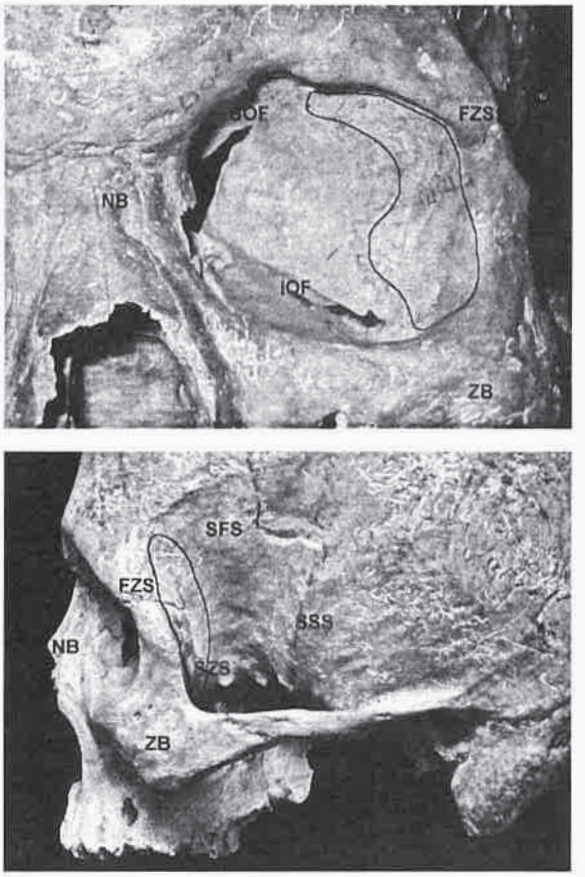

Figure 3: Bony speciments from a dry skull.
Abbreviations: SOF: superior orbital fissure, NB: nasal bone, FZS: fronto-zygomatic suture, IOF: inferior orbital fissure, ZB: zygomatic bone, SFS: spheno-frontal suture, SSS: spheno-squamous suture, SZS: spheno-zygomatic sture.
3a: Figure demonstrates the left orbita from anterior view. Drawing indicate the area that can be reached by lateral approach to the orbit.
3b: Figure demonstrates the left orbita from lateral view. Drawing indicates the area that is removed by lateral approach to the orbit after skin incision and temporalis muscle dissection.

removal. Special attention should be taken to preserve these structures.

We were dissected lateral rectus muscle from surrounding structures and retracted superior or inferiorly with silk sutures during surgery. In our series, transient impairment of eye movement and lateral gaze palsy were seen in two patients postoperatively and improved within two month follow-up period. It was thought that transient impairment of eye movement was due to retraction of lateral rectus muscle. Although retraction of lateral rectus muscle is required to expose the intraconal compartment, it should be keep on mind that traumatic retraction of the lateral rectus muscle may results in permanent lateral gaze palsy (4).

Enophthalmos as a result of removal of the lateral wall of the orbit was not seen in our series. We also avoided to damage the frontal branches of the facial nerve during the skin incision with the skin incision described above. Therefore, the operative results were also cosmetically satisfactory in our series.

As summary, the lateral orbital approach is less invasive than transcranial approach to the orbit and can be safely applied to intraorbital lesions which locate in the lateral, superior, and inferior compartments of the orbit.

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DETECTION RATE OF URETER STONES WITH US: RELATIONSHIP WITH GRADE OF HYDRONEPHROSIS

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SUMMARY

The aim of this study was to investigate the relation of hydronephrosis grade and the detection rate of ureteral stones with ultrasonography and call attention to the value of a careful sonographic study of these patients. Sonographic reports of 184 ureteral stones that has been followed in our clinic during 2000-2001 is evaluated. The detection rate of ureteral stones with US was 65. 9 %, 78 %and 95 % for grade1, 2 and 3 hydronephrosis respectively. US, as a noninvasive modality, is useful for detection of ureteral stones, with high detection rates in patients with grade 2 and detection rates comparable to spiral CT in grade 3 hydronephrosis.

Key Words: Hydronephrosis, Ultrasound, Ureteral Stone

ÖZET

Ultrasonografinin Üreter Taşlarını Saptama Oranı: Hidronefroz Düzeyiyle İlişkisi

Ultrasonografi (US) ile üreter taşlarının saptanmasında düşük tanısal doğruluk oranları bildirildiğinden, üreter taşı şüphesi olan hastalar diğer görüntüleme yöntemlerine yönlendirilmektedir. Bu çalışmanın amacı, üreter taşlarının US ile saptanma oranlarının hidronefroz düzeyiyle ilişkisini inceleyerek, bu grup hastalarda dikkatli bir sonografik incelemenin değerine dikkat çekmektir. 2000- 2001 yılları süresince kliniğimizde tedavi ve takibi yapılan 184 üreter taşının US bulguları değerlendirmeye alınmıştır. US' nin üreter taşlarını saptama oranı grade 1 düzeyinde hidronefrozda %65.9, grade 2 düzeyinde %78, grade 3 düzeyinde ise %95 dir. US, üreter taşlarını saptama oranları, grade 2 hidronefrotik böbreklerde yüksek; grade 3 hidronefrotik böbrekli olgularda ise spiral BT'ye karşılaştırılabilir düzeyde olan, noninvaziv ve kullanışlı bir yöntemdir.

Anahtar Kelimeler: Hidronefroz, Ultrasonografi, Üreter Taşı

Patients with renal colic are traditionally evaluated with intravenous urography (IVU) (1). In recent years, due to the reported high sensitivity and specificity, spiral computerized tomography (CT) is accepted as the best diagnostic modality for ureteral stones (1, 2). However, ultrasonography (US) is still used as a noninvasive and universally available modality (2). But, as US is considered to be of limited value in demonstrating ureteral pathology, patients with a suspect of ureteral stone are generally directed to other imaging modalities instead of US (3-5). In many articles it has been reported that US has nearly 90% sensitivity in obstructed collecting systems (3, 6, 7). We aimed to determine the correlation between the hydronephrosis grade and the success rate of US for detecting ureteral stones, in order to determine the effect of hydronephrosis on stone detection rate.

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** *Presented At; Uroradiology Symposium, April 2002, Çeşme- Turkey*

Received: *July 17, 2002* Accepted: *March 21, 2003*

Materials and Methods

172 patients (58 women, 114 men, age; 18-85 years) with 184 ureter stones have been evaluated in our department during a two year period. The ultrasonographic findings of all patients are noted in which the ureteral stone diagnosis is confirmed by IVU, spiral CT or intraoperatively. As this is a retrospective study, only confirmed ureteral stones are included, so there are no false (+) and true (-) groups and only the detection rates are calculated.

US examinations have been performed by the same radiologist with a SSA 250A Toshiba system using a 3.5 MHz abdominal probe.

The grade of hydronephrosis is classified as follows; mild pelvicalyceal system (PCS) dilatation: grade 1, moderate PCS dilatation without parenchymal loss: grade 2, severe PCS dilatation with parenchymal thinning: grade 3 hydronephrosis (8).

The localizations of ureteral stones are classifed as; proximal ureter if between the ureteropelvic (UP) junction and iliac cross, iliac cross localized, distal ureter if between the iliac cross and ureterovesical (UV) junction and UV junction localized.

The cases haven't been classified up to acute or chronic obstruction, only hydronephrosis grade in the presence of an ureteral stone is evaluated.

Results

US detected 136 of 184 (73.9 %) ureteral stones. In the grade 1 hydronephrotic group; US detected 60 of 91 (65.9 %) ureteral stones. The stones which could not be detected with US were localized at; 51.6 % (n: 16) distal ureter, 25.8 % (n: 8) proximal ureter, 19.3 % (n: 6) iliac cross and 3.3 % (n: 1) UV junction.

In the grade 2 hydronephrotic group; US detected 57 of 73 (78 %) ureteral stones. The stones which could not be detected with US were localized at; 56.25 % (n: 9) distal ureter, 25 % (n: 4) proximal ureter, 18.75 % (n: 3) iliac cross.

In the grade 3 hydronephrotic group; US detected 19 of 20 (95 %) ureteral stones. The stone which could not be detected with US was localized at distal ureter.

The detection rates of US does not show statistically significant difference between grade 1-2 and grade 2-3 hydronephrosis ($p > 0.05$), but there is statistically significant difference between grade 1 and 3 hydronephrotic groups ($p < 0.05$).

Of the 48 stones that could not be detected by US; 54.17 % (n: 26) was located at distal ureter, 25 % (n: 12) proximal ureter , 18.75 % (n: 9) iliac cross and 2.08 % (n: 1) UV junction.

Discussion

Until recent years the preferred diagnostic modality for the patients with suspect of ureteral stone was IVU (1). But, as intravenous contrast medium is used and patients are exposed to radiation, search for new diagnostic modalities has continued and spiral CT with its nearly 100 % sensitivity and ability to also detect extraureteral pathologies, has been accepted as the gold standart modality (1,2, 9). Spiral CT is less invasive than IVU as it doesn't require contrast medium, however patients are exposed to more ionizing radiation than IVU, during spiral CT (10).

As US is radiation free and doesn't require contrast medium, it is the modality of choice for the initial evaluation, especially for children and pregnant women (1, 11). Furthermore, it is inexpensive, universally available, has acceptable sensitivity and specificity and is not effected by the renal functions (2, 3, 5).

