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2. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

3. Lawton J, Ahmad N, Hallowell N, Hanna L, Douglas M. Perceptions and experiences of taking oral hypoglycaemic agents among people of Pakistani and Indian origin: qualitative study. BMJ 2005;30:1247-51. doi:10.1136/bmj.38460.642789.EO

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Cryopreservation triggers DNA fragmentation and ultrastructural damage in spermatozoa of oligoasthenoteratozoospermic men

Kriyopreservasyon oligoastenoteratozoospermik erkeklerde DNA fragmantasyonunu ve ultrastrüktürel hasarı tetikler

Pınar TURAN, Gözde ERKANLI ŞENTÜRK, Feriha ERCAN

ABSTRACT

Objective: Oligoasthenoteratozoospermia (OAT) is one of the causes of male infertility. Cryopreservation is valuable for the management of infertility. This study aimed to reveal the effects of cryopreservation on sperm motility, morphology, ultrastructural details and DNA fragmentation in patients with OAT.

Materials and Methods: In this observational study, ejaculates were collected from 40 male volunteers of whom 20 were OAT and 20 were normospermic. The ejaculates were stored in liquid nitrogen at -196°C and analysed following thawing after 1 or 3 months. Ejaculates were evaluated in terms of motility, morphology and DNA fragmentation before and after thawing.

Results: Sperm motility and morphology were both affected by cryopreservation in samples from both groups. After thawing, spermatozoa with morphological abnormalities were increased significantly in both groups. Ultrastructural investigations showed alteration in integrity of the membranes and increased acrosomal defects. Post-thaw investigations revealed prominent increases in the number of DNA fragmented cells in both groups when compared to the results before freezing. OAT groups revealed a significantly higher number of DNA fragmented spermatozoa compared to the normospermic group for both time periods.

Conclusion: Cryopreservation produces ultrastructural damage and induces DNA fragmentation in both normospermic and OAT groups. The damage is more severe in the OAT group.

Keywords: Cryopreservation, Oligoasthenoteratozoospermia, DNA fragmentation, Ultrastructure

ÖZ

Amaç: Oligoastenoteratozoospermi (OAT) erkek infertilitisi sebeplerinden biridir. Kriyopreservasyon infertilite yönetimi için değerlidir. Bu çalışmanın amacı, OAT'lı hastalarda sperm motilitesi, morfolojisi, ultrastrüktürel detaylar ve DNA fragmentasyonu üzerine kriyopreservasyonun etkilerini ortaya çıkarmaktır.

Gereç ve Yöntem: Bu gözlemsel çalışmada, gönüllü 20 normospermik ve 20 OAT'lı hastadan ejakulatlar toplanmıştır. Ejakulatlar, -196°C sıvı nitrojende saklanmış ve çözdürmeden üç ve altı ay sonra analiz edilmiştir. Ejakulatlarda, motilite, morfoloji ve DNA fragmantasyonu dondurma öncesi ve sonrasında değerlendirilmiştir.

Bulgular: Her iki grupta da sperm motilitesi ve morfolojisi kriyopresvasyondan etkilenmiştir. Her iki grupta da çözdürmeden sonra morfolojik değişiklikler anlamlı artmıştır. Ultrastrüktürel incelemeler membran bütünlüğünün değiştiğini ve akrozomal hasarın arttığını göstermiştir. Her iki grupta da, dondurma öncesi ile karşılaştırıldığında, çözdürme sonrasında DNA kırıkları olan hücrelerin arttığı gözlenmiştir. Her iki dondurma-çözme periyodunda, kontrol grubu ile kıyaslandığında OAT grubunda DNA kırıkları olan spermatozoa sayısının anlamlı olarak daha yüksek olduğu gözlenmiştir.

Sonuç: Kriyopreservasyon, hem normospermik hem de OAT gruplarında ultrastrüktürel hasar yapmaktadır ve DNA hasarını indüklemektedir. Bu hasar OAT hastalarında daha ciddidir.

Anahtar kelimeler: Kriyopreservasyon, Oligoastenoteratozoospermi, DNA fragmantasyonu, ultrastrüktür

Introduction

Sperm cryopreservation is more frequently used in assisted reproductive techniques for the management of infertility. Donor semen is cryopreserved where the reproductive capacities of men with different malignancies will be affected by necessary surgery, radiotherapy or chemotherapy. However, for infertile men with different degrees of oligoasthenoteratozoospermia (OAT), the use of frozen and

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thawed spermatozoa has also been suggested for assisted reproduction [1]. Many of the procedures of cryopreservation produce important functional and morphological changes that reduce the fertilization potential [2]. During the cryopreservation process spermatozoa sustain physical and chemical stresses. It has been reported that the lipid structures of the cell membranes are damaged and this is relevant to the decreased motility and altered morphology of spermatozoa produced by the cryopreservation process [3, 4].

Apoptosis balances the generation and reduction of cells during their lifespans. The most significant alterations concerning apoptosis are phosphatidylserine production, DNA fragmentation and the creation of apoptotic structures [3]. Decreased sperm quality, increased DNA fragmentation and mitochondrial dysfunction have been reported in OAT patients [5]. It was also shown that cryopreservation-thawing techniques reduced motility, viability and fertilizing ability of sperms in teratospermic patients more than in normospermic men [6]. DNA integrity, morphology and ultrastructure of sperm after cryopreservation-thawing have not been studied in OAT patients with different time periods.

The aim of this study is to evaluate effects of freezing in different time periods on sperm motility, morphology, DNA fragmentation and ultrastructure in OAT and normospermic men.

Materials and Methods

In this observational study, two groups were formed as normospermic (n=20, mean age 29.1 years), and OAT (n=20, mean age 35.6 years) groups. The study was approved by Istanbul No: 2 Clinical Researches Ethical Committee (2009/2530-13, 09.09.2009). Ejaculates were obtained from volunteers. All volunteers gave informed consent prior to participation in the study. All microscopical analysis was done by blind observers.

Selection criteria

The selection criteria for the normospermic and OAT groups were being between age 25 to 50 years; having no history of any inflammatory, systemic or other diseases that could affect reproductive functions; having no history of testicular injury; not using any medications (medicine for tension pills, kidney diseases, antidepressants, alcohol and smoking etc.); and being normospermic in terms of criteria of the WHO 2010 laboratory manual for the evaluation of human semen [7]. Infertile patients with OAT were selected as follows: having a sperm density of < 15 million/mL; having progressive (PR) and nonprogressive (NP) motility (PR+NP) of 40% with a decrease in progression of 32% and abnormal morphology < 4%. Ejaculates were put into sterile containers after a 3-day sexual abstinence.

Sperm motility

Sperm motility was evaluated by a light microscopic (Olympus BX51, Tokyo, Japan) observation before freezing and after thawing at 1 or 3 months following the freezing. A Makler Counting Chamber (Sefi Medical Instruments, Haifa, Israel) was used. Sperm motility was labelled as "PR", "NP" or "immotile (IM)" according to WHO 2010 laboratory guidelines.

Sperm freezing and thawing procedures

We applied the sperm freezing and thawing procedure as described in a previous study [8]. Each ejaculate was divided into three equal parts after a liquefaction process lasting for 45 min at 37 ^{o}C. One part of the ejaculate was evaluated just after the liquefaction. The remaining parts of the ejaculate were frozen for 1 or 3 months respectively, and evaluated after thawing. Semen samples and cryoprotectant (MediCult, Origio, Malov, Denmark: Sperm freezing medium, 10670010) were mixed at the rate of 1:1 for the freezing procedure and were collected into cryovials (Simport Scientific, Beloeil, QC, Canada: CryoVial®, T308-2A) with a maximum volume of 1 ml. Before being placed in liquid nitrogen (-196 ^{o}C), cryovials were kept for 10 min at room temperature, and for 30 min in nitrogen vapor. Thawing procedure was carried out at the room temperature for 5 min after the cryovials were removed from the liquid nitrogen. After thawing of ejaculates, a washing solution (Vitrolife, Englewood, CO, USA: G-Sperm™ Plus, 10107) was added and the samples were centrifuged at 1200 rpm for 2 x 10 min. Then, after removal of the cryoprotectant, the superrnatant was collected. After thawing processes, spermatozoa were evaluated for their motility, morphology, DNA fragmentation and ultrastructure.

Histological preparation

Sperm morphology was evaluated in smear slides stained with Diff-Quick kit (Medion Diagnostics, Gräfelfing,

Germany) at x100 magnification with a photomicroscope (Olympus BX51, Tokyo, Japan) according to Kruger's strict criteria.

TUNEL assay

DNA fragmentation was evaluated by a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit (in situ cell death detection kit, Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the manufacturer's guidelines. The air-dried smears were fixed in 4% paraformaldehyde at room temperature rinsed in phosphate buffered saline (PBS), pH 7.4, and then permeabilized with 2% Triton X-100. The TdT-labeled nucleotide mixture was added to each slide and incubated in a humidified chamber at 37 °C for 60 min in the dark, then rinsed in PBS and counterstained with 10 µl 4',6-diamidino-2-phenylindole (DAPI) II (Abbott Laboratories, Salt Lake City, UT, USA, 06J50-001). For each slide, approximately 200 cells were analysed with a fluorescence microscope (Olympus BX51, Tokyo, Japan). Each spermatozoon was classified as either a normal (blue nuclear fluorescence due to DAPI II) or fragmented DNA (green nuclear fluorescence). The final percentage of spermatozoa with fragmented DNA was referred to as "% TUNEL-positive cells" for each sample.

Ultrastructural preparation

For ultrastructural evaluation, the ejaculates were incubated in a washing solution after liquefaction, centrifuged at 1500 rpm for 10 min and the supernatant was removed. The resulting pellet was prefixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.2) at 4°C for 4 hours, washed with PBS, post-fixed with 1% OsO_4 (0.1 M, pH 7.2) for 1 hour, and then processed for routine electron microscopical study. The ultrastructure of the spermatozoa were examined by a JEOL 1200 EXII transmission electron microscope (TEM, Tokyo, Japan) and photographed by a side mounted digital camera (Olympus Morada Soft Imaging System).

Statistical evaluation

Statistical evaluations were done using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Paired Student's t-tests and one-way analysis of variance (ANOVA) tests were used for comparison of normospermic and OAT groups. For all statistical evaluation, all data were calculated as mean±standard deviation (SD) and $P < 0.05$ was considered significant.

Results

Motility rate

According to the WHO criteria PR+NP motility rate was significantly decreased in the OAT group compared to the normospermic group before freezing ($P < 0.001$). PR+NP motility and IM rates were significantly altered in both the normospermic and the OAT groups after thawing for 1 month or 3 months compared to before freezing in the similar time periods respectively ($P < 0.001$). When the 1 month or 3 month post-thaw OAT groups were compared to those of the normospermic group, PR+NP motility rate and IM rate was decreased and increased, respectively (Table I).

Histological evaluation

In both the normospermic and OAT groups; head, neck, tail and mixed spermatozoa defects relative to normal spermatozoa were observed in pre-freezing, and 1 month and 3 months post-thawing periods (Figure 1). The percentage of normal spermatozoa before freezing was 7.40% (± 0.27) in the normospermic group and 2.10% (± 0.27) in OAT group before freezing. The number of spermatozoa (thawed either 1 month or 3 months later) with normal morphology significantly decreased compared to the pre-freezing group in normospermic and OAT groups ($P < 0.001$). Post-thawing sperm damage increased significantly in both the normospermic and the OAT groups ($P < 0.001$, $p < 0.01$, respectively) compared to the pre-freezing groups. Moreover, comparisons of 1 month or 3 months post-thaw OAT groups with those of the normospermic groups showed that rates of normal spermatozoa and abnormal spermatozoa had decreased and increased, respectively (Table I).

Evaluation of DNA fragmentation

The spermatozoa that contain fragmented DNA were observed as green and normal spermatozoa as blue with florescence microscopy (Figure 2). The rates of DNA fragmentation of spermatozoa were significantly increased in the OAT group ($P < 0.001$) when compared to the normospermic group before freezing. In both normospermic and OAT groups there was a significant increase in the rates of DNA fragmentation of spermatozoa after 1 month (38.35% and 72.95%) and 3

	Motility rate (%)						Morphological defects (%)					
	Before Freezing		AfterThawing				Before Freezing		AfterThawing			
			1st Month		3rd Month				1st Month		3rd Month	
	PR+NP	IM	PR+NP	IM	PR+NP	IM	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Normospermic group (n=20)	65.62 ±2.03	34.90 ±1.62	13.70 ±1.80 +++	86.10 ±1.33 +++	5.05 ±1.05 +++	95.20 ±0.86 +++	7.40 ±0.26	92.70 ±1.01	3.50 ±0.21 +++	96.20 ±1.03 +++	3.20 ±0.28 +++	97.22 ±1.24 +++
OAT group (n=20)	34.80 ±5.31 ***	65.21 ±4.04 ***	7.30 ± 2.12 ααα	92.25 ±1.88 ααα	1.70 ±0.84 ααα	97.80 ±0.78 ααα	2.10 ±0.23 ***	97.95 ±1.23 **	0.50 ±0.11 ααα	99.9 ±1.16 ααα	0.40 ±0.08 ααα	99.95 ±1.23 ααα

Table I: Sperm motility rate and morphological defects in normospermic and OAT groups. $^{**}P<0.01$, $^{***}P<0.001$ and $^{+++}P<0.001$: compared to normospermic group before freezing; $^{ααα}P<0.001$: compared to OAT group before freezing, PR: progressive, NP: nonprogressive; IM: Immotile. Mean age is 29.1 and 35.6 in normospermic and OAT men, respectively. Values are means±SD.

months (53.60% and 84.52%) freezing periods compared to results before freezing (5.20% and 58.60%, $P < 0.001$). On the other hand, there was no statistically significant difference in the rates of DNA fragmentation between the samples of each group that had been frozen for either 1 month or 3 months in both groups. However, comparison of the 1 month or 3 months post-thaw samples of the OAT groups with those of the normospermic group, showed that the rates of DNA fragmentation of spermatozoa were significantly increased ($P < 0.001$, Figure 3).

Ultrastructural evaluation

The spermatozoa in normospermic and OAT groups were evaluated ultrastructurally before freezing and after thawing. Normal spermatozoa with typical head shapes, regular internal and external acrosomal membranes and electron-dense acrosomal ultrastructure were observed more in normospermic than in the OAT groups before freezing (Figure 4). It was also observed that spermatozoa with disturbed head morphology and cytoplasmic residues in the neck region were present in both groups but there were more in the OAT group. Disrupted spermatozoa were more common in the OAT group before freezing. Decreased numbers of normal spermatozoa, a high number of spermatozoa with degenerated acrosome structures, subacrosomal swellings, acrosomal losses, vesicle formation, separation and loss in plasma membrane and chromatin condensation disorders were observed at 1 month or 3 months after freezing in the OAT groups. Moreover, ultrastructural changes were qualitatively quite severe in the OAT group compared to normospermic group after 1 month and 3 months freezing periods.

Discussion

In the present study, the spermatozoa of normospermic and OAT groups were compared both before freezing and after freezing and thawing regarding sperm motility, histology, DNA fragmentation and ultrastructural features. In both groups, there was a decrease in PR+NP motility rate, an increase in structural damage and DNA fragmentation after the freezing and thawing procedures. Number of TUNEL positive cells after the freezing and thawing procedure in the OAT groups at 1 month and 3 months were significantly increased compared to those of the normospermic groups for similar time periods. The ultrastructural examinations qualitatively showed that most of the spermatozoa had membrane disintegration, acrosomal and tail defects in the OAT groups before and after freezing.

Sperm cryopreservation is wide-ranging clinical application but present procedures are not perfect, and especially semen samples from OAT patients there is an increased vulnerability to cryoinjury [1]. It is also important to demonstrate the ratio of functional sperms after thawing in OAT patients, as it has been reported that sperm from OAT patients have more DNA damage, mitochondrial dysfunction and chromosomal aneuploidy than normospermic men [5].

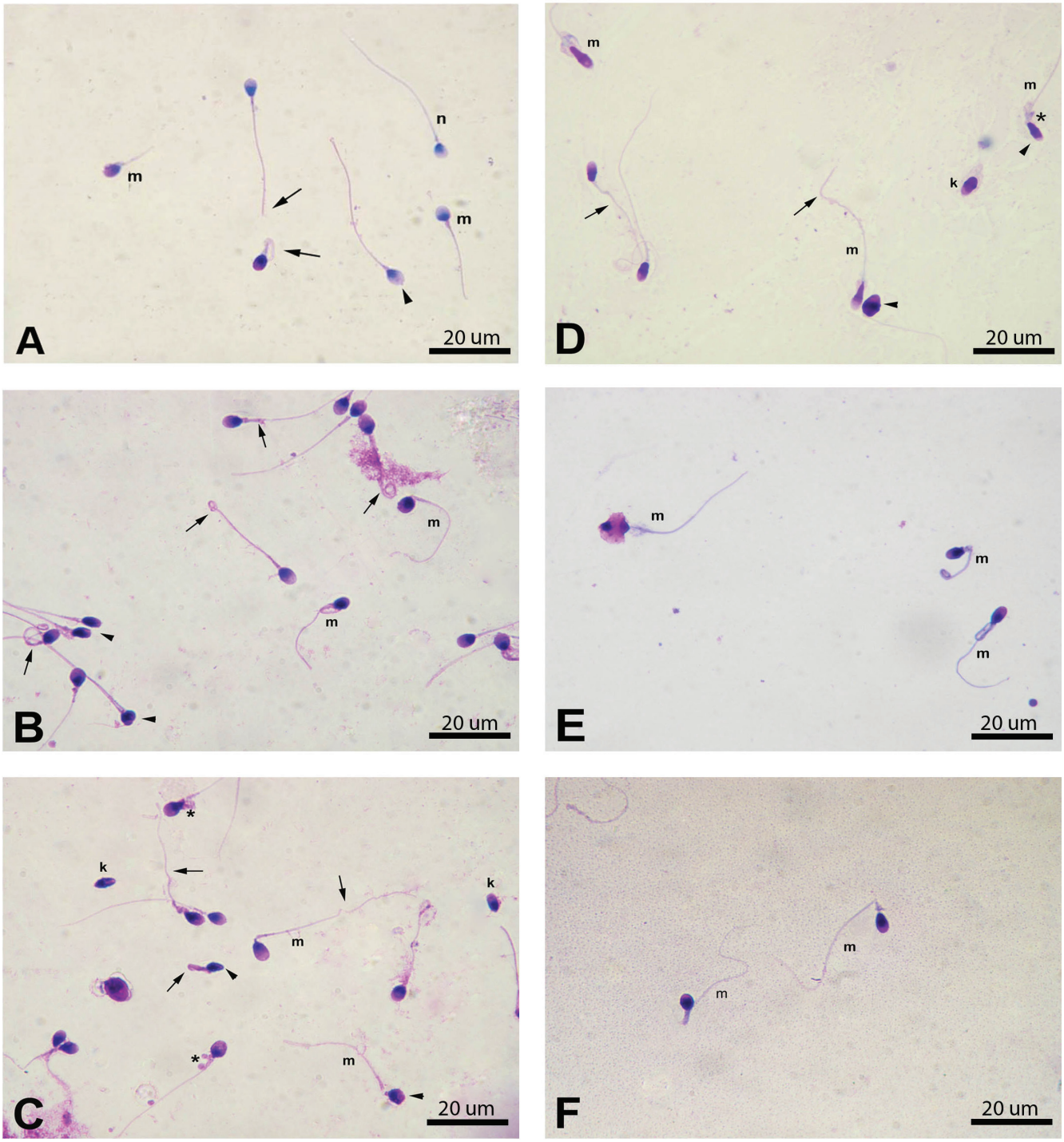

Figure 1. Represantative light micrographs of normospermic group (A, B, C) and OAT group (D, E, F) before freezing (A, D) and thawing after 1 month (B, E) and 3 months (C,F) are seen. Normal spermatozoon (n), head (arrowhead), ruptured head (k), cytoplasmic droplet (*), tail (arrow) and mixed (m) defects are seen in experimental groups. Diff-Quick staining.

Extracellular and intracellular ice crystal generation, osmotic shock and cellular dehydration have been reported after freezing and thawing of human sperms [9]. Cooling damage can also change the integrity and structure of cellular membranes [10, 11, 12]. Both the plasma membranes and the mitochondrial membranes have this sensitivity to cryopreservation [13]. A modification of the liquidity of membranes of mitochondria may also cause a modification in mitochondrial membrane potentials and the release of reactive oxygen species [14].

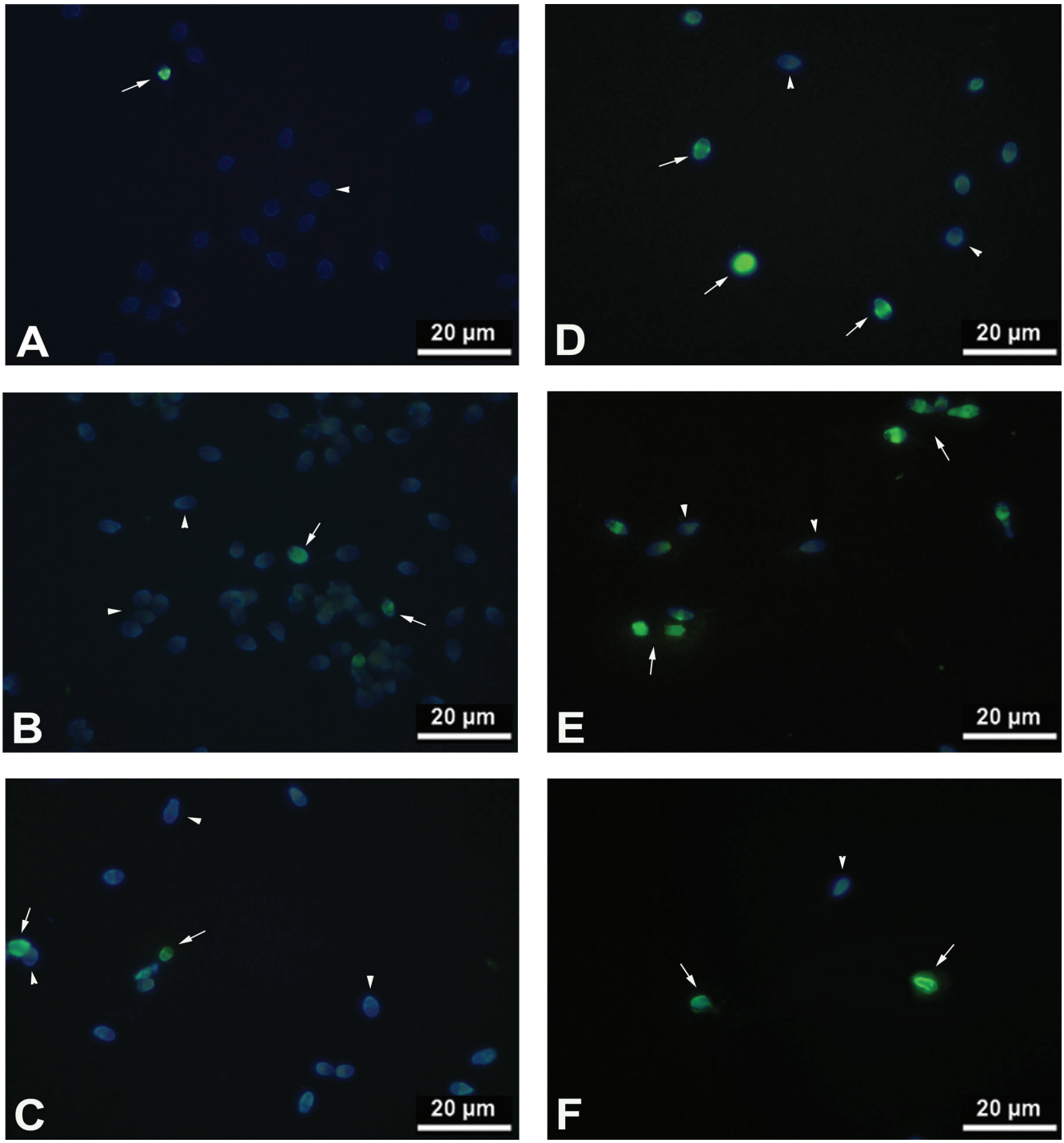

Figure 2. Represantative light micrographs of DNA fragmented spermatozoa of normospermic group (A, B, C) and OAT group (D, E, F) before freezing (A, D) and thawing after 1 month (B, E) and 3 months (C, F). TUNEL-positive sperms (green) (arrow) and TUNEL-negative sperm (blue) (arrowhead) are seen in experimental groups. TUNEL kit and DAPI II staining.

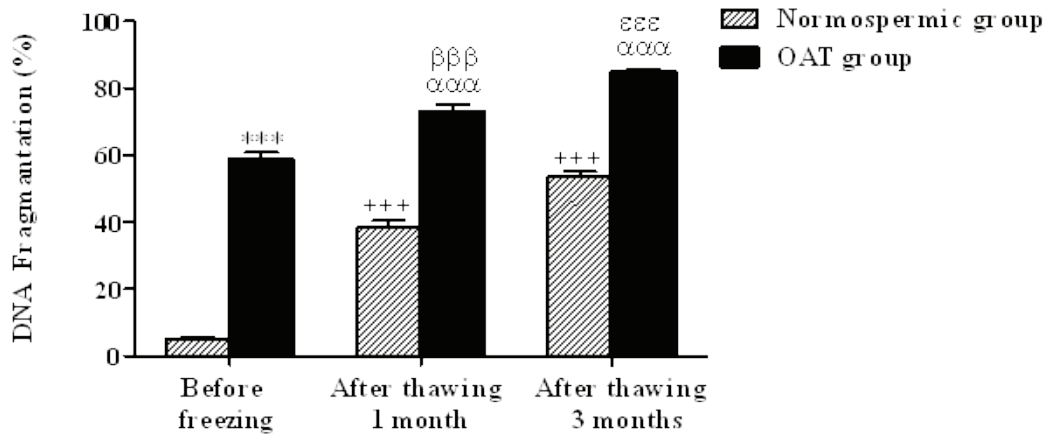

Figure 3. Assessment of DNA fragmented cells ratio. $^{***}P<0.001$ and $^{+++}P<0.001$, compared to normospermic group before freezing; $^{\alpha\alpha\alpha}P<0.001$, compared to OAT group before freezing; $^{\beta\beta\beta}P<0.001$, compared to normospermic group thawing after 1 month, $^{\varepsilon\varepsilon\varepsilon}P<0.001$, compared to normospermic group thawing after 3 months.

The peroxidative impairment induced by increased concentrations of reactive oxygen species is related to the detoriation of the axonemal structure and damage to the spermatozoa membrane [15]. It has also been shown that cryopreservation reduces the antioxidant activities of the spermatozoa, making them more sensitive to reactive oxygen injuries [16]. Elevated concentrations of reactive oxygen species and decreases of antioxidant enzymes cause cell death after cryopreservation [17, 18]. It has been reported that normospermic samples have higher levels of polyunsaturated fatty acids than do oligospermic and asthenospermic samples and that the total antioxidant capacity was higher in normospermic samples than the oligospermic and oligoastenospermic groups before freezing. It was described that the fatty acid constitution of the spermatozoa or seminal plasma has been described as directly related to sperm viability and motility after freezing and thawing [19].

It has been reported that cryopreservation causes structural disorganisation such as spiral tails, degenerated acrosome and plasma membranes [20], cell death and reduced numbers of vital spermatozoa [21]. Cryopreservation also causes an increase in DNA damage [22]. In the present study we have observed a reduction in numbers of normal spermatozoa and an elevation in abnormal spermatozoa having head, neck or tail defects in both the normospermic and OAT groups thawed after 1 or 3 month-long freezing periods. The number of spermatozoa with multiple signs of damage was increased in both groups after thawing. Sperm with normal morphology and sperm with abnormal head morphology, with a disorganised chromatin condensation or cytoplasmic residues in the neck region were observed in the ultrastructural material fixed before freezing.

It had earlier been shown that cryopreservation induces subacrosomal and acrosomal swelling and vesiculation, as well as separation in the cellular membranes [23, 24]. Parallel to this, we also saw that sperm with degenerated acrosomal structures, vesicle formation and loss of the acrosome, subacrosomal swellings, detoriation of plasma membranes and disturbed mitochondrial organisation in the neck region. In an earlier study, similar ultrastructural alterations were also observed after thawing in samples from fertile smoker volunteers [8].

In our study, there were significant increases in multiple head damage in the OAT groups compared to normospermic groups after thawing by light and electron microscope levels. This indicated that the degeneration of membrane, chromatin and acrosome were more severe in the OAT groups. Ultrastructural studies showed injury mechanisms related to spermatozoa in OAT patients after thawing.