Sensitivity of US for ureteric stones has been reported to be 37- 64 % in different articles, but it has also been reported that these rates rises to 74- 95 % in obstructed collecting systems (1, 4, 5, 6, 7). Sommer et al reported that they had high success rates for detecting ureteral stones by US when there is minimal hydronephrosis and that the false (-) rates are higher if there is no hydronephrosis (12). So one can mention that as the grade of hydronephrosis rises, the detection rate of ureteral stones with US also rises. In our study, 136 of 184 ureteral stones are detected by US, giving a detection rate of 73.9 %. When we

evaluate the cases in our study up to the grade of hydronephrosis, it is seen that the detection rate of US rises from 65.9 % in grade 1 to 78 % in grade 2 and 95 % in grade 3 hydronephrosis. The detection rate of US between grade 1 and 3 hydonephrotic groups shows a statistically significant difference ($p< 0.05$). The 95 % detection rate in grade 3 hydronephrosis is close to spiral CT but 78 % in grade 2 hydronephrosis is clearly less (1). Spiral CT has reported to have 91- 95 % sensitivity and 95-100 % specificity for detecting uretral stones in different articles (1, 2, 13). Yılmaz et al reported that CT was found to be the best modality for depicting ureteral stones with an accuracy of 95 %, while IVU had 66 % and US 45 % accuracy values (13). But Patlas et al found US and spiral CT equally sensitive in detection of ureteral calculi with 93 % and 91 % sensitivity respectively (2). In one study, IVU could not depict 58 % of calculi that were depicted by spiral CT (14). Several investigators reported US as a good alternative to IVU with sensitivities of 95-100 % for the detection of urinary tract obstruction, however other studies suggested less US sensitivities of 74- 85 % for ureteral stone detection (3, 4, 6, 7). In Sheafor's study, the sensitivity of US for direct depiction of ureteral stones was significantly lower than that of CT (61 %- 96 % repectively) (1). But none of these studies had classified the value of imaging methods up to the grade of hydronephrosis. Thus the reported low values of US for detecting ureteral stones may be related to nonobstructed or low grade hydronephrotic kidneys. Our results also suggest that the detection rates rises from 65.9 % to 95 % with the increase of hydronephrosis grade.

It is reported that, acute obstructions may not cause dilatation of collecting system and there is no clear correlation between hydronephrosis grade and ureteric dilatation (9, 15). In our study, we focused just on the grade of hydronephrosis and didn't evaluate the ureteral dilatation and as the cases which are hydronephrotic but without ureteral dilatation are included in our study group, our detection rates decreases, but even so, this comparable detection rate with spiral CT is achieved in grade 3 hydronephrosis.

Saita et al determined the success rates of US according to the localization of the stone and they reported success rates of 82.2 % in the proximal and 68 % in the distal ureter (16). In our study, the rate of ureteral stones that couldn't be detected by US was 54.17 % in distal ureter, 25 % in proximal ureter, 18.75 % in iliac cross and 2.08 % in UV junction. Our results are in agreement with Saita and we have determined that the stones located in the distal ureter (between the iliac cross and the UV junction) are the most difficult to visualize by US because of intestinal artefacts. The detection rate of US for this location is 55.5 % in grade 1, 70 % in grade 2 and 75 % in grade 3 hydronephrosis in our study.

In summary, stones located in the distal ureter, or in the ureters of grade 1 or 2 hydronephrotic kidneys are more difficult to detect with US, but US has high sucess rates comparable to spiral CT for detecting ureteral stones in grade 3 hydronephrosis. US, as a noninvasive modality, must be the first imaging choice for suspect of ureteral stones and when hydronephrosis is seen, a careful US examination must be done to evaluate the ureters before directing patient to spiral CT. Spiral CT can be reserved only for cases which US fails to provide information.

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JOURNAL OF ANKARA MEDICAL SCHOOL Vol 24, No 4, 2002 187-196

CRYOPRESERVATION: BASIC KNOWLEDGE AND BIOPHYSICAL EFFECTS

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SUMMARY

It had been a great dream of humankind to be able to pause the time or stay young forever. Not the whole body yet; but some cells and even tissues can be conserved by the help of cryotechnology now. In practise, mostly, embryos are being frozen for the situations occur in assisted reproductive techniques, by means of slow cooling or vitrification. In this review the biophysical effects, that take place on cells during cryopreservation are discussed. Especially, the importance of cooling rates and the fate of water content of a frozen cell are emphasized. A historical backround as well as thawing methods and cryoprotectant solutions are also mentioned.

Key Words: *Cell, Cryopreservation, Physics, Vitrification*

ÖZET

Soğukta Koruma: Temel Bilgiler ve Biyofiziksel Etkiler

Zamanı durdurmak ya da sonsuza kadar genç kalmak eskiden beri insanlığın büyük bir hayali olmuştur. Bugün, kriyoprezervasyon teknolojisi ile henüz tüm vücut olmasa da, hücreler hatta dokular korunabilmektedir. Günümüzde en sık olarak uygulanan, yardımcı üreme tekniklerinde gametlerin, gonadların ve özellikle de embriyonların yavaş soğutma ve vitrifikasyon kullanılarak dondurulmasıdır. Bu derlemede soğukta koruma uygulanan hücrelerin maruz kaldığı biyofiziksel etkiler ele alındı. Özellikle soğuma hızlarının önemi ve donmuş hücrelerdeki su içeriğinin akıbeti üzerinde duruldu. Tarihsel zemin ve çözme metodlarının yanı sıra soğukta koruyan çözeltiler de özetlendi.

Anahtar Kelimeler: *Fizik, Hücre, Kriyoprezervasyon, Vitrifikasyon*

From the moment of fertilization onwards, life processes run by the rhythm of a biological clock. The technology of cryopreservation gives us the possibility of interfering with the clockwork by stopping biological time. Cryopreservation of a cell therefore involves the cooling of a cell and storage at a temperature where all metabolic processes are arrested. In practice, frozen cells are stored at the temperature of -196°C in liquid nitrogen. The freezing of single cells is considered cryobiologically the simplest, since only the physicochemical characteristics of one specific type of cell must be taken into account in predictions of the response to freezing.

Paradoxically, sperm cells and oocytes are single cells but seem rather sensitive to cryopreservation stress. This may be related to their highly specialized structure and function of reconstituting an entire organism from the fusion of two single cells. Today, the most common procedures are cryopreservation of the embryos, spermatozoa and some somatic cells and tissues.

Cryopreservation methods can be described under a few titles:

Cryopreservation at -196°C

The viability of biological material stored at -196°C (liquid nitrogen temperature) is essentially independent of the period of storage. The oldest sample available, bovine spermatozoa that has been stored for over 50 years, shows no reduction in viability. The stresses associated

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Received: *March 10, 2003* Accepted: *March 25, 2003*

with cryopreservation are not mutagenic: millions of cattle have been produced from frozen sperm and the incidence of abnormalities is identical to that observed with non-frozen sperm.

Frozen storage at temperatures above -196°C

Storing biological material in a conventional freezer is more convenient than using liquid nitrogen and a number of freezers are available which maintain temperatures down to -130°C.

However, unless biological material is stored at temperatures below -135°C the viability decreases during long-term storage. Whilst, this may be appropriate with microbial suspensions or with mammalian tissue cultures where large numbers of cells are frozen and some loss of viability may not cause practical problems, storage at these temperatures would not be acceptable with embryos, oocytes etc.

Freeze drying

It would undoubtedly be very convenient if mammalian cells could be freeze dried to retain viability because all the costs and difficulties associated with frozen storage and the maintenance of cold chains would be avoided. Recently it has been demonstrated that freeze dried mouse sperm, when injected directly into mouse ova could support normal development. However, the evidence from yeasts and bacteria is that the freeze drying process is highly mutagenic and that viability would be expected to decrease with storage time: these two factors would preclude the use of freeze dried spermatozoa in IVF.

Vitrification

An alternative approach to cryopreservation is vitrification. Conventional methods of cryopreservation have been developed to accomodate the consequences of ice formation.

Vitrification is a process which, by combining the use of concentrated solutions with rapid cooling, avoids the formation of ice. Samples reach low temperatures in a glassy state, which has the molecular structure of a viscous liquid and is not crystalline. This method has the potential advantages of being rapid to carry out and does not require controlled rate cooling apparatus. Good survival of mammalian embryos has been demonstrated by vitrification in a number of laboratories, however this approach is still experimental and the high levels of additives that are necessary to achieve vitrification are potentially cytotoxic. A further practical problem arises from the tendency of vitrified solutions to devitrify, i.e. crystallise into ice, during storage or thawing leading to loss of viability. (1)

Historical Background of Cryopreservation

More than two centuries ago, in 1776, Spallanzani was the first to report the maintenance of motility of human spermatozoa after exposure to low temperatures.

Mantagazza in 1866 suggested sperm banks for frozen human sperm. In 1949 Polge and colleagues discovered the effectiveness of glycerol as a cryoprotective agent for fowl spermatozoa. Freezing of human spermatozoa was reported shortly afterwards by Sherman (1953), observing that human spermatozoa, after freezing on dry ice, were able when thawed to fertilize and to produce normal embryonic development and offspring. The first birth after freezing human spermatozoa with glycerol in liquid nitrogen vapor was described by the same group.

The development of human sperm cryobanks in the 1970s gave rise to a growing need for standardization. Especially in the United States commercial and university sperm banks were established to permit later fertilization after vasectomy. The first association of human sperm banks was set up in France, the Center d'Etude et de Conservation du Sperme, in 1973, followed by the creation of the American Association of Tissue Banks in 1976, which also covered cryopreservation of gametes. The growing use of cryopreserved human semen led to the First International Meeting on Human Semen Cryopreservation, held in Paris in 1978.