Stability of the DNA content of the spermatozoa is definitely crucial due for the paternal contribution to fertilization and for avoiding early embryonic death [25] and the normal progression of the pregnancy [26]. It has been reported that, oxidative stress leads to degeneration of cellular membranes and impairs the DNA integrity of spermatozoa. Damage to DNA caused by reactive oxygen species expedites the death of spermatozoa [27]. In the present study, we detected the DNA fragmentation of spermatozoa by the TUNEL method before freezing, and

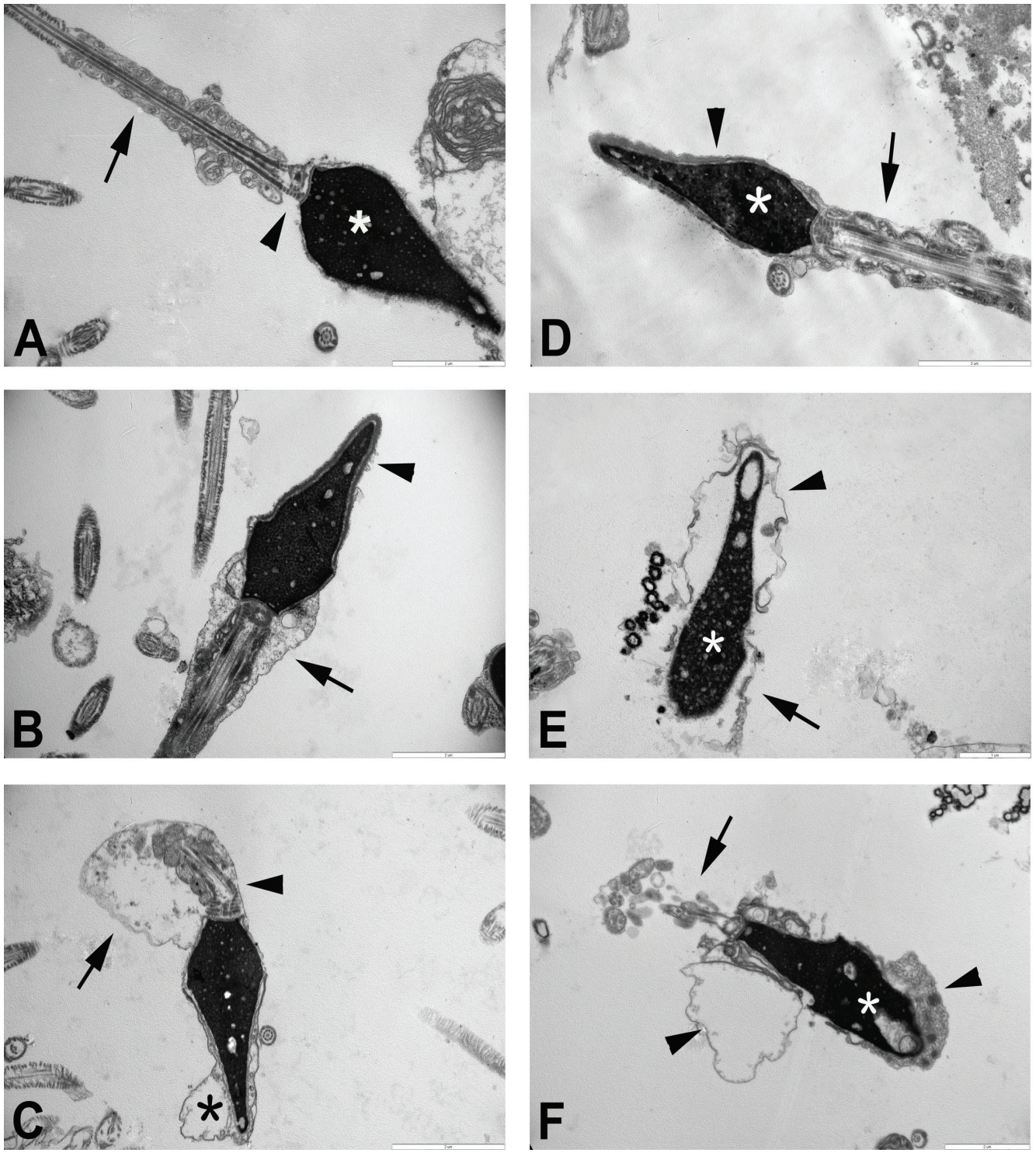

Figure 4. Represantative transmission electron micrographs of normospermic group (A, B, C) and OAT group (D, E, F) before freezing (A, D) and thawing after 1 month (B, E) and 3 months (C, F). (A) Normal spermatozoon with entire head (*), neck (arrowhead), and midpiece structure (arrow) before freezing; (B) rather normal spermatozoon with entire head (arrowhead), neck (arrow), and membranes thawing after 1 month; (C) disintegration of membranes of acrosome (*), degenerated neck (arrowhead) and tail (arrow) thawing after 3 months in normospermic group. (D) Normal spermatozoon with entire head (*), acrosome (arrowhead), membranes and midpiece ultrastructure (arrow) before freezing; (E) disintegration of chromatin condensation (*), acrosomal swelling (arrowhead) and degeneration of membranes (arrow) thawing after 1 month; (F) vacuole formation in head (*), acrosomal swelling and degeneration (arrowhead), degenerated mitochondria (arrow) and loss of membranes with tail defect thawing after 3 months in OAT group.

after thawing at 1 month and 3 month in the normospermic and OAT groups. There was a significantly higher DNA fragmentation rate in the OAT groups compared to normospermic groups before the freezing procedure. This is probably due to altered oxidant/antioxidant composition of semen/spermatozoa of the OAT patients.

Seminal plasma preserves the sperm from adverse factors such as oxidative stress, although these protective effects were altered in infertile patients [28]. However, seminal plasma has also been shown to contain cells such as aging sperms, leukocytes and epithelial cells that can generate reactive oxygen species and can lead to oxidative and apoptotic injury during the freezing and thawing procedure [14]. Another study reported that sperm selection by density gradient centrifugation before freezing allows the selection of a better functional sperm population with more resistance to the cryopreservation processes [29]. In the present study, we used spermatozoa obtained by density gradient centrifugation and washing to eliminate the leukocytes and epithelial cells that might produce oxidative stress. However, an increase in rates of DNA fragmentation was statistically significant in the thawed sperms of both normospermic and OAT groups after the 1 and 3 months freezing periods. This might be due to increases in the impairment of spermatozoa morphology and membrane permeability caused by physical and chemical stress based on cryopreservation. For both the 1 month and 3 months freezing periods, DNA fragmentation in the OAT group was more severe than the corresponding normospermic groups. Approximately, 70% of spermatozoa after 1 month and 85% of spermatozoa after 3 months freezing in the OAT group were TUNEL-positive. These results should be related with storing of spermatozoa in artificial chilling condition, and it made spermatozoa of OAT patients more sensitive than the normospermic men. These results demonstrate that the freezing and thawing process and the period of freezing are critical to save functional sperm populations in OAT patients.

Conclusion

Cryopreservation causes DNA fragmentation and damage in sperm ultrastructure of both normospermic and OAT groups; however these results were more severe in the OAT group. In order to detect abnormalities of spermatozoa, use of histochemical and electron microscopic techniques supply more elaborate results than the use of routine histological techniques. The severe decrease in functional sperms and the increase in DNA fragmentation following cryopreservation in the OAT group compared to the normospermic group after 1 month and 3 months freezing indicates that improved methods are needed. The mechanisms of DNA fragmentation after cryopreservation techniques in sub fertile men should also be studied in detail so that assisted reproduction can be more effective. New techniques are needed for cryopreservation of sperm from OAT patients. For minimizing the formation of physical and chemical stresses on sperm structure during cryopreservation, the method needs to be improved by the use of chemical agents such as antioxidants that prevent the formation of reactive oxygen species.

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Influence of rosuvastatin treatment on airway inflammatory markers and health related quality of life domains in asthmatic patients

Astımlı hastalarda rosuvastatin tedavisinin havayolu inflamatuvar belirteçleri ve yaşam kalitesi göstergeleri üzerine etkisi

Sehnaz OLGUN YILDIZELI, Derya KOCAKAYA, Baran BALCAN, Aygun IKINCI, Rengin AHISKALI, Berrin CEYHAN

ABSTRACT

Objectives: Statins are lipid lowering agents which have pleiotropic and anti-inflammatory effects. Beneficial effects of statins have been shown in many inflammatory and asthmatic diseases. However, the results are still not consistent. The aim of this study is to determine the clinical and anti-inflammatory effects of rosuvastatin in asthmatic patients.

Patients and Metods: A case control study among asthmatic patients was conducted. One hundred and thirty-six participants were screened. Seventy-four patients were eligible. Fifty-one patients have completed the trial. Twenty-five patients with blood levels of low-density-lipoprotein (LDL) above 130mg/dL, were treated with rosuvastatin 40mg for 8 weeks in addition to their standard treatment for asthma; 26 asthmatic patients were followed as control group. In both groups baseline and 8th week evaluation were recorded including pulmonary function test (PFT), bronchial provocation test (PD20), induced sputum and serum inflammatory markers, asthma control test (ACT) and quality of life scores (QoL).

Results: Statin group showed improvement in FEV1/FVC (pp) (85.8±11.1% vs 90.2±8.8% P<0.043), FEF 25-75 % (63.6±7.8 % vs 74.6±8.4 %, P<0.0001) and FEF25-75(L/sc) (3.51±0.4 vs 4.1±0.4 P<0.05) and no change was seen in non-statin group (P>0.05) at the end of the 8-week treatment. Treatment with rosuvastatin resulted in decreased sputum eosinophilia percentage (P<0.05); IL-6 and TNF-alpha levels (P<0.05) however, bronchial challenge test, ACT and QoL domains did not change in both groups (P>0.05).

Conclusion: An 8-week treatment with 40mg rosuvastatin in asthma decreased the peripheral eosinophilia, total IgE levels and inflammatory markers in the induced sputum samples. Beneficial effects in PFT have also been observed. However, ACT and QoL domains were not affected. The implication of this study is that rosuvastatin could potentially have anti-inflammatory effects on asthmatic airways. Prospective randomised trials to evalute the clinical effects of rosuvastatin are warranted.

Keywords: Asthma, Airway inflammation, Remodelling, Rosuvastatin, Statin

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ÖZ

Amaç: Statinler kan lipid düzeylerinde azalma sağlamakla beraber aynı zamanda pleiotropik ve anti-inflamatuvar etkileri olan ilaçlardır. Statinlerin bir çok inflamatuvar hastalıkta yararlı etkileri gösterilmiş olmasına karşın sonuçlar halen tartışmalıdır. Bu çalışmanın amacı astım hastalarında rosuvastatin tedavisinin klinik ve anti-inflamatuvar etkilerini incelemektir.

Hastalar ve Yöntem: Astım hastalarında vaka-control çalışması planlanmıştır. Toplam 136 astım hastası incelenmiş ve 74 hasta çalışmaya uygun bulunarak dahil edilmiştir. Elli bir hasta çalışmayı tamamlayabilmiştir. Hastalar başlangıçta yapılan kan tahlilinde serum düşük dansiteli lipoprotein (LDL) seviyesine göre iki gruba ayrılmıştır, LDL düzeyi 130mg/dL üzerinde olan 25 hasta mevcut tedavilerine ek olarak 8 hafta boyunca 40mg rosuvastatin tedavisi alırken, 26 hasta kontrol grubu olarak almakta oldukları tedaviye devam etmişlerdir. Her iki gruptaki hastalar, başlangıçta ve 8. haftanın sonunda solunum fonksiyon testi (SFT), bronş provokasyon testi (PD20), indükte balgamda serum inflamatuvar belirteçleri, astım kontrol testi (AKT) ve yaşam kalitesi skorları (QoL) ile değerlendirilmişlerdir.

Bulgular: Statin kullanan grupta 8. hafta sonunda SFT'de FEV1/FVC (pp) (%85,8±11,1 vs .%90,2±8,8 P<0,043), FEF % 25-75 (% 63,6±7,8 vs %74,6±8,4, P<0.0001) ve FEF25-75(L/sc) (3,51±0,4 vs 4,1±0,4 P<0,05) düzelme saptanırken statin kullanmayan grupta anlamlı fark saptanmamıştır (P>0,05). Rosuvastatin tedavisi ile indükte balgam eosinofilisinde (P<0,05); IL-6 ve TNF-alfa seviyelerinde azalma (P<0,05) saptanırken, AKT ve QoL skorları ve bronş provakasyon testinde anlamlı değişiklik saptanmamıştır.

Sonuç: Bu çalışmada, astım hastalarında rosuvastatin 40mg ile 8 haftalık tedavi sonrasında periferik eosinofili, total IgE seviyesi, indükte balgamda inflamatuvar belirteçlerde anlamlı düzeyde azalma saptanmıştır. Yine SFT parametrelerinde düzelme izlenmiştir ancak buna karşın AKT ve QoL skorlarında değişim saptanmamıştır.Bu sonuçlara göre rosuvastatin tedavisinin astım hastaları üzerinde bir takım potensiyel anti-inflamatuar etkileri bulunmaktadır. Rosuvastatinin klinik etkilerinin saptanması için daha fazla sayıda randomize çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Astım, Havayolu inflamasyonu, Remodeling, Rosuvastatin, Statin

This study was presented as a poster in the European Respiratory Society Congress in 2011 Barcelona and presented as an oral presentation in Turkish Thoracic Society Annual Meeting in 2011 Antalya

Introduction

Asthma, a common global health problem, remains as one of the most prevalent chronic inflammatory airway diseases with a trend of increase in both prevalence and annual cost. It has been strongly associated with bronchial hyperreactivity and airway remodelling. Frequent hospitalization, emergency department visits, and early deaths still have been reported despite guideline-depended treatments. It is the fact that novel managements are needed to control asthma.

The statins have been known to decrease the blood cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A which is the rate-limiting enzyme of cholesterol synthesis [1]. Additionally, it has been demonstrated that statins exhibit anti-inflammatory and immunomodulatory effects. The pleiotropic anti-inflammatory actions have been shown in several diseases. Previous studies have reported that statin use in asthmatic population decreases sputum eosinophilia [2], use of asthma medications, asthma related hospitalization and emergency department visits [3,4]. Furthermore, some studies have reported that statin therapy improves quality of life (QoL) scores in asthmatics, [5], whereas some others have failed to replicate these results [6,7] thus the potential therapeutic role of statins related to anti-inflammatory action in asthma remains an open question.

In the current study, we aimed to assess the impact of statin treatment on pulmonary function test (PFT) including bronchial challenge test inflammatory and allergic markers, asthma control test (ACT) and QoL.

Patients and Methods

Subjects

This case-control study was conducted between December 2009 and March 2011 at the Asthma Clinic of our hospital among asthmatic patients as defined by the global initiative for asthma (GINA) guidelines [8]. The study was approved by the University Ethics Committee (MAR-YC-2009-0009) and all patients provided written informed consent prior to participation.

Inclusion criteria were; age of patients between 18-80 years old, known asthmatic patients according to the GINA criteria; positive methacholine challenge test and no history of anti-hyperlipidemia treatment. The exclusion criteria were; pregnancy, any medical contraindication for usage of statins, diagnosis of chronic obstructive pulmonary disease (COPD), history of cancer and history of smoking >10 pack-years. As shown in Figure 1, 136 consecutive patients with known asthma were eligible. Among them, 24 were excluded due to negative methacholine test, and 38 were

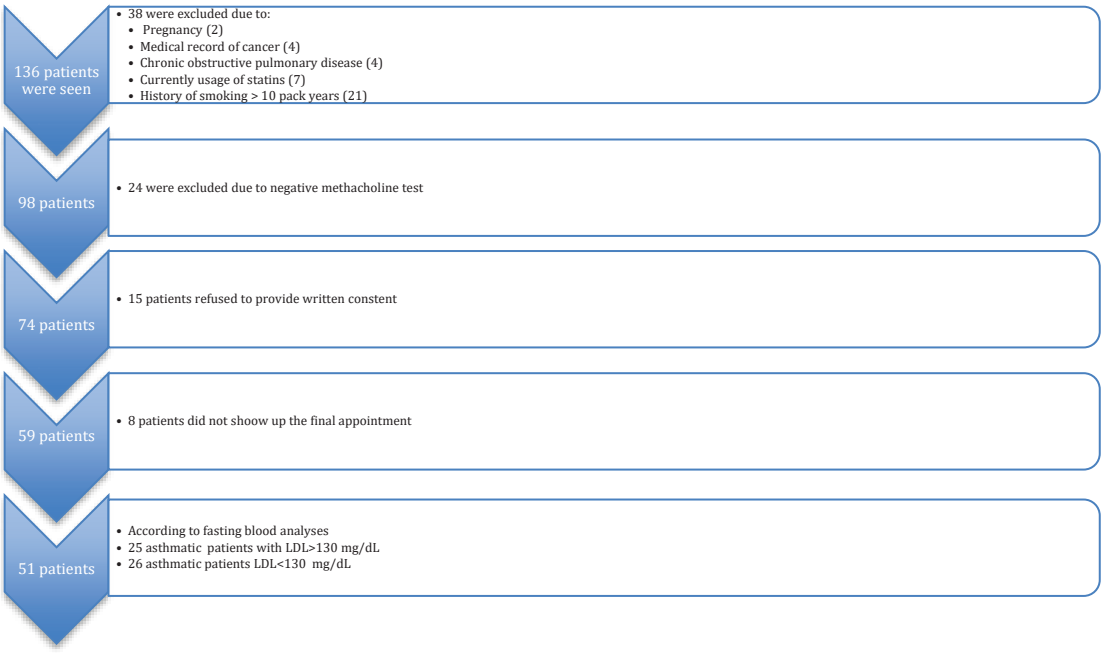

Figure I: Consort diagram of subjects

excluded due to the other exclusion criteria described above. Fifteen individuals refused to provide an informed consent and 8 asthmatics did not come to the final appointment. Thus, 51 patients constituted the final study population (Figure I).