The pioneers of oocyte cryoconservation set out their goals almost 50 years ago. It is therefore

surprising that now, in the turn of the twenty-first century, there is still no successful method for the cryoconservation of the human oocyte. In 1957 Lin, Sherman, and Willet demonstrated that the mouse oocyte can survive cooling to -5°C in a 5% glycerol-containing medium. In 1958 Sherman and Lin reported the birth of live young following in vitro fertilization (IVF) of mouse oocytes that had been "frozen" at -10°C in a 5% glycerol-containing medium. However, no oocytes survived exposure to a temperature of -20°C. The question with regard to these experiments is whether the oocytes were really frozen at -10°C, or whether they were reduced to a "supercooled" state (see below).

It was not the human oocyte but the human embryo that was the first to be successfully cryopreserved. Indeed, over a period of approximately 20 years (1960-1980) cryobiology had been raised from the level of pragmatism to science by pioneers of mathematical cryobiology such as Mazur (1970) and Leibo (1977, 1978, 1980). These authors had setup mathematical models for the freezing of biological systems based on the physicochemical characteristics of cells, such as membrane permeability and surface-to-volume ratio. In 1972 Whittingham and coworkers applied mathematically based cryopreservation protocols to mouse embryos, so leading to the first report of the birth of live young after storage of mouse embryos at -196°C in liquid nitrogen. The protocols that had been shown to work well with mouse embryos were largely copied for the human embryo, and in 1983 the first paper on a pregnancy from a cryopreserved human embryo was published by Trounson and Mohr (1983). Unfortunately a live birth did not ensue, and it was the Dutch group of Zeilmaker and colleagues (1984) who reported the first recorded live birth after cryopreservation of human embryos in 1983. It was again Wittingham (1977) who reported on the first successful cryoconservation of the mouse oocyte at -196°C in liquid nitrogen followed by the birth of live young. Despite the early successes oocyte cryopreservation did not find widespread clinical application. The reasons for this were that the efficiency of oocyte cryopreservation was low due to low survival rates, and that alarming reports questioning the genetic safety appeared in the literature. It was found that the degree of polyploidy increased significantly after cryopreservation of the mouse oocyte and of human oocytes. Almost all clinical teams stopped oocyte cryopreservation around 1987; and only a few research teams continued to investigate the effects of cryopreservation on the oocyte before considering moving oocyte cryopreservation back to the clinic again. (2)

Physics in Cryopreservation

Since liquid water is considered essential to the structure and function of living cells, it is not surprising that the solidification of water by freezing is usually lethal. Yet paradoxically freezing can also preserve cells for long periods of time in a viable state. It can slow or stop some biochemical reactions, but it accelerates others. Contrary to the usual impression, the challenge to cells during freezing is not their ability to endure storage at very low temperatures; rather it is the lethality of an intermediate zone of temperature (~–15 to –60°C) that a cell must traverse twice –once during cooling and once during warming. No thermally driven reactions occur in aqueous systems at liquid N_2 temperatures (–196°C), the refrigerant commonly used for low temperature storage. One reason is that liquid water does not exist below –130°C. The only physical states that do exist are crystalline or glassy, and in both states the viscosity is so high (>10^{13} poises) that diffusion is insignificant. Moreover, at –196°C, there is insufficent thermal energy for chemical reactions.

The only reactions that can occur in frozen aqueous systems at –196°C are photophysical events such as the formation of free radicals and the production of breaks in macromolecules as a direct result of "hits" by background ionizing radiation or cosmic rays. Over a sufficiently long period of time, these direct ionizations can produce enough breaks or other damage in DNA to become deleterious after rewarming to

physiological temperatures, especially since no enzymatic repair can occur at these very low temperatures. The dose of ionizing radiation that kills 63% of representative cultured mamlian cells at room tempreature is 200-400 rads. Because terresterial background radiation is some 0.1 rad/year, it ought to require some 2,000-4,000 years at –196°C to kill that fraction of a population of typical mammalian cells. Besides this proportional calculation, there is no evidence that storage at –196°C results in the accumulation of chromosomal or genetic changes. (3)

One of the most important things is the fate of intracellular water during freezing. At this stage it may be useful to go over some terms. It is necessary to know about the term "supercooling", to understand the freezing mechanisms of cells better.

Supercooling is the ability of an aqueous solution, to cool down it's normal freezing point, without changing it's state; from liquid to solid (ice). At this situation, if the aqueous solutions or water are touched by a crystal or a metal object, the crystallization will occur and temperature will immediately return to the freezing point. This process is called "seeding" and is used to obtain a manually controlled freezing. Crystallization can be induced by the particules in the medium, mechanical vibration or the rough surfaces. Supercooling is a situation, that takes place in the atmosphere frequently.

Water and aqueous solutions have a strong tendency to cool below their melting point before nucleation of ice occurs. For example, whilst 0°C is the melting point of ice, the temperature of water may be reduced significantly below 0°C before ice formation occurs, and in carefully controlled conditions water may be cooled to approximately –40°C before ice nucleation becomes inevitable.

Following ice nucleation and initial crystal growth the temperature rises to its melting point and remains relatively constant at that temperature during the subsequent phase change to ice ('latent heat plateau'), when the temperature then changes more rapidly to the environment temperature (fig. 1). The tendency of a system to supercool is related to a number of factors including temperature, rate of cooling, volume, exclusion of atmospheric ice nuclei and purity of particulates. In cryopreservation of cells and tissues in IVF systems, there is thus a strong tendency for supercooling to occur. To avoid the damaging effects of supercooling on cells and in particular embryos ice formation is initiated in a controlled manner. This is commonly referred to as 'seeding' -although, strictly speaking, this term refers to the introduction of a crystal to an under

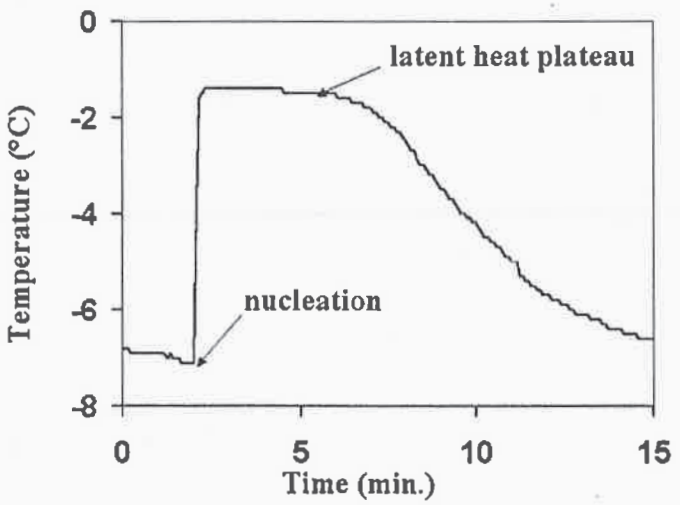

Figure 1: Temperature changes during the freezing of an aqueous solution of glycerol

supercooled solution. Controlled ice formation during freezing is recognized to be a key factor in determining the viability of embryos following freezing and thawing. In a carefully controlled series of experiments, samples which were nucleated below -9°C had a low viability, whilst nucleation (seeding) at higher subzero temperatures of -5°C to -7.5°C resulted in much higher viability (fig. 2). Normal practice is to cool straws to a temperature of approximately -7°C, hold at this temperature for thermal equilibration, and then initiate ice formation in the straw by touching the outside of the straw with cold forceps etc. The temperature of the straw rises to the melting point of the solution, and then following ice formation the temperature returns at a rate of 2.5°C/min to -7°C. Cellular dehydration then occurs during subsequent slow cooling. (1)

Down to –5°C, the cells and their surrounding medium remain unfrozen both because of supercooling and because of the depression of the freezing point by the protective solutes that are frequently present. Between –5 and –15°C, ice forms in the external medium (either spontaneously or as a result of seeding), but the cell contents remain unfrozen and supercooled, presumably because the plasma membrane blocks the growth of ice crystals into the cytoplasm. The supercooled water in the cells has a higher chemical potential than that of water in the partly frozen solution outside the cell, and in response to this difference in potential, water flows out of the cell and freezes externally.

The subsequent physical events in the cell depend on cooling velocity. If cooling is sufficiently slow (fig. 3, upper right), the cell is able to lose water rapidly enough by exosmosis to concentrate the intracellular solutes sufficiently to eliminate supercooling and maintain the chemical potential of intracellular water in equilibrium with that of extracellular water. The result is that the cell dehydrates and does not freeze intracellularly. But if the cell is cooled too rapidly (fig. 3, bottom and center right) it is not able to lose water fast enough to maintain equilibrium; it becomes increasingly supercooled and eventually attains equilibrium by freezing intracellularly. Intracellular ice formation leads to cell death.