Group assignment

The cases were the asthmatic patients with LDL≥130mg/dL (n=25), who were given 40mg rosuvastatin treatment daily in addition to standard asthma therapy. The control group was the asthmatic patients with LDL <130mg/dL (n=26) who were given standard asthma treatment. Both groups were evaluated at baseline and after 8 weeks.

Measurements

Patients underwent physical examination and spirometry according to the American Thoracic Society criteria [9] in order to assess the forced expiratory volume in 1 second (FEV_1) and other parameters of PFT (MIR Spirolab II Italy). A methacholine bronchial provocation test was performed with Koko dosimeter (Devilbiss 626) using a standardized technique [10]. We also assessed current medication use (inhaler corticosteroids [ICS]), long-acting beta-2 agonists, leukotriene receptor antagonist and oral steroids) and whether the patient had been hospitalised or treated in the emergency department for asthma in the previous year.

Positive skin prick test was defined as a wheal diameter ≥ 3mm in response to aeroallergens (SAY ILAC, Turkey). IgE levels were evaluated using the chemiluminescent immunometric method in Immulite 2000 (Siemens Healthcare Diagnostics, USA). A total IgE level >100 IU/L was defined as high. Peripheral eosinophilia was determined on peripheral blood smear (patients categorized as having eosinophilia if ≥3%).

We assessed asthma control and life quality using the Turkish translation of the ACT and QoL Questionnaire. In the ACT, a score of 19 or less was used to define poor control [11]. A change at least 0.5 in the QoL Questionnaire was of clinical relevance.

Sputum induction and processing

The induced sputum was assessed according to European Respiratory Society guideline criteria [13]. All subjects produced an adequate aliquot of sputum, which was produced within termination of the induction. Cell smear preparations were made using a cytospin cytocentrifuge (500g force, two minutes). Gram staining was used to differentiate cells and counts within the sputum cytospin slides, conducted by a cytologist to identify 400 non-squamous cells. The supernatant was stored -80 degrees C for subsequent assay for IL-2 (Dia Source KAP1241, Nivelles- Belgium), IL-4 (Dia Source KAP1281, Nivelles-Belgium), IL-6 (Dia Source KAP1261, Nivelles- Belgıum), IL-8 (Dia Source KAP1301, Nivelles- Belgium), IL-10 (Dia Source KAP1321, Nivelles- Belgium), TNF-alpha (Dia Source KAP1751, Nivelles-Belgium). Concentrations were determined using the Enzyme Amplified Sensitivity Immunassay method.

Statistical analysis

Changes were described as delta within groups (8 weeks vs baseline) and compared with paired t-test, respectively. Delta changes between groups were compared with unpaired t-test. All descriptive variables between groups were compared with the chi-square test. Due to the wide range of PD20 values of methacholine provocation test, logarithmic (log10) transformation was performed. For the evaluation of association between inflammatory cells and cytokine levels in sputum and lung function parameters, ACT and QoL scores, Spearman correlation test was used. A p value of less than 0.05 was considered significant. Primary sample size calculation was based on assumption that mean FEV_1 would increase by approximately 0.10 L/sec (± 0.12 L/sec) with 23 patients in the statin group with 80% power at the 0.05 level.

Results

Demographic findings

As illustrated in Figure 1, 51 patients constituted the final study population (25 statin group vs 26 non-statin group). Female-to-male ratio was 23/2 and 25/1, and mean age was 45.6 ± 9.1 in statin group and 42.8±9.4 in non-statin group (P>0.05). Asthma severity (severe 3.9%, moderate 33.7% and mild 62.7%) was comparable between groups. There was no statistical difference between the groups regarding, comorbidities and medication (Table I).

Table I; Patient demographics, disease characteristics and medication

	Statin group (n=25)	Non-statin group (n=26)	*P* Value
Diagnosis of comorbid diseases	6	4	>0.05
Positive skin prick test	14	16	>0.05
Mild asthma	15	12	>0.05
Moderate asthma	9	12	>0.05
Severe asthma	1	2	>0.05
ICS**	3	2	>0.05
ICS+LABA***	22	24	>0.05
Oral Steroid	1	1	>0.05

*: Congestive Heart Failure and/or Diabetes Mellitus and/or Chronic Renal Failure
**: Inhaled corticosteroid
***: Inhaled corticosteroid + Long acting beta-agonist
****: Leukotriene receptor antagonist

Pulmonary function test

As shown in Table II, there was no statistical difference between the groups at baseline regarding the PFT results. No significant change in FEV_1, but significant improvements in FEV_1/FVC (%) (85.8±11.1 vs 90.2±8.8), FEF_{25-75} pp (%) (63.6±7.8 vs 74.6±8.4 and FEF_{25-75} (L/sec) (3.51±0.4 vs 4.1±0.4) were observed in the statin group at the end of 8 weeks (Table II). No significant changes were observed in the non-statin group. Between-group differences regarding delta change from baseline reached the significance level for FEF_{25-75} pp and FEF_{25-75} (L/sec).

Bronchial challenge test

Mean PD20 provocation dose increased from 4.1±2.8mg/ml to 5.1±3.4mg/ml (P>0.05) in the statin group and decreased from 6.4±3.7mg/mL to 5,2±3mg/mL in the non-statin group (P>0.05) however, these changes were not significant (Table III).

Sputum cells and peripheral eosinophilia

Baseline sputum eosinophil percentages were similar between groups, totally 62% of the patients had sputum eosinophilia >%3. Mean peripheral eosinophilia in the statin group showed a significant decline from 3.7+/-1.2% to 2.2+/-1.1% (P<0.05) while in the non-statin group there was no significant change (baseline: 2.9+/- 1.2% vs 8th week: 3 +/-1.5%, P>0.05). These changes showed statistically significant differences between the two groups (statin group **Δ=-**1.5±1.4% vs non-statin group **Δ=** + 0.7±1.3 %) (P<0.05).

Allergy parameters

Total IgE levels showed a significant decline in the statin group (P<0.05). However, in the non-statin group no significant change was observed. Prick test results did not change in both groups (P>0.05), (Table III).

Cytokines

A significant decrease in the level of IL-6, and TNF-α was observed in the statin-group (P<0.05) moreover, comparison of delta changes in both groups revealed a significant decrease in vascular endothelial growth factor (VEGF) and TNF-α levels (P<0.05) (Table IV).

ACT and QoL

Both asthma control questionnaire and QoL questionnaire did not show any significant change at the end of the study period in both groups (Table III).

There was no documented side effect of statin.

Table II; PFT results in statin and non-statin groups.

	Statin group				Non-statin group				
	Before statin treatment	After statin treatment	Δ change	p value	Baseline	8 weeks later	Δ change	*P* value †	*P* value ††
FVC (L)	3.02±0.6	3.04±0.6	1.6±9.8	0.4	2.8±0.9	2.8±0.9	0.02±0.1	0.4	0.2
FEV_1(pp)%	90.4±11.1	89.8±10.7	-0.5±66	0,6	80±18	80,2±20.8	0.1±6.1	0.9	0.6
FEV_1 L/sec	1.85±0.2	1.82±0.2	-0.1±1.1	0.5	1.83±0.37	1.82±0.4	-0.1±0.9	0.6	0.6
FEV_1/FVC (%)	85.8±11.1	90.2±8.8	4.3±10.1	0.043	89.3±13	88.8±12.5	-0.4±10.6	0.8	0.1
FEF25-75 (pp)	63.6±7.8	74.6±8.4	11.±6.6	0.0001	61.7±13	60.8±13.5	-0.8±12.8	0.7	0.000
FEF25-75 (L/sec)	3.51±0.4	4.1±0.4	0.4±1.1	0.05	3.42±0.51	3.39	-0.1±1.4	0.6	0.001

Δ change: Baseline vs 8 weeks statin treatment † p : Within-group comparison of data at baseline vs 8th week (paired t-test),
† † p: Between-group comparison of Δ change in statin group vs non-statin group (unpaired t-test)

Table III; ACT,quality of life scores and allergy test results.

	Statin group				**Non-statin group**				
	Before statin treatment	After statin treatment	Δ change	*P* value	Baseline	After statin treatment	Δchange	*P* value †	*P* value ††
ACT	1±09	1±0.7	-0.06±1	0.7	1.3±0.7	1±1.6	-0.2±0.9	0.2	0.4
QoL	127.7±31.5	130.1±28.4	2.4±22	0.5	122.6±25	124.8±29	3±16	0.3	0.9
Total IgE (IU/L)	215.3±334.0	182.6±320.0	-32.6±233	0.01	125.9±117.0	135.3±142	9.4±40.7	0.7	0.015
Peripherl eosinophilia (%)	3.7+/- 1.2 %	2.2+/-1.1 %	- 1.5±1.4	<0.05	2.9+/-1.2 %	3+/-1.5%	0.7±1.3 %	>0.05	<0.05
PD20 (mg/ml)	4.1±2.8	5.1±3.4	5±19	>0.05	6.4±3.7	5.2±3	-3±11	>0.05	>0.05

Δ change: Baseline vs 8 weeks statin treatment, † p :Within-group comparison of data at baseline vs 8th week (paired t-test)..
† † p: Between-group comparison of Δ Change in statin group vs non-statin group (unpaired t-test)

Table IV; Inflammatory markers in group I and II (baseline, 8th week and change)

	Statin Group				Non-Statin Group				
	Baseline	8 weeks after statin treatment	Δ Change	P value †	Baseline	8 weeks after statin treatment	Δ Change	P value †	P value ††
IL-6 (pg/ml)	18.7 ± 23.4	9.9 ± 37.2	-8.7±40.5	0.000	24.3 ± 23.5	6.7 ± 8.7	-17.5±24	0.09	0.3
IL-5 (pg/ml)	5.2 ± 10.6	8.5 ± 21.5	3.2±19.9	0.2	10.5 ± 32.8	4.4 ± 14.5	-6±31.9	0.6	0.2
IL-8 (pg/ml)	187,3 ± 131	295,3 ± 339	107,9±356	0,4	272,2 ± 321	272,9 ± 257	,8±395	0,3	0,3
IL-4 (pg/ml)	106,9 ± 40.9	140.5 ± 104.2	33.1±112.9	0.8	113 ± 38.4	142.6 ± 74.8	29.5±78.9	0.3	0.8
IL-10 (pg/ml)	20.8 ± 26.7	13 ± 19	-8.0±32.3	0.5	14.6 ± 21.6	14.4 ± 23.5	-0.2±27.2	0.7	0.3
TNF – alpha (pg/ml)	0.91 ± 1.1	0.22 ± 0.7	-0.7±0.8	0.000	0.38 ± 1.2	0.15 ± 0.4	- 0.22±1.37	0.5	0.007

† † *P*: Between-group comparison of Δ Change in statin group vs non-statin group (unpaired t-test)

Discussion

In this study, we evaluated the effects of statins in asthmatic patients. To our knowledge, this is the first study addressing the clinical and immune-modulatory effects of rosuvastatin together in asthmatics patients . Our study results have shown that a daily dose of 40mg rosuvastatin for a duration of 8 weeks is effective in decreasing the level of inflammatory markers such as TNF-α, IL-6 in induced sputum samples, and it improved pulmonary function indices such as FEV_1/FVC (%), FEF_{25-75} (pp) and FEF_{25-75} (L/sec), peripheral eosinophilia and total serum IgE in asthmatic patients. However, we did not find any statistically significant change in ACT, QoL assessments and bronchial challenge test with 8 weeks statin treatment.

Previous publications have reported the clinical efficacy of statin treatment in asthmatic patients. Inhibition of airway remodelling which is secondary to airway smooth muscle proliferation and contraction, goblet cell hyperplasia and blockage of inflammatory cell influx with depleted cytokines release are the most significant effects of statins on asthmatic airways [4]. According to Kwak et al., diminished amounts of eosinophils and macrophages after statin treatment are the proof of decreased airway inflammation [3]. The main surrogate markers of efficacy in these studies were change in respiratory symptoms and PFT values. However, these results are inconsistent. Several randomised controlled studies assessed the effects of statin treatment in asthmatic patients, and six of them did not show any improvement on PFT indices [2,5,14-17). On the other hand, a minor improvement in FEV_1 (P<0.01) with simvastatin treatment and improvement in FEV_1 and all day symptoms with atorvastatin therapy in severe asthmatics have been reported [18]. A recent retrospective study did not show a significant change in PFT of severe asthmatics with the usage of statins [4]. Moreover, mild-moderate asthmatic patients showed no significant change (FEV_1 and FVC) with atorvastatin use [17]. However, patients in our study receiving statin treatment showed improvement in FEV_1/FVC (%), FEF_{25-75} (pp) and FEF_{25-75} (L/sec). The discrepancy between previous and

present findings can be attributed mainly to the difference in the clinical status of participants. Some of these studies showed that statins significantly improved clinical outcomes in severe asthmatics, however, the participants were mostly mild to moderate asthmatics in these studies similar to our study [20]. We also evaluated the effect of statin according to severity of the disease but improvement of PFT did not show any difference statistically, probably this would be related with inconvenient dispersal number of cases in each group.

Another point is the type of the statins that may affect the results; in our study we used rosuvastatin, which has been known to have more powerful pleiotrophic effects than other statins. Differences in dose and duration of drugs make it difficult to conclude that whether statins can improve lung functions or not. Additionally, we identified no significant effect of rosuvastatin treatment on mean methacholine provocation dose. Similar results have been reported with 20 mg atorvastatin treatment for 4 weeks in 22 adult asthma patients compared with placebo group [21]. Contrary to these results, Menzies et al., showed increased metacholine provocation dose levels after 4 weeks simvastatin therapy in the absence of inhaled corticosteroids treatment, but no other improvement in PFT[15].

Asthmatic symptoms evaluated with ACT and QoL using 40mg atorvastatin for 4 weeks have shown improvement in scores, however this was not maintained at the 8th week in a study [5]. Moreover, a recent study has identified higher adjusted mean ACT scores in severe asthmatics with the use of statins [4]. In contrast, these findings were not supported by others [2, 17, 18]. Similarly, in our study we also did not find any significant change in ACT and QoL scores after the 8 week statin therapy. This discrepancy may be due to usage of different statins and different duration period as well as different asthma severities.

Previously, it has been shown that statins have resulted in structural changes in IgE receptor, decreased IgE secretory functions, increased levels of anti-IgE activity and decreased mast cell degranulation [22,23]. The effect may differ based on the type of statin. Interestingly, a decrease in histamine levels were seen with cerivastatin and atorvastatin while it was not seen with simvastatin and pravastatin in a human mast cell line study [24]. Animal studies have also shown a decrease in IgE levels and inflammatory cells in bronchoalveolar lavage (BAL) specimens of asthmatic rats with simvastatin treatment [25]. Although, several studies did not show any effect of statin on peripheral eosinophilia [4,15,17] we found a decline of peripheral eosinophilia in asthmatic patients by using rosuvastatin. There is only one study which evaluated skin prick test and other atopic findings reported partly similar results with our results. They reported that statin usage did not effect IgE levels, skin prick test results and nasal polyp formation [26]. However, we have found a substantial effect of statin treatment on IgE levels. It is also possible that these discrepancies may result from differences in statins used and study duration. Overall, it is likely that statin may have a role in atopism, however, the clinical implications are not clear yet.

The underlying mechanism of statins on asthma is not clear. However, there are some possible mechanisms that may cause reduction in inflammation. Downregulation of gene expression and decreasing the stability of lipid raft formation both result in decreased release of cytokines and chemokines [2]. This proinflammatory cytokines IL-4, IL-5, IL-6, IL-13 and TNF-α, trigger airway inflammation with different pathways [27]. IL-4 effects B cells with the production of IgE which is the important feature of mast cell activation and allergic inflammation, IL-5 increases the eosinophils production and IL-13 has a central role in IgE synthesis [28]. It is well-known that TNF-α has an important role in activation of neutrophils and eosinophils. It increases the cytotoxic ability of eosinophils and smooth muscle contractibility and potential to stimulate fibroblast and mesenchymal cells which is important for airway remodelling in asthma [29]. Studies on patients with asthma exacerbation have shown increased levels of TNF-α in BAL specimens also inhalation of TNF-α in healthy volunteers resulted in increased amounts of cytokine release and neutrophil in sputum samples [30]. Studies targeting TNF-α blockage resulted in the improvement of PFT and asthma symptom scores [31]. Epithelial remodelling in asthma is greatly influenced by TNF-α and IL-1 [32]. Recent studies have shown that statins have suppressive effects on macrophages, antigen presenting cells and natural killer cells (NK) also inhibiting leukocyte extravasation and cause decline in IL-1, IL-6 and TNF-α levels in cell lines [3]. A decrease in the levels of TNF-α in cell-lines with atorvastatin has been previously shown [33]. Further animal studies using ischemia models described the immune-modulatory effects of rosuvastatin by a decrease in the levels of TNF-α, IL-6 and IL-1 in serum and related organ tissues. Researchers proposed that increased levels of IL-10 were related with its anti-inflammatory effects [34]. Similar to the previous studies, we have found a decrease in the levels of IL-6, TNF-α with rosuvastatin in sputum sample of asthmatic

patients. However, an increase in IL-10 levels was not observed. The discrepancy between our study, animal and in vitro studies may be related with the experimental antigen sensitization, and asthma model created mechanisms in animals differ from humans and different dosage in animal studies may be too high for humans. Also, in animal studies, inhaled form of statins are used that directly effect airways but in human studies statins given orally and extensively are absorbed by the liver [35]. In animal studies, despite short-term statin treatment, positive results have been considered to be related with the drug doses and administration route of statins. Finally, patients were classified only according to their blood cholesterol levels in our study, but PFT, PD20 for metacholine provocation, total IgE levels, specific IgE numbers, eosinophil count and skin prick test results were similar at baseline. Therefore, it may be interpreted that serum cholesterol levels have no effect on the mentioned parameters and these results are in accordance with the previously mentioned large population screening study [35]. In this study, because of paucity of studies evaluating the effects of statins according to asthma phenotypes we did not classified patients as allergic, non-allergic, late onset or obese asthmatic.

Limiting factors of our study can be listed as higher rate of females and small number of severe asthmatics being included. Additionally, the lack of a group of patients with normal blood cholesterol levels being treated with statins is another drawback. Furthermore, the anti-inflammatory effect of statins are potentially due to the cholesterol lowering effects in hyperlipidemic subjects, is another source of debate. However, large population studies have shown no association of serum cholesterol levels with atopy [36]. Although, the anti-inflammatory effects of statins appear before their anti-hyperlipidemic effects, the time interval for anti-inflammatory effects of statins on airways has yet to be defined. In our study, the treatment duration of 8 weeks may not be sufficient for the all anti-inflammatory effects to be revealed. The heterogeneity of the study groups, medications, and doses in previous studies as well as their designs do not allow us to comment on which statin has the best effect.

Conclusion

In conclusion, an 8-week treatment with 40mg rosuvastatin in asthma decreased the peripheral eosinophilia, total IgE levels, and inflammatory markers seen in the induced sputum samples of asthmatics. Furthermore, beneficial effects in PFT have also been observed. However, these results did not translate to any improvement in ACT and QoL scores. Understanding the immune-modulatory effects of statins in asthma by studies designed in large number of patients may lead to the development of novel targeted interventions. A further long-term larger study with placebo control is warranted.

Conflict of Interest: None

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Learning styles and learning approaches: How closely are they associated with each other and do they change during medical education?

Öğrenme stilleri ve öğrenme yaklaşımları: Birbirleri ile ilişkili mi ve tıp eğitimi sırasında değişiyor mu?

Ayşe Hilal BATI, Nilüfer Demiral YILMAZ, Tahir YAĞDI

ABSTRACT

Objectives: This study aims to determine medical students' learning styles and approaches and to evaluate the relationship between them, as well as observe whether any changes occur in these during the course of their education.

Methods and Materials: This research was carried out on students who were enrolled in 2008-2009 and was conducted in their first, second and fourth years. The study group consisted of students who had fully completed the scales of measurement used for this research in all years. Learning styles and approaches were determined for each period.

Results: An increase in the convergent and divergent styles and a decrease in the assimilator style, determined over the years were not statistically significant. The decline in the deep learning approach scores was important. Evaluating the relationship between the learning styles and approaches, we found that the deep learning approach scores with the divergent style were lower in the first two years; however, no relationship was observed in the fourth year.

Conclusions: The learning style follow-up study indicates a trend among students who will try to understand the whole by giving importance to details, to focus on problem solving, and to move away from traditional learning. The decrease in deep learning approaches may be linked to the nature of assignments and testing systems.

Keywords: Learning, Learning styles, Learning approaches, Undergraduate medical education

ÖZ

Amaç: Araştırmanın amacı, Ege Üniversitesi Tıp Fakültesi öğrencilerinin eğitim süreçlerinde öğrenme stil ve yaklaşımlarının belirlenmesi ve öğrenme stilleri ile öğrenme yaklaşımları arasındaki ilişkinin değerlendirilmesidir.

Gereç ve Yöntem: Araştırma, 2008-2009 akademik yılında Ege Üniversitesi Tıp Fakültesinde eğitime başlayan öğrencilerin eğitimlerinin 1., 2. ve 4. yıllarında gerçekleştirilmiştir. Araştırmada kullanılan ölçekleri üç akademik yılda da eksiksiz olarak dolduran öğrenciler araştırma grubunu oluşturmuştur. Öğrencilerin her dönemdeki öğrenme stilleri ve öğrenme yaklaşımları belirlenmiştir.

Bulgular: Öğrencilerin ilerleyen eğitim yıllarında belirlenen, ayrıştıran ve değiştiren öğrenme stillerindeki artış ve özümseyen stildeki azalma istatistiksel önem taşımamaktadır. Öğrencilerin yıllar içinde derin öğrenme yaklaşım puanlarındaki azalma önemlidir. Öğrenme stilleri ile öğrenme yaklaşımları arasındaki ilişki değerlendirildiğinde ilk iki yılda değiştiren öğrenme stiline sahip öğrencilerin derin yaklaşım puanlarının daha düşük olduğu, 4. yılda ise öğrenme stil ve yaklaşımları arasında ilişki olmadığı saptanmıştır.

Sonuç: Ege Üniversitesi Tıp Fakültesi öğrencilerinin üç farklı dönemdeki öğrenme stili izlemleri, detayları önemseyerek bütünü anlamaya çalışan, sorun çözümüne yönelen, geleneksel öğrenmeden uzaklaşan bir yönelimi işaret etmektedir. Derin öğrenme yaklaşımlarındaki azalma sınav sistemine bağlanabilir.

Anahtar kelimeler: Öğrenme, Öğrenme stilleri, Öğrenme yakaşımları, Mezuniyet öncesi tıp eğitimi

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Introduction

The ever-growing burden of information and the need to change and update this information mean that educational programs have become highly intensive. Under this burden, students tend towards memorizing rather than learning, and as a result, they graduate before having acquired the tools necessary for them to learn how to learn, or having gained an adequate grasp of the philosophy of lifelong learning. To solve these problems, the aim should be to train students in such a way that they learn how to learn, and acknowledge responsibility for self-learning. Hence, it is crucial that individuals should recognize their own learning characteristics and approaches, strengths and weaknesses.

There is a general consensus that the way individuals approach a learning situation is an influence in reaching performance and learning outcomes [1]. The learning style and learning approach that define the learning characteristics of individuals are two different concepts in close proximity to each other, which gain meaning when they are defined and known together. If they are used in a relevant manner by educators and students, they will lead to a more efficient learning process. The terms learning styles and learning approaches are generally used interchangeably. Although they are similar, these two concepts differ on many levels. While learning style indicates the ways in which the learner deals with the reception and processing of information, learning approach defines the method preferred by the learner as he or she is addressing a learning task [2].

Every human being uses different and unique ways of preparing, learning and remembering new information and this is referred to as a learning style [3,4]. Learning styles do not depend on the learning ability of the learners and do not determine how to improve learning ability [2].

The learning model is defined from two perspectives, as functioning at the comprehensive level (concrete experiences and abstract conceptualization) and at the conversion level (reflective observation and active experimentation). This is an ideal learning process in which the learner functions on a feeling, monitoring, thinking and doing basis, and in which he or she questions the process of learning and what is learnt [5]. Kolb describes the learning styles of the individuals according to these learning methods in four groups [6,7]:

Assimilators: In this style the individuals consider their educators as the most important source of information. They are internalizing individuals with a tendency to learn by listening and watching and prefer to receive systematic information that has been structured in a sequential, logical and detailed manner.

Convergers: The individuals who have a convergent learning style are good at issues such as systematic planning and deductive reasoning. These individuals, who try to understand the whole by giving importance to details, learn by doing and thinking, and they are more successful in tests where there is only one answer.

Divergers: These individuals, who are patient and learn carefully, prefer to deal with technical issues and problems rather than with social and interpersonal issues. Their tendencies to learn by doing and feeling enable them to look at tangible situations from different angles, to make observations in the face of events, and to focus on situations presenting different ideas.

Accommodators: These individuals, who make use of their previous experience in the learning process, welcome new practices and experiences. In problem solving, they prefer to use information derived from others rather than analyzing and thinking.

No matter what the learning style is, an evaluation of it as good or bad is not under discussion here. Individuals adopt a learning style appropriate to the learning context. In the educational field, learning styles are used as a means of developing the application of teaching methods and structures; however, they are not an indicator of the ability of the individual to understand content and context.

The learning processes were first classified by Marton and Saljo in 1976 at two basic levels, as superficial and deep-processing. Later, the term “approach” was put forward by Enwistle et al., in 1979 in order to identify the differences in these two learning methods. Unlike learning styles, learning approach is linked with the level of understanding of the learner rather than his or her way of comprehending and processing the information. The quality and quantity of learning is determined by the learning approach adopted by the student. The learning approach consists of a motivation-strategy set that comprises the strategies adopted by students to attain the motivation and learning objectives that are necesssary to achieve the desired learning outcome [8,9]. Motivation indicates why the students want to learn, whereas strategy indicates how they learn [10].

It is known that learning approach is affected by many factors, such as the characteristics of the teaching, the intensity of the educational program, the nature of the educational environment, testing methods and

teaching methods [11,12]. Learning approaches measure the relationship between two conscious and deliberate approaches of the student in order to understand the content and context for a learning task. The level of understanding and exact learning appear as a result of the approach chosen for learning [2]. The learning approach indicates the harmony between the purpose, motivation and learning strategy of the students [13].

In the literature, three learning approaches are described as superficial, deep and strategic:

Deep approach: The students try to associate new information related to the learning topic with what has already been learnt, use the evidence and make logical evaluations. They examine the different aspects of the material in order to see the whole picture, and explore the points of connection between daily life and personal experience [9,13].

Superficial approach: The students tend to choose the fastest way to achieve success. They prefer learning by memorizing without understanding, without asking detailed questions, by studying in a linear form and by dealing with the subject at the minimal level [14].

Strategic approach: This is an approach in which students display purpose and motivation in order to be successful and achieve the highest possible grades by effectively organizing their time and the learning environment. A strategic approach can be combined with deep or superficial learning approaches [13]. It has emerged as a term that defines students who have the ability to adopt both the deep and superficial learning approaches. Those students who are academically more successful adopt a strategic or performance-oriented approach [2]. Kember et al., in 1999 have reported that the learning approaches of students can be described with a model consisting of two main factors. Each main factor has a strategy indicator characterised principally by the presence or absence of the intention to understand the material. [15].

The ever-growing importance of lifelong learning and individual learning requires the recognition and development of concepts such as learning styles and approaches. While some researchers and educators believe that learning styles are of a nature that is flexible and more amenable to change depending on the learning environment, others maintain that they are difficult to change. [16]. Kolb argued that the learning styles of individuals are not constant and may change over time [6]. The learning style of each individual is in a relatively stable structure in accordance with their personal characteristics, and displays compliance with learning status [2]. The learning approaches considered as a reaction to the learning environment, however, are dynamic and open to change [17-19]. Learning approach may change with prior knowledge, capabilities, educational program (together with measurement-evaluation, course structure and content), teaching methods, the learning climate of learning and learning outputs [20]. There is evidence indicating that the deep learning approach can be taught and developed through education and practice [8,17].

The purpose of this study is to determine the learning styles and approaches of the students at Ege University, Faculty of Medicine, where an integrated education model is implemented, to investigate the change in subsequent educational periods, and furthermore, to determine whether or not there is a relationship between the learning styles and learning approaches.

The questions that will be attempted to be answered by this research are as follows;

1. What are the learning styles and approaches of the students who were enrolled at Ege University, Faculty of Medicine in the academic year 2008-2009?

2. Is there a change in the learning styles and approaches of the students in the ensuing years of education?

3. Is there a meaningful relationship between the learning styles and approaches of the students?

Materials and Methods

Study Group

This research was carried out by following the 354 students who started their education at Ege University, Faculty of Medicine, in the academic year 2008-2009 during the course of their educational process. In the first year, the status of the students was determined prior to undergoing medical education; in the second and fourth years, however, their status was monitored at pre-clinical and clinical stages. The educational program of Ege University, Faculty of Medicine has been carried out by providing vertical integration in the first three years since the academic year 2003-2004 after the horizontal integration practice that started in 1997, and in the fourth and fifth years as from the academic year 2011-2012. The educational program incorporates horizontal and vertical integration arranged with a spiral approach, aimed at

graduation targets and covering the priority health issues of the community. At Ege University, Medical Faculty, organ-system based themes in ten blocks are formed by many different disciplines in the first three years. Additionally, in these blocks, small group activities and simulated patient problem sessions are implemented. The students here are assessed by questions requiring short answers in each block and by multiple-choice question (MCQ), simulated patient problems, assignments and a portfolio at the end of each block. Similarly, six clinical internship blocks are formed by many different disciplines in the fourth and fifth years. The students here are assessed by MCQ and oral exams at the end of each block.