In the course of time it is needed to formulate these physical events and to see what kind of factors are effective on cryopreservation. More specifically, the proposal was that the rate of exosmosis of water during freezing can be described by four simultaneous equations. The first relates the loss of cytoplasmic water to the chemical potential gradient expressed as a vapor pressure ratio; second expresses the change in this vapor pressure ratio with temperature

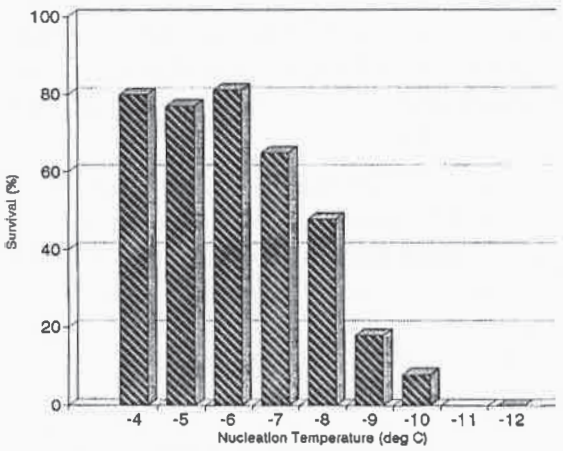

Figure 2: The survival of mouse cell embryos after seeding at various subrero temperatures

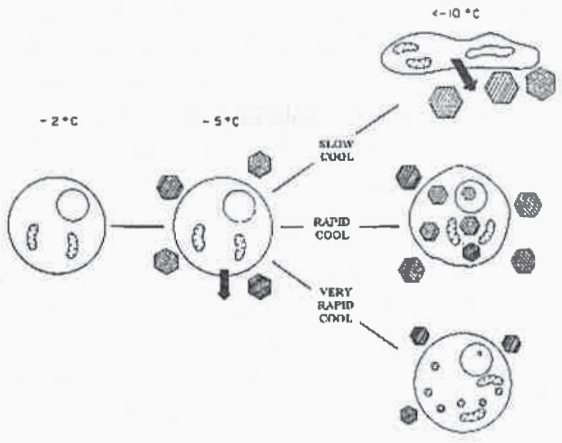

Figure 3: Schematic of physical events in cells during freezing. *Cross-hatched hexagons, ice crystals.*

(Clausius-Clapeyron equation and Raoult's law). Third equation is about time and temperature which are related by the cooling rate (B). And finally the fourth one is related to the activation energy and temperature (3).

1. $dV/Dt = (L_p ART \ln P_e/P_i)/V_i°$
2. $d\ln(P_e/P_i)/dT = L_f/RT^2 - [N_2V_1°/(V+N_2V_1°)V]dV/dT$
3. $dT/dt = B$
4. $L_p = L_p^g \exp [-E/R`(1/T-1/T_g)]$

According to these equations, the "optimum" rate of cooling may be considered to be the fastest rate of cooling at which intracellular ice formation does not occur. Whilst the existence of an optimum rate of cooling has been reported for a wide range of cell-types, a clear optimum does not seem to exist with some cells of importance to IVF. The response of cells to the hypertonic conditions encountered during freezing is determined by a number of biophysical factors:

1. Cell volume (V) and surface area (A)
2. Cellular permeability to water (Lp)
3. Arrhenius activation energy (E)
4. Type and concentration of cryoprotective additives
5. Cooling rate (B)

Thus to avoid the probability of intracellular ice damage to embryos, which have low surface area to volume ratio and low water permeability, slow rates of cooling are required. Sperm, with a large surface area to volume ratio and a higher value for water permeability may be cooled faster before the intracellular compartment becomes significantly supercooled (4).

An increase in diameter (D) proportionally reduces the cooling rate required to produce a given probability of intracellula freezing because the fractional water loss during cooling is proportional to the ratio of the cell surface (D^2) to cell volume (D^3). The development of cryomicroscopes has permitted experimental tests of the presumed relation between cooling rate and the occurence of intracellular freezing. It has been seen that the ovum cooled at 1.2°C/min dehydrates without evidence of internal freezing, but the ovum cooled at 32°C/min undergoes no discernable shrinkage and freezes intracellularly at –40°C as evidenced by its sudden opacity.

To avoid intracellular freezing, the ova must be cooled slowly enough to permit their water content to approach the equilibrium value before they have reached their ice-nucleation temperature. The available evidence indicates that when ~90% of cell water is removed, the residual ~10% cannot freeze at any temperature. (3)

Effects of Freezing on Cells

Cells are exposed to a continuously increasing hypertonic solution medium during freezing. It is essential to remove a particular amount of water from cell osmotically, to avoid intracellular freezing, before and –partially- after the ice nucleation. This is something related to the cooling rate. At slow rates of cooling, cells try to remain in equilibrium with the external solution. The external solution freezes before the intracellular medium because of the protective effects of cell membranes. Thus, outside of the cell becomes more hypertonic and a water-flow from inside to outside (exosmosis) occurs. This is like a knife that cuts both ways for the cell. Removal of too much water may lead to increase in the solute load that means the disturbance of equilibrium. As the cooling rate is increased there is less time for water to move from the cell, which becomes increasingly supercooled and eventually intracellular ice formation occurs that is inevitably lethal.

So, an optimum rate of cooling results from the balance of these two phenomena. At rates of cooling slower than the optimum, cell death is due to long periods of exposure to hypertonic conditions. At rates of cooling faster than the optimum, cell death is associated with intracellular ice formation.

Cells in suspension are not punctured by ice crystals, nor are they mechanically damaged by ice. (1)

Stress Encountered	Potential Cellular Response
Reduction in temperature	Membrane lipid phase changes Depolymerisation of the cytoskeleton
Increase in solute concentration	Osmotic shrinkage
Increase in ionic concentration	Direct effects on membranes, including solubilisation of membrane proteins
Dehydration	Destabilisation of the lipid bilayers
Precipitation of salts and eutectic formation	Not known
Gas bubble formation	Mechanical damage to membranes and the cytoskeleton
Solution becomes extremely viscous	Diffusion processes, including osmosis may become limited
Changes in pH	Denaturation of proteins etc.
Cells become closely packed	Membrane damage

Thawing & Post Thaw Handling

During thawing of cryopreserved samples the physical processes which occur during freezing will be reversed. The solid system will partially melt and cells will become again suspended into hypertonic solutions which will become more dilute during thawing. Cryoprotective additives and water may be transported across cell membranes and any intracellular ice may grow before it finally melts. In most cases examined, rapid rates of thawing are generally better than slow rates of warming.

The measured rates of warming of the samples are relatively high between -196°C and approximately -10°C, in this temperature range the amount of ice to be melted is relatively small. The warming curve is then observed to flatten off between -10°C and the melting point of the solution, within this temperature zone a large amount of ice needs to be melted -this is the reverse of the latent heat plateau.

Conventionally, material cryopreserved in straws is held in air for 40 seconds, during which time the temperature will rise rapidly to approximately -50°C, before the straw is transferred to a water bath held at 30°C for 1 minute. The step of holding the straw in air was originally employed to allow the boiling off of any liquid nitrogen trapped within the straw because immersion of straws containing entrapped liquid nitrogen into warm water would lead to rapid boiling of the liquid nitrogen with possible fracture of the straw or violent expulsion of the plug. Development in plugs for straws now make it unlikely that nitrogen can leak into straws.

Upon thawing the cryoprotective additives are diluted out either in a single step or in two steps. Following freezing and thawing cells will contain cryoprotective additive and upon exposure to normal growth medium will tend to expand. To prevent swelling of cells, shrinkage is induced by using wash out solutions which contain hypertonic sucrose (0.2 M), this sucrose is then diluted away by washing with growth medium.

Cryoprotectants

In dilute aqueous solutions such as growth media the increase in ionic composition following ice formation is dramatic and by -10°C the ionic concentration reaches approximately 3 molar which is, not surprisingly, lethal to cells.

A number of compounds, so called cryoprotective additives, are employed to reduce cellular damage following freezing and thawing. The general properties of cryoprotective compounds are that they have low molecular weight, are non toxic and can permeate cells. Cryoprotective additives achieve their protective effects by increasing the unfrozen fraction at a

given temperature and thereby reducing the ionic composition. This effect is illustrated here with glycerol but the other commonly employed additives (propanediol, dimethlysulphoxide etc.) act in the same manner. Cells are exposed to a high concentration of the cryoprotective additive during freezing rather than a high ionic concentration, which is less damaging. Cells can be permeated by all of the commonly employed cryoprotective additives used in IVF, and it is standard practice to "incubate" cells in the cryoprotective additive before freezing commences to allow them to attain an equilibrium intracellular concentration.

When compared at the same concentration (molar), all cryoprotective additives have a very similar effect to that described above. However the protective efficiency of these compounds may vary from cell-type to cell-type: for example it has been found that human embryos are best frozen with propanediol whilst human blastocysts are optimally frozen with glycerol. This may be related to the relative cellular toxicity or the differing permeability of these additives to differing cell-types, but because experiments to compare them have been mostly carried out at a single freezing protocol, the explanation may be more complex than these experiments suggest.

Sucrose is employed in the standard protocols for embryo and oocyte cryopreservation with a low level (0.1 M) used in addition to the cryoprotectant. To prevent swelling of cells on thawing, shrinkage is induced by using wash out solutions which contain hypertonic sucrose (0.2 to 0.3M). This sucrose is then diluted away by washing with growth medium. (4)

Conclusion

Today, by the help of progressing biotechnology, it is more often that we apply cryopreservation. While, there are still insufficient researches especially for the oocytes, studies on the cryopreservation of immunological memory lymphoid cells, aortic root allografts and osteoblasts for bone banking, are going on. (5-8). An updated, but discursive subject is postmortem human reproduction; and it currently has many ethical and legal problems awaiting to be solved. (9). Recently, it can be seen that vitrification is more preferable than slow cooling as it is cheaper and easier to handle. (10). Cryopreservation of cornea, umbilical cord and hematopoietic cells and sperm banking procedures are performed routinely. (11)

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JOURNAL OF ANKARA MEDICAL SCHOOL Vol 24, No 4, 2002 197-200

A CASE OF MULTIPLE MYELOMA AND AMYLOIDOSIS OF THE TONGUE

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SUMMARY

Multiple myeloma is a clonal plasma cell proliferative disorder. Ten to fifteen per cent of patients with multiple myeloma have associated primary amyloidosis. We describe a case of oral amyloidosis presented with macroglossia and characteristic nodular lesions which developed as a complication of multiple myeloma. Pathogenesis, diagnosis and treatment of oral amyloidosis are also discussed.