In this follow-up research conducted with ethical approval, it was aimed to reach all of the students in each year without determining a sample.

Instruments

In the literature, different scales are used in studies researching the learning styles and approaches of medical faculty students. In the determination of the learning style, it is in particular the Kolb Learning Style Inventory which is more commonly used [3,7].

One of the scales used most frequently in the evaluation of the learning approaches is the Approaches to Study Inventory (ASI) developed by the Lancaster group, and the Revised Approaches to Study Inventory (RASI), the revised form of ASI. Another, however, is the Study Process Questionnaire (SPQ) developed by Biggs. This scale was later reviewed, and its short and final form Revised Two Factor Study Process Questionnaire (R-SPQ-2F) was devised [9,21]. Apart from these, there are also scales that contain many articles, and that make multi-dimensional measurements. In this type of scale, easy applicability gains importance because answering takes a long time, participation in the survey is low and not all of the articles are answered. The measurement tools chosen for this research on the grounds of common usage and easy applicability are defined below:

1. Kolb Learning Style Inventory: The Kolb Learning Style Inventory which was adapted to Turkish and whose validity and reliability study was conducted by Aşkar and Akkoyunlu has been used to determine the learning styles [3]. In this scale with 12 questions, each of which contains four subordinate clauses, there are 48 subordinate clauses in total. For each question, the students' responses are scored in a way that will allot 4 points to the statement that suits them the best of the four subordinate clauses, and 1 to the statement that suits them the least. The results ranging between -36 and +36 obtained in the calculation made with this scoring are evaluated in the diagram developed by Kolb, and the learning styles are determined.

2. Revised Two Factor Study Process Questionnaire: Learning approaches were evaluated by using the new form of the R-SPQ-2F developed by Biggs and adapted to our language. The validity- reliability study was conducted by Bati et al. [9]. The R-SPQ-2F is a refined version of Biggs' original SPQ. It consists of 20 items scored on a five-point Likert scale and categorizes the students into two different approaches to learning (surface and deep), each with a motive and strategy. The scale point that can be taken for deep and superficial approach is between 10-50 points [21].

3. Survey form: A short questionnaire form consisting of independent variables (gender, class attended, choosing to study at the Faculty of Medicine of his/her own will, etc.) considered to have affected the learning styles and approaches of the students, was only applied in the first year of the research because the same students were monitored.

The scales were applied in the academic years 2008-2009, 2009-2010 and 2011-2012 by means of a single form.

Data Analysis

The data were evaluated by using the Statistical Package for Social Science (SPSS) version 21.0. Chi-square, McNemar, ANOVA and variance analysis in the repeated measurements tests were used in the statistical analyses.

Results

Three hundred and fifty-four students were attending the first year class in 2008. The scales used in the research were completed in full by 319 (90.1 %), 228 (64.4 %), and 178 (50.3%) of them in the academic years 2008-2009, 2009-2010 and 2011-2012 respectively. Monitoring was possible for the 154 (43.5 %) students who completed these scales in full in all three years.

It was determined that 49.4 % (76) of the students comprising the study group were females and that 94.8 % (146) had chosen the medical faculty of their own will.

When the students' learning styles were examined, the rate of students adopting the assimilator and converter

learning styles in all three years was higher. While this rate decreased relatively in the fourth year, the increase in the rate of the students with a diverger learning style attracted attention. The change of learning styles between the educational periods presented in Table I does not bear any statistical importance ($\chi^2_{\text{Mc Nemar-Bowker}}$: 2.452, *P*:0.874).

A meaningful relationship was not detected between gender and learning styles in any of the three educational periods. When the students' free choice of the faculty and their learning styles were compared, however, the relationship between choosing the faculty of their own will and the assimilator learning style was found to be meaningful only in the first year (X^2:8.288, *P*:0.040).

When the educational periods and the learning approach scores of the students were compared, while their superficial approaches did not change, it was observed that there was a decrease in their deep learning approaches over the years. It was found that the group that created a difference with Post Hoc LSD analysis were the first year students (Table II).

When an evaluation was made according to the components of the deep learning approach scores, it was seen that there was a statistically meaningful decrease in both the deep motivation (F:10.825, *P*:0.000) and deep strategy (F:17.549, *P*:0.000) scores. It was determined that the difference with Post Hoc LSD analysis resulted from the first year students.

When the students' free choice of the profession and the effect of their gender on learning approach were examined, the superficial approach scores of the students who were males and who did not choose the profession willingly were higher in all the educational periods. When the learning approach scores in the three years were examined in terms of gender, a difference between the deep approach scores of the female and male students was not detected. In spite of this, the superficial approach scores of the male students were found to be higher than those of female students at a meaningful level. This difference arose from the superficial motivation scores. While the deep approach scores did not

Table I. The learning styles of the research group according to the educational periods

	Learning Styles (n:154)										
	Assimilator		**Converger**		**Diverger**		**Accommodator**		**Statistical Analysis***		
Period	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**		**χ^2**	***P***
1st	87	56.6	48	31.2	9	5.8	10	6.5	**1-2 year**	7.994	0.239
2nd	77	50.0	58	37.7	9	5.8	10	6.5	**1-4 year**	6.876	0.332
4th	71	46.1	60	39.0	14	9.1	9	5.8	**2-4 year**	2.452	0.874

* χ^2 Mc Nemar-Bowker

Table II. The learning approach of the study group according to educational period

Period	**Learning Approach Mean Scores (n:154)**			
	Deep Approach		**Surface Approach**	
	Mean	**SD**	**Mean**	**SD**
1st	32.05	5.513	27.14	6.416
2nd	30.45	6.461	27.20	7.148
4th	29.33	6.003	26.97	6.562
Statistical Analysis *	**F** 17.768		0.094	
	P 0.000*		0.910	

* ANOVA for repeated-measures *P*<0.05 and Post Hoc LSD *P*<0.05, the group that created the difference 1st Year

display any difference in the first year in terms of freely choosing the profession, it was found to be higher in those who chose the profession freely in the second and fourth years. The high superficial approach level of the scores of those who did not freely choose the profession, however, created a statistical difference in all three years.

When the relationship between the learning styles and learning approaches was evaluated, it was detected that the deep approach (F: 3.047, *P*: 0.031) and deep motivation (F.2.950, *P*: 0.035) scores of the students with a diverger learning style were lower than those of students favouring another learning style in the first two periods. In the Post Hoc evaluation, the group that created a difference was determined to be the students with a diverger learning style. In the second year, in addition to this, in the Post Hoc analysis, it was found that the deep approach scores of the students who had a converger learning style were higher than in the other groups, whilst their superficial approach scores were lower (Table III).

In the fourth year, the number of students who had an assimilator and converger learning style was high and equal in both of the learning approaches. Despite this, the deep or superficial approach score averages of the students in terms of their learning styles did not display a statistical difference (Table IV).

Discussion

The profession of medicine, which must be engaged in with enthusiasm and devotion, necessitates the constant renewal of acquired knowledge together with lifelong learning, due to an ever-growing information load, even after a long and challenging educational process. No matter how

Table III. The relationship between gender, free choice of the profession and learning approaches

Period	Learning Approach	Gender		Statistical Analysis*		Choosing the Profession Willingly		Statistical Analysis *	
		Female (76)	**Male** (78)	t	*P*	**Yes** (146)	**No** (8)	t	*P*
1st	**Deep**	32.37 ± 5.54	31.74 ± 5.51	0.702	0.484	32.15 ± 5.44	30.25 ± 6.86	0.949	0.344
	Surface	25.53 ± 5.38	28.72 ± 6.97	-3.177	**0.002**	26.75 ± 6.19	34.38 ± 6.55	-3.384	**0.001**
2nd	**Deep**	30.71 ± 6.03	30.19 ± 6.88	0.496	0.620	30.69 ± 6.37	26.00 ± 6.93	2.020	**0.045**
	Surface	25.97 ± 6.50	28.40 ± 7.58	-2.128	**0.035**	26.87 ± 6.92	33.25 ± 9.05	-2.500	**0.013**
4th	**Deep**	29.30 ± 5.82	29.36 ± 6.22	0.292	0.954	29.60 ± 5.99	24.50 ± 3.96	2.373	**0.019**
	Surface	25.40 ± 5.94	28.47 ± 6.83	-2.946	**0.004**	26.71 ± 6.31	31.75 ± 9.47	-2.139	**0.034**

*Student's t test

Table IV. The relationship between learning approaches and learning styles

Period	Learning Approach	Learning Style												Statistical Analysis*	
		Assimilator			**Converger**			**Diverger**			**Accommodator**				
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	F	*P*
1st	**Deep**	87	32.33	5.22	48	32.50	5.50	9	26.78	7.26	10	32.20	4.69	3.047	**0.031**
	Surface	87	26.62	5.98	48	27.02	6.89	9	32.22	5.95	10	27.70	7.09	2.150	0.096
2nd	**Deep**	77	29.16	5.95	58	32.60	5.99	9	27.00	7.65	10	31.00	8.64	4.322	**0.006**
	Surface	77	28.94	6.70	58	24.50	6.57	9	29.78	6.46	10	27.20	9.96	5.030	**0.002**
4th	**Deep**	71	29.37	5.69	60	29.97	6.32	14	26.93	5.48	9	28.56	7.14	1.023	0.384
	Surface	71	26.52	6.86	60	26.27	6.28	14	30.21	5.52	9	30.22	5.89	2.274	0.082

*ANOVA $P<0.05$, Post Hoc LSD $P<0.05$, Difference is created by the students who have a learning style undergoing change in the first two years.

heavy the professional workload is in the process of work, access to new information becomes a part of life. Hence, the continuity of education is achieved with self-directed learning in which individuals undertake responsibility for their own learning. The awareness of individuals regarding their own learning styles bears special importance with regard to maintaining more efficient learning in the process of education and working life. It is known that learning styles and approaches stem from individual preference, and can change with factors such as learning environments, tasks, and test systems. The educational program which is carried out through multidisciplinary integration at Ege University, Faculty of Medicine, supports students' learning and constitutes a basis for future learning. In this study, which evaluates the learning characteristics of the students in the educational process in our Faculty, remarkable results have been achieved.

The learning style follow-ups of the students at our medical faculty during three different periods indicated a tendency amongst them of trying to understand the whole by giving importance to detail, of moving towards problem solving, and away from traditional learning.

In this study, in line with the researches carried out previously in Turkey and a research study in Sri Lanka, a decline in the deep learning approaches in the process was detected [14, 22-24]. It was thought that this change could be connected to the tasks/assignments in the process of education and the nature of the testing systems. In the study of Samarakon et al., however, it was determined that there was no difference between the first and final years in terms of learning approaches [25]. In the qualitative study of Ozolins et al., it was determined that the students used informal sources such as previous exam questions, the lecture notes of previous students, and the interviews carried out with them while they were preparing for the exams. The students underlined the fact that they obtained information to pass exams in a way that did not fit with their learning approach in the educational process, and that they were prepared for the exams with more superficial learning [26]. The change that we detected in the deep learning approach was supported by other studies which determined that students channel their learning towards exam performance in an effort to use their time efficiently as a consequence of highly intensive educational programs and workload concerns [27]. The decrease in the deep approaches of the students with a diverger learning style emphasized the problem-oriented approaches of these students. It can be said that because the learning styles do not display any change in the first three years, the density of theoretical knowledge that constitutes the basis for their professional education in the first years of the faculty playes a crucial role in the students' maintenance of learning styles adopted during the period of secondary education.

In our faculty, more time is allocated to profession-oriented practical training and given more space in the educational program especially in the second year. Besides this, the ever-increasing density of information and the lengthy courses, as with all medical faculties, present challenges in the use of time for students at an individual level. These factors can explain the decrease of students favouring the assimilator and converger learning styles and also the tendency of those using the diverger learning style to reduce the use of information for solving a particular problem.

Given the fact that learning approaches can change as a result of factors such as preliminary information, skills, educational program, teaching methods, learning climate or learning outcomes, the decrease in the deep approach scores of medical faculty students seems more understandable [8,12,28]. Medical faculty students come from secondary education institutions where different teaching approaches are applied, and they endeavour to adapt to the educational structure, environment and program of the faculty. The elevation of the deep approach level in this period depends on the level of intrinsic motivation [23]. Especially in the first year of faculty, students combine the topics that constitute a basis for professional education with their previously acquired learning styles and try to prepare for the clinical process. On the one hand, the students who enter the faculty with a high level of success after a stressful university entrance examination period must adapt to a challenging professional educational process, and on the other, they aim to maintain the high levels of success achieved. The regression in the motivation determining why students want to learn and strategy indicating how they learn outside the dimensions of deep learning approaches also indicates a the tendency to aim to succeed in mastering intensive learning topics in a short time. Lawson reported that Newstead stated in the research he conducted that frequency of formal measurement assessment may cause superficial learning [23]. Smith and Miller's study results indicated that assessment type had no significant influence on how students approached their learning [29]. Gijbels et al., also reported that the learning

approaches of the students might change depending on the assessment method that was used. They emphasized the fact that assessment was not a practice applied at the end of the learning process or a practice that was separate from education, but was a powerful tool that stimulated deep learning [30]. In spite of the fact that the presence of the formative interim assessments in the evaluation system in our faculty supports the learning process, it leads students to feel that they are subject to too many exams in addition to the already loaded content of education. For this reason, it is feasible that they opt for the superficial learning approach on a strategic basis and largely prefer to memorize most of the time in order to be successful. Learning, teaching and assessment integration bears a special importance. Besides this, it was emphasized that the other learning environment features such as the work load, feedback, regular and segmented information which affected the learning approaches of the students should also be taken into consideration [30].

In this research, whilst no relationship was determined between gender and learning styles and deep approach points in all three educational periods, it was found that the superficial approach scores of male students were found to be significantly higher than those of female students. In spite of the fact that the relationship between gender and learning approach was not among the purposes of this research, it was evaluated in the light of the data obtained. In the literature, there was no consistency between the results found in the studies addressing this issue. In Wickramasinghe and Samarasekera's study, it was determined that learning approaches were similar in both sexes, and that the male students preferred to obtain information via an instructor support, whilst the female students preferred to obtain information passively [18]. Similarly, the relationship between learning approach and gender had not been established in Shah's study [31]. In the researches of Duff, Gledhill and Van Der Merwe, it was reported that the deep approach scores of the female students were high in accordance with our research; however, Sadler-Smith's results were completely the opposite. Apart from these, results had been reported in many research studies as to the fact that gender was not a determinant [32,33]. In different sources, there were views to the effect that these inconsistent results could be affected by many factors such as statistical analysis, cultural differences and learning environments [19,34]. Carrying out qualitative studies would shed more light in order to be able to interpret the results.

Choosing or not choosing the profession willingly is a determinative factor for motivation. In this research, the fact that the students who did not choose the profession willingly had a higher superficial score in all the periods, can be explained by the fact that these students perceived this educational period as a duty to be completed, and that they adopted a superficial motivation with the goal of merely completing this task successfully.

The fact that the deep approach scores of students with a converger learning style were higher than those of the other groups and that their superficial approach scores were lower in the second year was compatible with the analytical and objective approaches of converger students and their characteristic of giving importance to detail. Thus it was an expected result.

Despite the fact that the deep or superficial approach scores of the students did not differ in the fourth year in terms of their learning styles, the number of students with an assimilator and converger learning style was high for both learning approaches. The number of students with an assimilator learning style was high in each period, can be considered as an outcome of the primary and secondary education system in our country. No matter to what extent the complex structure of medical education affects the learning characteristics of the individuals, the intensive educational process and time restrictions lead students to a superficial approach which is more simplistic. In addition to this, concern for the Specialty Exam in Medicine that must be passed after graduation and is carried out via a centralized system, is a significant factor in students developing a superficial learning approach that focuses on this exam, consisting as it does of MCQ. In spite of these issues, the relative increase in the number of students with a converger learning style in our faculty can be evaluated as an outcome of the laboratory and practical training which play an extensive role in our educational program.

This study was essential in that it was a piece of research in which learning styles and approaches were assessed with a three-year follow-up and a high answer rate. On the other hand, it had two basic limitations. The first of these was that several very important confounding factors such as educational program and learning environment features, assessment methods and teaching characteristics had been ignored because the research was carried out only on the students in a medical faculty. The second limitation was that the social factors regarding the students such as place of accommodation, family-friend features, were left out of

the assessment during the follow-up. It would be beneficial to answer these research questions in medical faculties adopting a different educational program model with qualitative research that pays regard to the limitations that we have mentioned.

Conclusions

Learning styles and approaches play an important role in the learning process. Determination of the learning styles and approaches of medical students is helpful in evaluating teaching and assessment-evaluation strategies. This can enable favorable changes to be made to improve medical education programs as well as giving support in particular to students experiencing academic difficulties.

The effect of the educational environment on shaping the learning characteristics of students is observed in this research as well as in many other studies. The applications that support life-long and self-directed learning are particularly vital in structuring medical education programs. Students should be encouraged to use their learning approaches over a wide range in order to strengthen their cognitive skills and individual development. For this purpose, it would be useful to develop student-centered applications as well as supporting the measurement and evaluation systems in a way that would motivate learning.

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The role of endoscopic mucosal resection in gastrointestinal precancerous lesions

Gastrointestinal prekanseröz lezyonlarda endoskopik mukozal rezeksiyonun rolü

Suleyman ORMAN, Orhan GULTEKIN

ABSTRACT

Objectives: Endoscopic mucosal resection (EMR) has been widely used in early gastrointestinal malignancies and precancerous lesions. We aim to analyze the outcomes of EMR for gastrointestinal precancerous lesions.

Materials and Methods: A total of 1,518 cases were retrospectively analyzed. Of these, 59 patients had undergone EMR for gastrointestinal lesions. The lesions were divided into two subgroups according to size; smaller than 20 mm and equal or larger than 20 mm. The mucosal aspect (Kudo pit pattern), Paris classification of the resected lesions and recurrences that were determined during close follow-up were recorded.

Results: A total of 94 polyps were resected in 59 patients. En-block resection was performed in 42 (71.2%) patients and piecemeal resection in 17 (28.8%). There was no significant difference between two modalities; regarding age, gender, histopathology, complications, recurrence or macroscopic type (P>0.05). However, the occurrence of Kudo type 4 lesions and adenomatous polyps (P=0.001) was significant in the >20 mm group (P=0.03).

Conclusion: Endoscopic mucosal resection is a safe and feasible procedure for precancerous lesions, with low complication and acceptable recurrence rates. Additionally, the Kudo pit pattern should be taken into consideration on a routine basis when determining the risk of cancer.

Keywords*:* Endoscopic mucosal resection, Gastrointestinal precancerous lesion, Pit pattern, Recurrence

ÖZ

Amaç: Gastrointestinal maligniteler ve prekanseröz lezyonlarda endoskopik mukozal rezeksiyon (EMR) yaygın olarak kullanılmaktadır. Bu çalışmada amacımız gastrointestinal prekanseröz lezyonların tedavisinde EMR'un etkinliğini değerlendirmektir.

Araç ve Gereçler: Binbeşyüz onsekiz hasta retrospektif olarak incelendi. Toplamda 59 hastaya EMR uygulandı. Lezyonlar 20 mm'den büyük ve 20 mm ve küçük olmak üzere 2 gruba ayrıldı. Mukozal görünüş (Kudo pit patern yapısı) ve Paris sınıflamalarına göre lezyonlar kaydedildi. Hastalardan iki işlem denemesine rağmen polipleri tam çıkarılamayanlar cerrahiye yönlendirildi ve bu çalışmaya alınmadı. Yakın takipler esnasında saptanan tekrarlar kaydedildi.

Bulgular: Elli dokuz hastadan toplam 94 polip rezeke edildi. Hastaların 42 (%71,2)' sine enblok, 17 (%28,8)'sine ise piecemeal rezeksiyon uygulandı. İki yöntem arasında yaş, cinsiyet, histopatoloji, komplikasyonlar, tekrar ve makroskobik tipler açısından farklılık gözlenmedi (P>0,05). 20 mm'den büyük poliplerde Kudo tip 4 görünüş ve adenomatöz polipler (P=0,001) yaygın olarak izlendi (P=0,03).

Sonuçlar: Prekanseröz lezyonlarda EMR işlemi düşük komplikasyon ve kabul edilebilir tekrar oranları ile güvenli ve kullanışlı bir işlemdir. Kudo pit patern yapısı kanser riski değerlendirilmesinde rutin olarak kullanılmalıdır.

Anahtar kelimeler*:* Endoskopik mukozal rezeksiyon, Gastrointestinal prekanseröz lezyon, Pit paterni, Nüks

Introduction

The use of endoscopic mucosal resection (EMR) was pioneered in Japan for the treatment of early gastric cancer and has been widely used in treating other early gastrointestinal malignancies and precancerous lesions [1-4].

Early endoscopic detection and removal of gastrointestinal precancerous lesions reduce the incidence of

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gastrointestinal malign neoplasias [5]. It has recently been shown that colonoscopic polypectomy significantly reduces the incidence of colorectal cancer associated with mortality [6]. Cancer screening programs, which are currently broadly implemented, can facilitate the detection of cancers at a curable stage, also help to diagnose more precancerous lesions. The increase in the detection of precancerous lesions, brings with it the need for more treatment facilities and optimal treatment techniques to facilitate cost-effective care. Endoscopic treatment of gastrointestinal precancerous lesions may prevent the need for surgical interventions such as segmental resection, wedge resection, hemicolectomy, eosophagectomy and gastrectomy with their associated complications. The resection of polyps can be performed en-bloc or piecemeal, according to the size and location of the lesion. Large adenomas require treatment beyond simple loop polypectomies or single-piece EMR, with the piecemeal method being the most common endoscopic resection technique used for them. However, piecemeal resection often leads to high rates of residual adenoma found during the follow-up period [7]. Two recently published prospective studies on the piecemeal EMR of colorectal adenomas, report residual adenoma rates as 20–38%, although, late recurrence was eventually diagnosed in less than 5% of these patients [8,9]. A previous systematic review, including 20 studies on EMR of large (>2cm) colorectal adenomas, described an early recurrence rate of 11.2% (after a single attempt), where the lesions were in fact residual adenomas rather than actual recurrences. If those adenoma remnants were re-treated within 6 months, the late recurrence rate then dropped to 1.5% and complication rates were 3.8% [10].

In many countries, EMR is considered a complex endoscopic procedure and is usually only performed at specific centers to preserve its effectiveness and safety [11]. However, the complication rate associated with EMR is low [12]; the most frequent adverse effect is bleeding [13-17], followed by perforation [3,18,19], as well as recurrence [15-17,20]. Consequently, the use of EMR for gastrointestinal precancerous lesions has become increasingly popular in Western countries and has been found to be a safe and efficient treatment. This technique is not commonly practiced in rural hospitals in Turkey and most reports come from large tertiary referral practices. The aim of this present study is to retrospectively analyse the outcomes of EMR for gastrointestinal precancerous lesions with a particular focus on procedural complications and recurrence rates in two centers in Turkey.

Material and Methods

After being approved by the ethical board of Istanbul Medeniyet University, Göztepe Research and Training Hospital (2015/0140), a total of 1,518 patients who were referred to Istanbul Medeniyet University, Göztepe Research and Training Hospital and Istanbul Gastroenterology Centers for upper and lower gastrointestinal endoscopy between January 2011 and January 2015, were retrospectively analyzed. Only those patients with lesions suitable for EMR were included in the study. EMR procedures were performed on 59 patients by two endoscopists. Before the EMR, Kudo pit pattern type and Paris classification of all lesions were recorded [21,22]. Lesion size was endoscopically estimated using open biopsy forceps or a resection snare, although as some lesions were resected using the piecemeal method, an accurate size could not be calculated. Lesions were divided into two subgroups: those smaller than 20mm (<20mm) and those equal/larger than 20mm (≥20mm). Patients were excluded from the study if the mucosa was non-lifting or if there was any suspicion of submucosal invasion during diagnostic medical tests including computed tomography, magnetic resonance imaging or endoscopic ultrasonography. Patients that fulfilled all criteria were advised to undergo endoscopic treatment, additionally routine informed consents were obtained before each procedure.

Resection method

Before the endoscopic procedure, a split dose bowel preparation was used for the lower gastrointestinal area. Anticoagulant therapy was normally discontinued three days prior to the procedure. At the endoscopist's discretion, either conscious or deep sedation was administered using midazolam or propofol. Diagnostic endoscopes such as GIF-H180 or GIFHQ180: Olympus, Tokyo, Japan and VP4400 with XL 4400: Fujinon, Tokyo, Japan were preferred for the procedure. EMR was performed with polypectomy snares. Standard large (30mm: Boston Scientific, Natick, Massachusetts, USA); barbed (20mm: Olympus, Tokyo, Japan); or standard small (13 mm: Boston Scientific, Natick, Massachusetts, USA) snares were used in the piecemeal resection type. After proper endoscopic visualization of a lesion, it was lifted by injecting a large volume (10-100 mL) of a pre-mixed solution submucosally, using a saline solution of 0.9%, 1mL methylene blue and 1:10,000 units of adrenaline. Lesion margins were not routinely marked with mucosal cautery. The open snare was placed around the lesion and was gently pressed against the mucosa. The

aim was to resect the lesions in one single piece rather than piecemeal, if possible. After the snare excision, air was insufflated to visualize the area of resection and, if needed, any further residual tissue was removed with a similar technique. Snare resection was continued until the lesion was macroscopically entirely removed, and the blue colored submucosa was visible. The settings used were "auto-cut" at a set power of 120 W, along with the "endo-cut" mode. After resection, adjuvant heater probe coagulation therapy was used to remove any tiny visible remnants of lesions. There was no prophylactic treatment of visible vessels in the EMR defect; that were not bleeding and polypectomy sites were not closed with clips. Patients were discharged after the procedure.

Histopathological evaluation

After retrieval, whenever possible the size of the lesions was estimated by comparison and using open biopsy forceps. All removed tissue was retrieved using a retrieval basket, grasper or through the suction channel. All retrieved lesions were examined and classified by several experienced pathologists at the respective histopathology departments of each center. Polyps were classified as adenoma, intramucosal carcinoma or non-adenoma (e.g. hyperplastic polyps, leiomyoma, neuroendocrine tumour, lipoma, gastric mucosa). Adenomas were further classified for grade of dysplasia (i.e. low, or high-grade) using a combination of variables, including tubule configuration, nuclear polarity, orientation and structure, mucin content and location, etc, according to the Konishi-Morson system. [23]. The diagnosis of intramucosal carcinoma included adenomas with neoplastic cells invading the lamina propria mucosa [24,25]. It was impossible to evaluate the resection margins in piecemeal resection specimens;thus, only the basal margins, lesion type, and degree of dysplasia were assessed in these cases. When submucosally invasive carcinoma was diagnosed in the resection specimen, the necessity for additional treatment was discussed by a multidisciplinary team, and the patient was excluded from the study.

Follow-up and recurrence

Surveillance endoscopies were performed at various intervals of the follow up period. All patients underwent repeat endoscopies 3 months later and surveillance endoscopies were scheduled at 6, 12, 24 and 36 months after EMR. During endoscopic follow-up, any alterations in the mucosa of the resection area (scarring, retractions of mucosa, etc.) were biopsied. If the scar appeared visibly normal, without the presence of adenomatous tissue, random biopsies were taken from its center and its edges. Recurrence was defined as the presence of adenomatous or polypoid tissue in a single follow-up endoscopy. All remnants and recurrences were treated endoscopically, if practical.

Statistical analysis

Data analysis was performed using Number Cruncher Statistical System (NCSS) 2007 (NCSS, LLC Kaysville, Utah, USA). Descriptive statistics for continuous variables were shown as mean ± standard deviation or median (minimum-maximum), and categorical variables were shown as both the number and percentage of cases. Significant differences between the groups; in terms of mean value were analyzed with Student's t-test while median value differences were analyzed with the Mann-Whitney U test. Categorical variables were assessed by Pearson's chi-square test, Fisher's Exact test and Fisher Freeman Halton test. A P value less than 0.05 was considered statistically significant.

Results

In total, 94 polyps in 59 patients were treated. Of these, 30 polyps were large gastrointestinal precancerous lesions (mean diameter >20mm) with 19 patients having more than one lesion. 55 patients (76.3%) had lesions localized in the colon and rectum area; 14 patients (23.7%) had lesions in the stomach. En block resection was performed in 42 (71.2%) patients; piecemeal resection in 17 (28.8%). Macroscopically, lesions were protruding in 47 patients (79.7%), superficial in 8 patients (13.6%), and lateral spreading in the remaining 4 patients (6.8%). According to Paris classification grading, 1p, 1s and 2a grades were seen in 50 (84.7%), 8 (13.6%), and 1 patients (1.7%), respectively. Kudo pit patterns were as follows: type 4 - 24 patients (39.0%); 3L - 21 patients (35.6%); 2 out of 11 patients (18.6%) had type 2; and 4 patients (6.8%) had 3S. The characteristics of the patient population, including the polyp size and location, are presented in Table I. Complete resection was achieved in 56 patients (95%) while incomplete polyp resection in 3 patients required repeated sessions. One patient had significant bleeding during polypectomy and after no response to hemostatic interventions, underwent right hemicolectomy.

Table I: Demographics and clinical characteristics

Age (years),	62.54±12.31	
Duration of process (min)	13 (2-66)	
	n	**%**
Gender		
Male	24	40.7
Female	35	59.3
Pathology		
Non-adenomatous	16	27.1
Adenomatous	43	72.9
Type of adenomatous polyp		
Tubular Adenoma	17	39.5
Tubulovillous Adenoma	20	46.5
Villous Adenoma	6	14.0
Localization		
Stomach	16	27.1
Colon	28	47.5
Rectum	15	25.4
Complication	1	1.7
Recurrence	2	3.4
Dimension of lesion		
< 20 mm	29	49.2
≥20 mm	30	50.8
Macroscopy		
Superficial	8	13.6
Protruding	47	79.7
Lateral Spreading Type	4	6.8
Technique		
En-block	42	71.2
Piecemeal	17	28.8
Paris classification		
1p	50	84.7
1s	8	13.6
2a	1	1.7
Kudo classification		
2	11	18.6
3L	21	35.6
3S	4	6.8
4	23	39.0

The median follow-up period was 12 months (6-36 months). Local recurrence was recorded in two patients (3.4%), at the sixth month with a histopathological pattern of low-grade displasia. Remnant adenomatous tissue was found during follow-up in 2 of the 59 patients (3.4%).