Key Words: *Amyloidosis, Tongue, Multiple Myeloma.*

ÖZET

Bir Multipl Myeloma ve Dilde Amiloidozis Olgusu

Multiple myeloma klonal plazma hücre proliferasyonu ile karakterize bir hastalıktır ve multipl myelomalı hastaların %10-15'inde primer amiloidozis gelişmektedir. Bu makalede multiple myelomanın komplikasyonu olarak dilde makroglossi ve karakteristik nodüler amiloidosis lezyonları meydana gelen bir olgu sunulmuş ve oral amiloidozis patogenezi, tanısı ve tedavisi kısaca gözden geçirilmiştir.

Anahtar Kelimeler: *Amiloidozis, Dil, Multipl Myeloma*

Multiple myeloma is a malignant disorder which is characterized by an uncontrolled proliferation of plasma cells in bone marrow. Primary amyloidosis can either arise idiopathically or can be associated with plasma cell discrasia (1,2). Here we present a patient with amyloidosis of the tongue which developed as a complication of multiple myeloma.

Case Report

A 73-year-old man admitted to our clinic with macroglossia and asymptomatic multiple ulcerated nodular lesions on his tongue which first appeared 4 months ago and enlarged gradually. He complained of difficulty in speech and swallowing solid foods. His past medical history revealed multiple myeloma which was diagnosed one year ago and he was still being treated with pulsed courses of vincristine, adriamycine, dexamethasone and pamidronate disodium.

His dermatologic examination revealed slight macroglossia and multiple shiny, reddish-purple, ulcerated nodular lesions on the lateral borders of his tongue (Figure 1).

Apart from slight anemia and high erythrocyte sedimentation rate, laboratory examinations including complete blood count, serum biochemistry, urine analysis and protein electrophoresis were all normal.

An incisional biopsy was made from one of the nodular lesions under the diagnostic possibilities of hemangioma, lymphangioma, plasmasitoma and amyloidosis. Dermatopathological examination of the biopsy

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Received: *Dec 07, 2001* Accepted: *March 11, 2002*

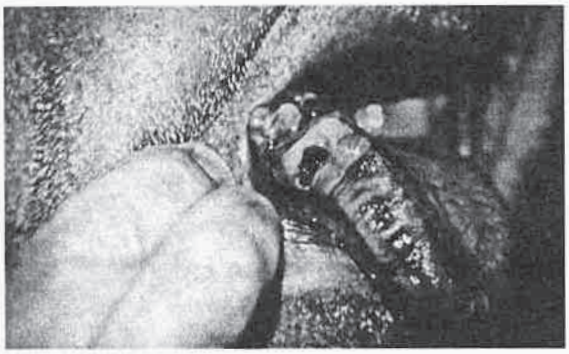


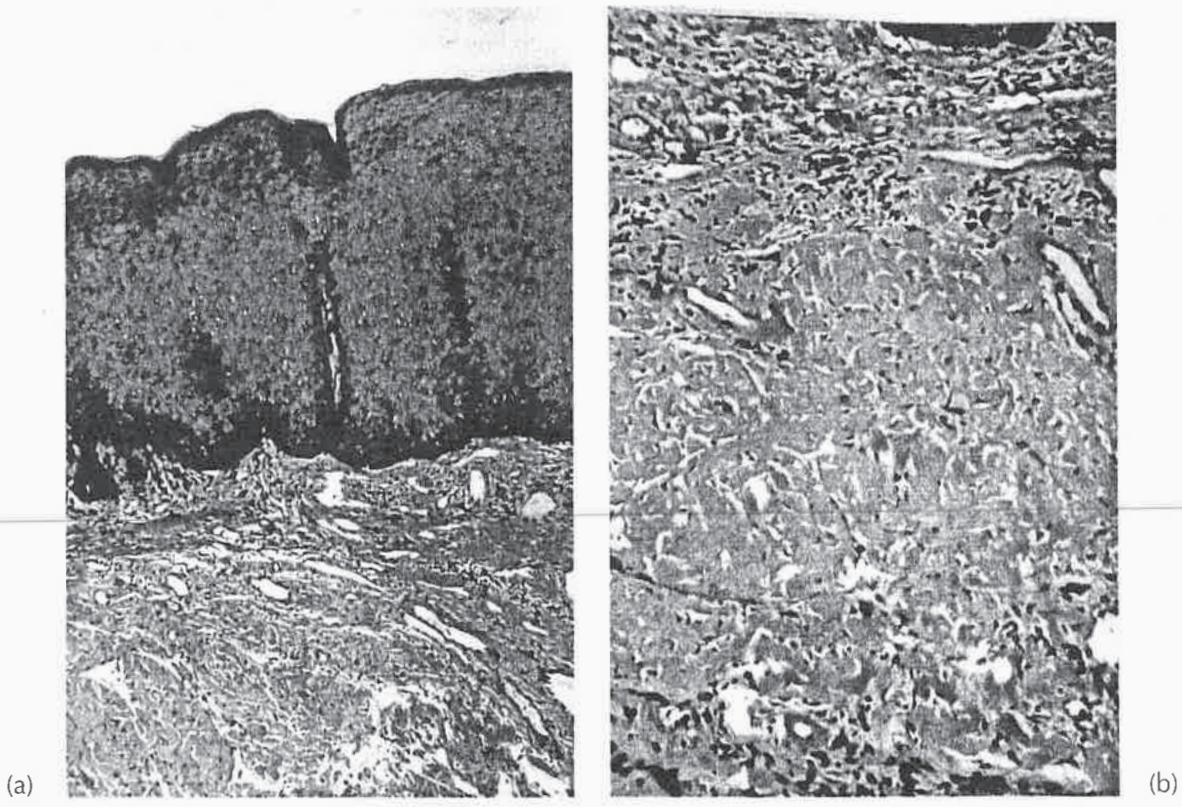
Figure 1. Macroglossia and multiple shiny, red-purple ulcerated nodular lesions on the lateral border of the tongue.

material showed massive eosinophilic amorphous material located in the reticular dermis and stained characteristically positive with Congo-red confirming amyloid deposition (Figure 2).

On the basis of these clinical and dermatopathological data the diagnosis of primary amyloidosis due to multiple myeloma was made. Since the lesions were not causing any significant oral disfunction surgical excision was not performed but regular control visits were planned for a close follow-up.

Riscussion

Amyloidosis is a rare, fatal metabolic disorder that leads to extracellular deposition of a sulphated mucopolysaccharide in various tissues and organs (1,3). Systemic amyloidosis is subdivided into immunocyte dyscrasia with amyloidosis (AL-fibril type), reactive systemic amyloidosis (AA-fibril type) and familial systemic amyloidosis. Primary systemic amyloidosis belongs to AL-type amyloidosis. It usually occurs in the setting of multiple myeloma, monoclonal gammopathies and macroglobulinemia.



(a) (b)

Figure 2. a) Massive eosinophilic amorphous material in the reticular dermis (Congo Red X 50). b) Amyloid deposits typically stained faint red (Congo Red X 100).

Secondary amyloidosis on the other hand mostly associates chronic inflammatory diseases or chronic infections and usually does not produce skin lesions (4,5). In our patient, primary oral amyloidosis was the result of multiple myeloma which was diagnosed 1 year ago.

Amyloid deposition in multiple myeloma associated systemic amyloidosis occurs as a result of plasma cell discrasia and is characterized by the presence of amyloid light chain in which the major protein component is the variable portion of immunoglobulin molecule (5,6). The abnormal monoclonal immunoglobulins are produced by the neoplastic cells. Amyloidosis occuring in multiple myeloma is characterized by the elaboration of light chains (Bence-Jones proteins) by the host. These light chains are converted to amyloid fibrils by proteolytic enzymes in macrophages and secreted to tissues. They can be deposited in connective tissues anywhere in the body and extensive deposition may cause disfunction (7).

Oral manifestations occur in nearly 39% of primary amyloidosis patients in which multiple myeloma associated lesions consist a small portion (1,6,8). Rarely oral amyloidosis may be the first symptom of multiple myeloma (9-11). The amyloid deposits in oral mucosa of primary amyloidosis patients presents as papules, nodules, plaques and macroglossia (1,2,6-8). These lesions may interfere with speech, chewing, swallowing and ability to close mouth. Amyloid deposition in the salivary glands may cause xerostomia. In late stages, lesions may even lead to oropharyngeal blokage (5). Eventhough macroglossia is known to be the most common manifestation, mucosal nodules are considered to be more specific signs indicative of amyloidosis of the tongue since tongue enlargement can also occur in the absence of amyloidosis (2).

Presence of amyloidosis in multiple myeloma patients is usually associated with poor survival. The median survival time in these patients is assumed to be about 4 months and death usually occurs as a complication of amyloidosis effecting major organ systems (12). We followed up our patient for 1 year and during this period his general status worsened although the size of the oral nodular lesions and macroglossia did not show significant difference. His survival time was relatively longer than the expected.

Since the presence of amyloid deposition in multiple myeloma patients is evaluated as a grave factor and since there are no biochemical or heamatologic parameters that associates amyloidosis in these patients, a routine histopathological examination is essential for every multiple myeloma patient with suspected oral lesions (2). Pyogenic granuloma, plasmasitoma and oral tumoral lesions such as lymphangioma, hemangioma and squamous cell carcinoma may also cause similar nodules in the oral mucosa but the diagnosis of amyloidosis can easily be made by typical histopathological findings. Light microscopic examination characteristically shows amorphous eosinophilic material which typically stains pale pink with Congo-red. The material also gives apple-green bi-refrigence under polarised light (1,2,4).

Treatment of oral amyloidosis lesions is nonspecific. Since multiple myeloma is a malignant neoplasm and development of primary amyloidosis shortens the survival, noninvasive and conservative treatments are primarily recommended for localized lesions, but surgical interventions can be inevitable for severe cases with extensive lesions compromising vital functions (1,13,14). In our case, we preferred to follow-up our patient since the lesions were not hindering vital functions.

Eventhough involvement of the oral mucosa in primary amyloidosis is a frequent entity; amyloid deposition on the tongue due to multiple myeloma is rare and indicates a poor prognosis. In this report we described a patient who developed macroglossia and characteristic multiple nodular amyloid deposits on his tongue approximately 8 months after the diagnosis of multiple myeloma and had a relatively long survival than the previously reported cases.

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PRİMARY PULMONARY NON-HODGKİN'S LYMPHOMA

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SUMMARY

Primary non-Hodgkin's lymphoma of the lung is a rare pulmonary tumor. We report herein a 69-year-old man who was found to have a primary pulmonary lymphoma of a low grade B-cell marginal zone type and present the clinical features of these tumors.

Key Words: Lymphoma, Primary, Lung

ÖZET

Akciğerin Primer Non-Hodgkin Lenfoması

Akciğerin primer non-Hodgkin lenfoması seyrek görülen bir tümördür. Low-grade B-cell marginal zon tipinde primer pulmoner lenfoma saptanan 69 yaşındaki erkek hastayı sunmayı ve bu tümörlerin klinik özelliklerini tartışmayı amaçladık.

Anahtar Kelimeler: Lenfoma, Primer, Akciğer

Non-Hodgkin's lymphoma and Hodgkin's disease are malignant neoplasms of lymphoid tissue [1]. Although the lung is a frequent metastatic site for a Hodgkin's or non-Hodgkin's lymphoma, primary pulmonary lymphoma of the lung is extremely rare. Primary pulmonary non-Hodgkin's lymphoma comprise of only 0.4% of all lymphomas [2].

We report herein a case of a primary pulmonary lymphoma with a histological diagnosis of low-grade marginal zone B-cell lymphoma and discuss the clinical features of these rare pulmonary tumors.

Case Report

A 69-year-old man was referred with the diagnosis of a bronchogenic squamous cell carcinoma obtained by bronchoscopic biopsy at another hospital. He presented with chest and back pain of two months duration. He has been a worker in a glass factory for 15 years and a smoker for 45 years. On physical examination, breath sounds were diminished on the left lower zones of the lung. In addition, he had an apparent pretibial edema on the right side, which was attributed to an angiography procedure, performed eight years before admission and hyperkeratotic lesions on the back of his foot. Laboratory data were within the normal limits. Chest X-ray showed a pneumonic consolidation on the left lower zone (Figure 1). On computed tomography (CT), a hypodense, solitary mass, measuring 6x4x3 cm was visible in the posterabasal segment of the left lower lobe (Figure 2). Metastatic work-up was negative. Bronchoscopy showed no endobronchial lesion.

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Received: *Nov 26, 2001* Accepted: *March 11, 2002*

Cytologic examination of the bronchoalveolar lavage and sputum was not definitive. A left lower lobectomy with mediastinal lymph node dissection was performed. Histological examination showed a primary pulmonary lymphoma without any involvement of either hilar or mediastinal lymph nodes (Figure 3). Immunohistochemical staining with CD20, CD23, CD 43, CD79a showed positivity on the lymphoid cells, and revealed a primary low-grade marginal zone B cell lymphoma of the lung (Figure 4). The patient was referred to medical oncology for further chemotherapy.

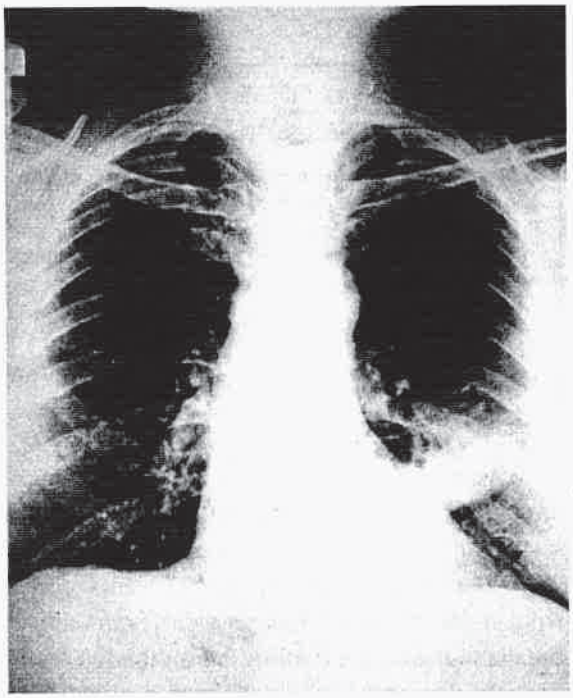

Figure 1. Chest X-ray showing an ill-defined mass in the left lower zone

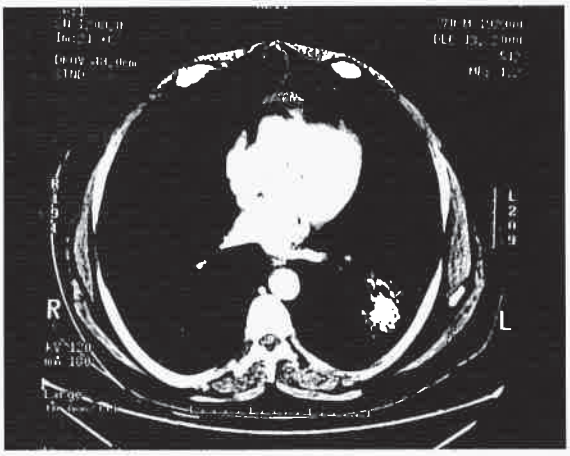

Figure 2. Computed tomography showing a heterogeneous mass in the left lower lobe with air bronchograms.

Discussion

Most primary lymphomas of the lung arise from the mucosa-associated lymphoid tissue (MALT) of the bronchus, which is believed to be an abnormal constituent of the human bronchial tree and an acquired tissue in response to long-term exposure to various antigenic stimuli such as smoking, infection, or autoimmune disorder [3]. Consistently, the finding that, MALT is not normally found in the stomach but is associated

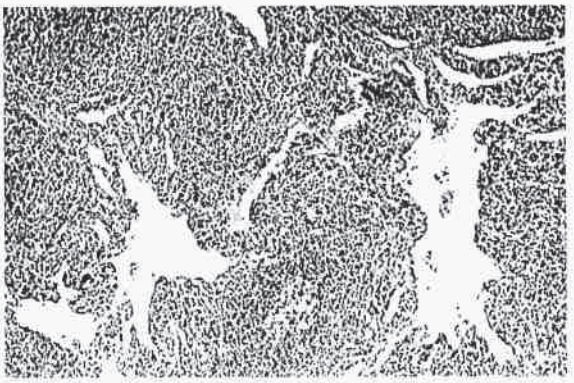

Figure 3. Peribronchial intense atypical lymphoid infiltration (HE x 50).

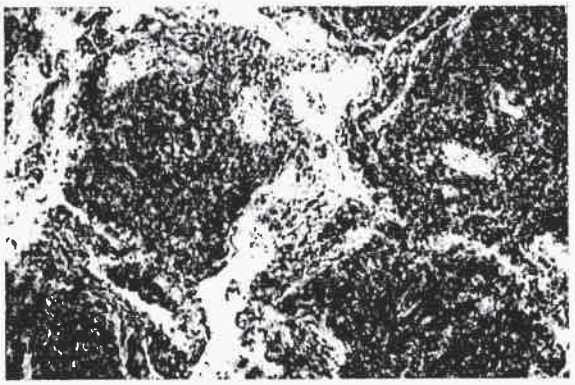

Figure 4. Atypical lymphoid cells showing positivity with B-lymphoid marker CD 79a (x 25).

with chronic Helicobacter pylori gastritis, supports this suggestion [4]. Reactive lymphoid proliferations such as pseudolymphoma, lymphoid interstitial pneumonitis, lymphomatoid granulomatosis, and follicular bronchiolitis are morphologically difficult to distinguish from primary malignant lymphoid tumors [3]. Recent work established that many of these lesions may actually be malignant lymphoma (5).

The advent of immunohistochemical techniques to detect monoclonality have resolved much of the controversy regarding the definition of these lymphoid tumors of the lung. A revised classification of lymphoid neoplasms including MALT lymphomas was proposed [6]. Currently, the staging classification used for these extranodal lymphomas is as follows [7]:

Stage IE : Involvement of lung only (can be bilateral)

Stage II 1E : Lung and hilar lymph nodes

Stage II 2E : Lung and mediastinal lymph nodes

Stage II 2EW : Lung and adjacent chest wall or diaphragm

Stage III : Involvement of lung and of lymph nodes below diaphragm

Stage IV : Diffuse involvement of one or more extralymphatic organs or tissues.

Most of the patients with primary pulmonary lymphoma are asymptomatic at presentation and the disease is often discovered on a screening chest radiograph. Symptoms, if present, are generally nonspecific except for a slight preponderance of respiratory abnormalities such as cough, dyspnea, chest pain, and hemoptysis [1]. Our case was symptomatic and presented with chest pain.

The roentgenographic appearance of pulmonary lymphoma is usually described as an alveolar mass or infiltrate with ill-defined margins and air bronchograms [8,9]. Although less common, rounded opacities or nodules [10] may appear as in the presented case. Thus, the roentgenographic findings are variable and can only suggest the possibility of lymphoma.

As a diagnostic procedure, bronchoscopy has a low diagnostic yield, because endoluminal lesions are quite rare. Analysis of bronchoalveolar lavage for tumor cell markers and with molecular techniques such as flow cytometry may be an aid in the diagnosis of pulmonary lymphoma [11,12]. Neither transthoracic needle biopsy nor mediastinoscopy is useful in the diagnosis [2]. Thus, surgical intervention, whether by thoracotomy or VATS, is required for diagnosis in the majority of patients seen with primary pulmonary lymphoma as in our case.

The role of surgery in the management of primary pulmonary lymphoma is to obtain a diagnostic yield and a therapeutic resection. Resectable tumors should be approached with an intent of a complete resection, whereas those, large and unresectable should be treated with limited resection such as wedge resection or even a biopsy procedure to obtain a sufficient tissue for histologic examination [2]. Hilar and mediastinal lymph node dissection should be carried out as a staging procedure. We have performed a curative resection as lobectomy in the presented case and hilar-mediastinal lymph nodes were free of tumor. The rate of local recurrence has been reported as high as 50% [13,14], and thus radical resections including pneumonectomies have been recommended [15]. However, extended resections or even postoperative chemotherapy do not offer better prognostic results [2].

A variety of histologic subtypes of non-Hodgkin lymphoma may manifest as primary pulmonary lymphoma. The most common histologic subtypes of primary pulmonary lymphoma are low-grade lymphoproliferative processes that are well-differentiated B-cell tumors that appear to arise from bronchus-associated lymphoid tissue (BALT). BALT forms part of the wider system of low-grade malignant lymphomas of MALT type such as those found in the gastric area. Pulmonary low-grade malignant lymphoma of MALT type tends to remain localized in the lung for long periods. This form may be referred as a subtype of marginal-zone B-cell lymphoma as in our case. The second most

frequent histologic type of non-Hodgkin lymphoma to involve the lung is diffuse large B-cell lymphoma [1].

Although optimal treatment has not been clearly defined, the prognosis of non-Hodgkin's lymphoma of the lung is favorable. Stage of disease or the presence of regional (hilar) lymph node metastases does not correlate with a worse prognosis [13,14], whereas histologic type of lymphoma should serve as a prognostic factor [16]. Malignant lymphomas arising from MALT remain localized until late in their natural history and thus carry a better prognosis compared with lymphomas arising in lymph node tissue of similar stage [8,13]. True lymphomas of MALT are low-grade tumors with a slow, indolent course, and long-term survival is very likely [8,13,16]. Non-MALT types of lymphoma of the lung are generally intermediate or high-grade tumors with a worse prognosis, which may show transformation to a large cell type.

In conclusion, primary non-Hodgkin's lymphoma is a rare entity of the lung. An open thoracotomy is very likely to provide a diagnosis as well as a therapeutic resection.

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ACUTE RENAL FAILURE DUE TO RHABDOMYOLYSIS SECONDARY TO SEVERE HYPEROSMOLALİTY: A CASE REPORT

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SUMMARY

A 80-year-old diabetic female with demans who developed acute renal failure resulting from rhabdomyolysis secondary to severe hypernatremia and hyperosmolality is presented. The patient recovered by means of rehydration, insulin and antibiotic treatment. The patient did not require renal replacement therapy during the course. Rhabdomyolysis due to hypernatremia and hyperosmolality and ARF is a rare condition therefore we have presented and discussed this case in the light of the literature.

Key Words: Hyperosmolality, Rhabdomyolysis, Renal Failure

ÖZET

Ciddi Hiperosmolalite Sonucu Gelişen Rabdomiyolize Bağlı Akut Böbrek Yetmezliği: Olgu Sunumu

Bu yazıda 80 yaşında diyabetik bir hastada hipernatremi ve hiperozmolariteye bağlı rabdomiyolize ikincil gelişen akut böbrek yetmezliği sunulmuştur. Hasta diyalize gereksinim duyulmadan rehidratasyon, insulin ve antibiyotik tedavisi ile tamamen düzelmiştir. Olgu nadir görülmesi nedeniyle literature bilgileri ışığında tartışılarak sunulmuştur.

Anahtar Kelimeler: Hiperosmolalite, Rabdomiyoliz, Böbrek Yetmezliği

Rhabdomyolysis is a syndrome characterized by muscle necrosis and the release of intracellular muscle constituents into the circulation. The severity of illness ranges from asymptomatic elevations of muscle enzymes in the serum to life-threatening cases associated with extreme enzyme elevations, electrolyte imbalances, and acute renal failure.

Most commonly, the cause of rhabdomyolysis is evident from the history or from the immediate circumstances preceding the disorder, such as postoperative surgical trauma, a comatose or postictal state, or extraordinary physical exertion. Occasionally, the precipitant will not be obvious. Some of these cases are due to muscle enzyme or electrolyte abnormalities, infections, drugs, toxins, or endocrinopathies.

Electrolyte imbalances, especially being frequent due to hypopotasemia and hypophosphatemia, hypernatremia may rarely give rise to rhabdomyolysis severe enough to cause acute renal failure (ARF)(1). Rhabdomyolysis, in case of delayed diagnosis and inappropriate treatment, may result in ARF. It constitutes approximately 5-9% of ARF cases. On the other hand, ARF develops in 15-30% of rhabdomyolysis cases (2). Rhabdomyolysis due to hypernatremia and hyperosmolality and ARF is a

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Received: *Oct 25, 2001* Accepted: *March 11, 2002*

rare condition (3). The case is considered to be worthy to report since it is a rare occurrence.

Case Report

A 80-year-old female with the diagnosis of diabetes mellitus type-2 and demans for 6 and 2 years respectively was brought to emergency department with the complaints of fever and loss of conscious. He was learnt to have experienced no convulsive attack. On admission her physical examination revealed an arterial blood pressure 110/70 mmHg, pulse rate 131/min-regular, respiratory count 30/min and body temperature 38.8 °C. She was unconscious, tongue was dry and skin turgor and tonus was decreased. Rest of physical examination didn't disclose any pathological finding. Laboratory data were as follows: white blood cell count 16100/mm^3, hemoglobin 14.6 gr/dl, platelet count 78000/mm^3, serum glucose 304 mg/dl, blood urea nitrogen 75 mg/dl, creatinine 2.6 mg/dl, serum sodium 177 meq/L, potassium 3.5 meq/L, aspartate transaminase (AST) 207 U/L, alanine transaminase (ALT) 79 U/L, lactate dehydrogenase (LDH) 2037 U/L, creatinine phosphokinase (CPK) 4512 U/L, calcium 7.3 meq/L, phosphate 6.0 mg/dl, blood osmolality: 361 mosm/kgH_2O, urinary osmolality 499 mosm/kgH_2O. Urinary sodium excretion was 40 meq/litre and fractional excretion of sodium was higher than 2%. Urinary dipstick analysis was 1+ positive for glucose, 3+ positive for protein and 3+ positive in hem test. Direct microscopic evaluation of urinary sediment showed a few red blood cells and some pigmented casts. Serum and urinary myoglobin levels were higher than 3000 ng/ml (normal upper limit is 72 ng/mL) in both samples. Urinary inoculation E. coli growth. Arterial blood gases analysis and coagulation parameters were within normal limits. Compiling all the data, she was considered as non-ketotic hyperosmolar coma secondary to urinary tract infection and dehydration leading to hypernatremia in turn, giving rise to rhabdomyolysis and ARF. On hospitalization she was rehydrated by 0,45% NaCl and 5% dextrose solutions meanwhile forced alkaline diuresis was applied and intravenous ciprofloxacine 200 mg twice daily was begun for fifteen days in conjunction with appropriate insulin treatment. In the follow up, fever was declined, she gained her conscious back and serum sodium, creatinine and glucose levels and blood osmolality were turned back to normal (Figure 1). On improvement of the general status of the patient, she was discharged. The decrease of serum sodium and CPK levels is shown in Figure 2.

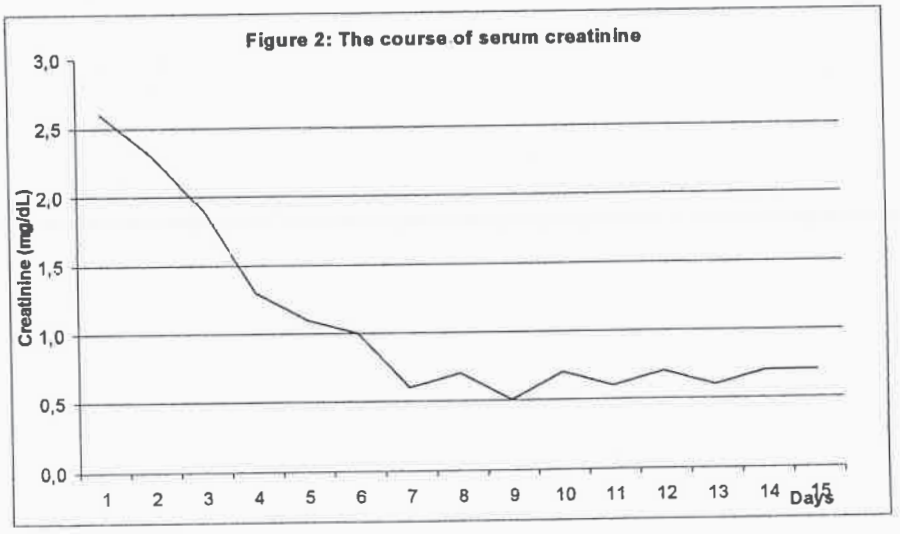

Figure 1: The course of serum creatinine

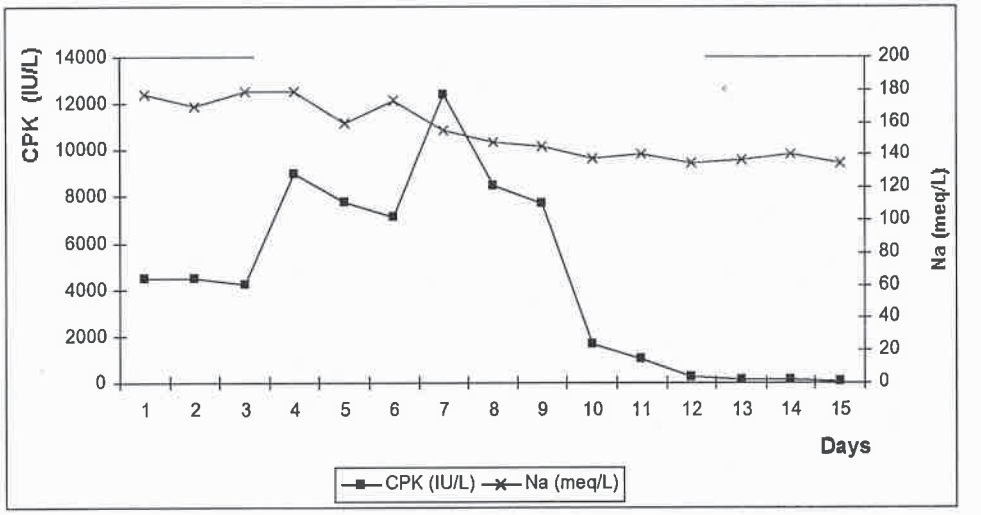

Figure 2: The course of the serum Na and CPK levels

Discussion

Rhabdomyolysis due to hypernatremia and hyperosmolality is a rare issue (3, 4). The mechanism is unknown. Grinstein et al (5) put forward that during the shrinkage of the cells in the hypertonic medium, normal cell volume is kept by means of Na^+/H^+ antiport and Cl^-/HCO_3^- exchange at the level of cell membrane. Meanwhile amount if intracellular calcium increases giving rise to activation of protein kinases and lysis of the cells. By means of cell lysis, muscle enzymes are liberated and rhabdomyolysis develops. Similarly the same hypothesis may also explain the pathogenesis of the rhabdomyolysis secondary to hypernatremia. Rhabdomyolysis is frequently observed in diabetics (6) and hyperosmolality secondary to high blood glucose level is proposed in the pathogenesis. In our case, in addition to hyperosmolality and osmotic diuresis resulting from high blood glucose level, decreased oral intake secondary to demans and fever contributed to the development of hypernatremia and rhabdomyolysis. Rhabdomyolysis, in case of delayed diagnosis and inappropriate treatment, may result in ARF.

As a conclusion hypernatremia is one of the rare but important causes of rhabdomyolysis. In the present case hypernatremia and hyperosmolality gave rise to rhabdomyolysis resulting in acute renal failure. Acute renal failure in our patient resolved with medical therapy alone and no renal replacement therapy was required.

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MYOCARDIAL BRIDGE SURGERY PERFORMED BY OFF PUMP TECHNIQUE: A REPORT OF TWO CASES

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Kaan Kaya**** ✣ Özlem Küçük***** ✣ Hakkı Akalın*

SUMMARY

Two patients, 43 and 51 years old, were admitted to our department with angina complaints. Coronary angiographies showed no atherosclerotic changes, but myocardial bridges located on the the left anterior descending artery (LAD) in both patients. Medical management started with beta-blockade drugs. In spite of this, no improvement was achieved, and anginal episodes lasting for 15 minutes insisted. Under these conditions, patients were submitted to coronary artery surgery without cardiopulmonary bypass, myotomy were performed to the involved segments.

Key Words: *Myocardial Bridge, Myotomy, Off Pump Surgery*

ÖZET

"Off Pump" Teknikle Yapılan Miyokardiyal Band Cerrahisi: Vaka Sunumu

Biri 43 yaşında diğeri 51 yaşında iki ayrı hasta göğüs ağrısı nedeniyle kliniğimize başvurdular. Koroner anjiyografileri, aterosklerotik değişiklikler olmadığını, fakat her iki hastada da LAD (sol anterior inen) arter üzerinde myokardiyal band olduğunu gösterdi. Beta-blokerler ile medikal tedavilerine başlandı. Bu tedaviye rağmen hiçbir gelişme sağlanmadı ve hastalarda 15 dakika süren göğüs ağrısı epizodları gelişti. Bu şartlar altında hastalar minimal invaziv koroner arter cerrahisine sevkedildiler ve kardiyopulmoner baypas kullanılmaksızın miyokardiyal band olan segmentlere myotomi operasyonu yapıldı.

Anahtar Kelimeler: *Miyokardiyal Band, Miyotomi, Off Pump Cerrahi*

Myocardial bridges, primarily described by Reyman in 1737 and Black in 1805 and result systolic compression to the epicardial coronary arteries are most probably randomly noticed (1). In postmortem studies, solitary or multiple bridges are reported in 5-86% frequency (2). These myocardial bridges are confronted in 0.5-33% rate (3). The majority are seen in LAD (1,2). Inspite of high frequency rate of their presence, these bridges hardly results to ischemia and symptoms. Although they are described as a simple variant of coronary artery anatomy, many complications such as angina, myocardial infarction, atrioventricular block or sudden death related to myocardial bridges were reported (2-4). In addition, Roul et al. reported a left ventricle dysfunction case due to the myocardial bridge (3). The importance of the myocardial bridges are still in debate. But these are generally accepted as benign lesions. We presented two myocardial bridge cases 43 and 51 years old with ischemic symptoms.

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Received: *Jan 07, 2002* Accepted: *April 08, 2002*

Case Report

Case I: 43 year-old female patient was admitted to our hospital with anginal complaints. It was informed that the angina was present for two years and increased its severity for the last 3 weeks. Serial electrocardiograms and myocardial enzymes studied excluded myocardial infarction. On the 2nd day of hospitalisation angina recurred and T inversion was observed during the anginal episode in the anterior derivations. In the coronary artery angiography performed because of these findings no atherosclerotic changes were observed in none of the coronary arteries, but an occlusion appearing in systole and disappearing in diastole located segmentally 2 cm long in the mid 1/3 LAD was noticed. Other vessels and left ventricle wall motions were evaluated normally. Septal hypertrophy was noticed in echocardiography. Although management was initiated with beta-blockade drugs, no improvement for the complaints was achieved and surgery was decided. A longitidunal myotomy on LAD was performed without cardiopulmonary bypass (off pump technique) via median sternotomy. After the operation, the patient has not complained of angina in her controls.

Case II: 51 year-old male patient was admitted to our hospital with recurring anginal complaints. Although no evidence for myocardial infarction was found following the results of the serial electrocardiograms and enzyme studies because of the frequent recurring complaints resistant to drug teraphy coronary angiography was performed. In coronary angiography, although no atherosclerotic changes were seen in none of the coronary arteries on LAD, beyond the first diagonal artery, nearly 1 cm long segmental occlusion appearing in systole and disappearing in diastole was observed. In echocardiography, left ventricle hypertrophy particularly profound in the septum was noticed. Beta-blockade teraphy was initiated, but no improvement was achieved

in the symptoms and the patient underwent operation. Longitidunal myotomy was performed by off pump technique via median sternotomy. The patient has not complained of angina in his controls after the operation.

Discussion

All myocardial bridges aren't symptomatic. In a study of Noble et al., symptoms appear when these bridges compress the coronary arteries over 75% and particularly following tachycardia due to the shortening of the diastolic phase the myocardial bridges may lead to myocardial ischemia symptoms (5). It was also reported that it is necessary for their resulting in symptoms the shortening of diastolic period (such as tachycardia) or accompanying cardiac anomaly (hypertrophy) (4). We observed profound septal hypertrophy in both cases by preoperative echocardiography and postoperatively performed myocardial perfusion scintigraphies. Besides, Morales et. al noted the importance of the length of the segments involved in the development of the symptoms (4). In our cases, it's seen that the length of the myocardial segments involved are about 2 cm.s. In the management of myocardial bridges, beta-blockade drugs, myotomy and intracoronary stant are the options. Although, beta-blockade drugs obtain a short term recovery, long term benefits could't be approved (6). The patients should be promptly evaluated clinically and angiographically before surgical decision. Untolerable angina, unsufficient response to the medical management, malign arrthymia, frequent syncope attacks should direct us to the surgery. We showed in these cases without cardiopulmonary bypass, myotomy ceases the ischemic symptoms of the patients.

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