Adenomatous polyps were found in thirty-seven patients (62.7%) with low-grade dysplasia in 26 patients (44.1%), and high-grade dysplasia in the remaining 11 (18.6%). There were twenty-two patients (27.3%) with non-adenomateous polyps: these comprised 11 (18.6%) hyperplastic polyps; 6 (10.2%) intramucosal cancers; 2 (3.4%) regular gastric mucosa; 1 (1.7%) neuroendocrine tumor; 1 (1.7%) leiomyom and 1 (1.7%) lipom.

Age, gender, histopathology, complications, recurrence or macroscopic type were not predictive values for the adoption of en-block or piecemeal resection (P>0.05).

However, en-block resection was more frequently used for lesions of the stomach, whereas piecemeal resection was associated with rectal lesions (*P*=0.042). Piecemeal resection usage was also significantly higher in lesions ≥20mm (*P*=0.001), (Table II), and the duration of this procedure was significantly longer (*P*=0.001).

No significant difference was found between en-block and piecemeal resection according to Paris and Kudo classifications (*P*>0.05), (Table III).

The prevalence of Kudo type 4 lesions and adenomatous polyps (*P*=0.001) was significantly associated with lesions ≥20mm (*P*=0.03). Kudo type 2 lesions were more frequent in the stomach with type 4 lesions more common in the rectum (*P*=0.001), but the distribution of colonic lesions according to Kudo types was not significantly different (*P*>0.05). Meanwhile, a significant association was found between the subgroups of adenomatous polyps and their Kudo classification type (*P*=0.02), (Table IV).

Table II: Comparison of the techniques

	En-block (n=42)	**Piecemeal (n=17)**	***P***
Gender			[a]0.088
Male	20 (47.6)	4 (23.5)	
Female	22 (52.4)	13 (76.5)	
Age (years)	62.79±12.93	61.94±10.98	[b]0.814
Pathology			[d]0.115
Non-adenomatous	14 (33.3)	2 (11.8)	
Adenomatous	28 (66.7)	15 (88.2)	
Localization			[c]**0.042**
Stomach	14 (33.3)	2 (11.7)	
Colon	21 (50.0)	7 (41.2)	
Rektum	7 (16.7)	8 (47.1)	
Complication			[d]1.00
Yes	1 (2.4)	0	
No	41 (97.6)	17 (100.0)	
Recurrence			[d]1.00
Yes	2 (4.8)	0	
No	40 (95.2)	17 (100.0)	
Dimension of lesion			[a]**0.001**
< 20 mm	29 (69.0)	0	
≥ 20 mm	13 (31.0)	17 (100.0)	
Duration of process (min)	2-45 (10.5)	3-66 (22)	[c]**0.001**
Macroscopy			[e]0.127
Superficial	6 (14.3)	2 (11.8)	
Protrude	35 (83.3)	12 (70.6)	
Spreading Type	1 (2.4)	3 (17.6)	

[a]Pearson Chi-Square Test, [b]Student T Test, [c]Mann-Whitney U Test, [d]Fisher Exact Test

[e]Fisher Freeman Halton Test,

Table III. Comparison of techniques according to Paris and Kudo classifications

	En-block (n=42)	Piecemeal (n=17)	*P*
Paris classification			[e]0.768
1p	36 (85.7)	14 (82.4)	
1s	5 (11.9)	3 (17.6)	
2a	1 (2.4)	0	
Kudo classification			[e]0.096
2	9 (21.4)	2 (11.8)	
3L	18 (42.9)	3 (17.6)	
3S	2 (4.8)	2 (11.8)	
4	13 (31.0)	10 (58.8)	

[e]Fisher Freeman Halton Test

Discussion

Several studies have been published regarding the safety and therapeutic potential of the endoscopic resection of gastrointestinal precancerous lesions. The most frequently reported adverse complication of EMR is bleeding, occurring in 1% to 45% of all cases [13-17] and the perforation rate has been reported as between 0.7% and 4% [3,18,19]. Our complication rate (intraprocedural bleeding) in this present study was 1.7%. Generally, complication rates of EMR are lower than those who undergo open surgery (2.3% to 6.3%) and comparable to those undergoing laparoscopic surgery (1.9% to 6.9%) [26]. Moreover, mean hospital stay for the patients who undergo colorectal surgery is 9.2-13.2 days and gastric surgery is 5.9-12.1 days, both significantly longer than EMR procedures which are usually performed as day cases [26,27]. Therefore, EMR is a good alternative to surgery in selected patients, especially as all lesions can frequently be removed in a single session, eliminating the discomfort for patients of repeated procedure. This was achieved in 95% of resections in the present study.

En-block resection is usually preferred to piecemeal resection, because it provides more accurate histological assessment and reduces the risk of local recurrence [28]. However, in the present study there were two recurrences (3.4%), with no significant difference found in the recurrence rate data between the two resection techniques used. Clearly, follow-up is essential due to the risk of recurrence; aggressive monitoring seems justified, as animal research has shown that residual tumor has a high re-growth rate [29]. All patients in the present study had follow-up upper and lower gastrointestinal endoscopy. A wide range of recurrence rates after EMR of colorectal polyps and gastric polyps has been previously reported: between 0% and 46% [15-17,30,20]. In the present study, our recurrence rate was 3.4% for gastrointestinal precancerous lesions. Several studies have suggested that the size of polyps is associated with recurrence [31-34]; however, no relation was found in the present study, suggesting that EMR can be carried out safely and effectively for gastrointestinal precancerous lesions. However, strict follow-up may be required for histologically advanced lesions due to a higher risk of recurrence.

This two-center study provides a faithful reflection of current daily practice in Turkey. Subsequent endoscopic follow up and treatment of adenoma remnants was possible in 100% of the patients. The recurrence and complication rates in our study compared favorably with previously published data on large gastrointestinal EMRs. Moss et al. demonstrated the feasibility of piecemeal EMR in colorectal adenomas, reporting very high case-volumes in a multicenter collaboration. Their results (early recurrence rate 20.4%; morbidity 7.7%) have established a sound standard for future endoscopists pursuing similar proficiency [9].

According to a recent study by Buchner et al., larger polyp size and the piecemeal nature of the resection were independent predictors of recurrence and complication [8]. In our study, we had just two recurrences and tumor size was not predictive of any recurrence or complication. This could be related to the prevalence of small sized lesions compared to large sized lesions and more frequent

Table IV. Association of Kudo classification with lesion characters

	Kudo classification				*P*
	2	3L	3S	4	
Dimension					***0.030**
<20 mm	7	14	2	6	
≥20 mm	4	7	2	17	
Localization					**0.001**
Stomach	7	8	1	-	
Colon	3	9	3	13	
Rectum	1	4	-	10	
Pathology					**0.001**
Adenomatous	4	14	3	22	
Non-adenoma	7	7	1	1	
Type of polyp					**0.022**
Tubular Adenoma	4	8	-	5	
Tubulovillous Adenoma	0	4	2	14	
Villous Adenoma	0	2	1	3	
Paris classification					**0.020**
1p	10	19	1	20	
1s	1	1	3	3	
2a	-	1	-	-	

*Fisher Freeman Halton Test

en-block resections. Non-adherence to protocols regarding the use of argon plasma coagulation (APC) has influenced recurrence rates in some studies; and previous studies have shown contradictory results regarding the effects of APC on adenoma recurrence [9,35]. In our study, a heater probe was used for the residue lesion areas.

Remnant or recurrent adenomatous tissue may be present as dysplasia in random biopsies of an apparently healed mucosectomy scar. This may be explained by the fact that chromoendoscopy or virtual chromoendoscopy-like narrow band imaging are not routinely used for the detection of dysplastic lesions located in the scar. Moreover, in the 2 recurrences we detected, the adenomatous tissue might well have been missed during the prior endoscopy due to insufficient imaging, poor bowel preparation or localization behind a fold. Undoubtedly, strict monitoring with high quality endoscopes is essential in the follow-up period of these patients.

Moreover, overall success of the total intervention strategy, including endoscopic surveillance and concurrent treatment of recurrence, can rectify a relatively high recurrence rate. Our two patients, whose remnant or recurrent adenomas were treated endoscopically, were free of recurrence in further monitoring endoscopies. All recurrences were low-grade displasia and no invasive cancers were detected during follow-up endoscopies.

Polyp morphology and the mucosal aspect may be predictive of submucosal invasion. Moss et al. identified the Paris 0-IIa+c classification as a risk factor, especially in combination with nongranular surface morphology [7]. However, none of the lesions included in the present study was classified as IIa+c. The mucosal aspect (Kudo pit pattern) of the resected adenoma is routinely assessed and defined for all our lesions. This may reflect the familiarity of endoscopists with dedicated classification systems such as the Kudo pit pattern type. Hence, endoscopists, especially

those resecting gastrointestinal precancerous lesions, should receive proper training in endoscopic pattern recognition.

In the present study, the small size sample and its retrospective nature present limitations. On the other hand, the predictive value of the Kudo classification, particularly Kudo type 4, in determining adenomatous polyps and cancer risk was analyzed in detail. Also, in contrast to other studies, besides adenomatous polyps, nonadenomatous precancerous lesions such as neuroendocrin tumors and leiomyoms were also analyzed.

In conclusion, we believe EMR to be a safe and feasible procedure for precancerous lesions, with low complication and acceptable recurrence rates. Clearly, the Kudo pit pattern should be considered in assessing cancer risk. Our low recurrence and complication rates also emphasize the importance of training and centralization of this procedure;optimal diagnostic work-up, the possibility of missed remnants, and recurrences during follow-up support this recommendation.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Determining the sample size in agreement studies

Uyum çalışmalarında örneklem büyüklüğünün belirlenmesi

Gülhan TEMEL, Semra ERDOGAN

ABSTRACT

Objective: Before beginning method comparison studies in clinical researches, all the researchers share a common problem. That is: how to determine the sample size. The aim of this study is to identify the sample size calculation steps for the consistency statistics used in the identification of the agreement between the raters/methods; and to present practical tables belonging to the minimum sample numbers required, before researchers start clinical trials.

Materials and Methods: In this study, the steps of sample size calculation have been given for cases where there is no information on neither the population nor the consistency among the raters. Tables have been formed for Cohen Kappa and Intra-class correlation coefficient. Besides, other steps of sample number calculation have been given by utilizing a common formulation used for all consistency statistics by Gwet and practical tables have been presented.

Results: When the tables are studied, no matter what the importance level and the test power is, the sample number increases while inconsistency rate between the two raters increases up to 0.50; and the sample number shows a symmetrical decrease while inconsistency rate between the two raters shows an increase from 0.50 through 1. Moreover, as the consistency value between the raters rise, no matter what the test power and the importance level is, the sample size to be included in the study decreases in direct proportion.

Conclusion: Before beginning a research study, with the exact determination of the minimum number of samples enough for the design of the study and the state of the final variable, besides proving reliability of the results of the study, sampling waste will also be prevented.

Keywords: Method comparison, Agreement between the raters, Sample size

ÖZ

Amaç: Klinik araştırmalarda metot karşılaştırması çalışmalarına başlamadan önce tüm araştırmacıların problem yaşadığı şey ne kadar örneklem büyüklüğü ile çalışılmasıdır. Bu çalışmanın amacı, değerlendiriciler / yöntemler arasındaki uyumun belirlenmesinde kullanılan uyum istatistikleri için örneklem büyüklüğünün hesaplanma adımlarını tanımlamak, klinik çalışmalar için araştırmacılara araştırmaya başlamadan önce gerekli olan minimum örneklem sayılarına ait pratik tablolar sunmaktır.

Gereçler ve Yöntemler: Bu çalışmada, populasyona ait bir bilgi olmadığı durumda ve değerlendiriciler arası uyum bilindiğinde örneklem büyüklüğünün hesaplama adımları verilmiştir. Cohen Kappa ve Sınıf içi korelasyon katsayısı için tablolar oluşturulmuştur. Ayrıca Gwet tarafından tüm uyum istatistikleri için kullanılabilecek ortak bir formülasyondan yararlanılarak da örneklem büyüklüğü hesaplama adımları verilmiş ve pratik tablolar sunulmuştur.

Bulgular: Tablolar incelendiğinde, önem seviyesi ve testin gücü ne olursa olsun iki değerlendirici arasındaki uyumsuzluğun oranı 0.50'ye kadar artış gösterirken örneklem büyüklüğü de artmakta, 0.50'den 1'e doğru artış gösterirken simetrik olarak bir azalış göstermektedir. Bunun yanı sıra, değerlendiriciler arasındaki uyum değeri arttıkça testin gücü ve önem seviyesi ne olursa olsun doğru orantılı olarak çalışmaya dahil edilecek olan örneklem sayısı da azalmaktadır.

Sonuç: Bir araştırma çalışmasının başlangıcında, çalışmanın tasarımına ve sonuç değişkeninin durumuna uygun olan yeterli minimum örneklem sayısının doğru olarak belirlenmesi ile, çalışma sonuçlarının güvenilirliği sağlanmış olmasının yanında, örneklem israfının da önüne geçilmiş olacaktır.

Anahtar kelimeler: Metot karşılaştırması, Değerlendiriciler arası uyum, Örneklem büyüklüğü

Introduction

Method comparison in clinical researches is, to assess whether the two different techniques handled, are in agreement or not, and whether the technique that can be used as an alternative to reference technique is valid

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or even superior or not. As the data obtained are based on a measurement, the measurement done or the new technique developed have to be shown to be valid and reliable to be used. Reliability is not only used in the comparison of measurement methods but also in the test of the compatibility between the measurements obtained from the repeated measurements of a single measurement method (the rater) or from two or more raters (method). The statistical method to be applied for the identification of the degree of reproducibility of the measurements, the agreement between the measurements between the methods or measurements taken by more than one raters depends on what kind of a variable the result of measurement has been expressed and the number of raters [1].

Agreement studies is quite common in studies in medical field as well as educational sciences. Besides, the most frequent problem all the researchers face with before starting such studies is required sample size they should study with; because the detection of the sampling size is one of the most important and even most difficult steps in the planning of clinical studies. Studying with enough samples is quite important in scientific, economical and ethical terms. Studying on a sample size large enough is the most important factor in guaranteeing the validity and reliability of the findings obtained from the study scientifically. In scientific studies, while studying with less than enough samples in number would decrease the power of the results of the study, studying with more than enough samples would lead to a futile effort and resource waste. Besides, as studying with more than necessary number of samples would lead to the exposure of many individuals to unnecessary harmful factors, that would create ethically inappropriate results. Many researchers and scientific publications get help from many guides and standards appropriate to the studies and developed accordingly in order to increase the quality and the reliability of the clinical studies. These kind of guides' having the question of "How the sampling size is determined" in their method parts show how greatly important the determination of the sampling size and power in clinical studies is [2, 3].

Before the determination of sample size, it is necessary that the population is known well and the effect size representing how much type 1 error, the test power and the estimated value obtained as a result of the study would digress is determined.

In agreement studies, if there are preexisting studies, sample size can be calculated with the help of the agreement statistics between the raters in these studies. Sometimes, while calculating the sample size, there may not be any information about the population or may not be a preexisting study. In this case, the researcher is advised to do a pilot study. However, if the researcher does not have enough time to do a pilot study, only when the type 1 error, the power of the test and a significance difference between the two raters (effect size) is known can the sample size be calculated. The formulations of power and sample size show difference for each clinical study. The aim of this study is to describe the sample size calculation steps for agreement statistics used in the determination of the agreement between the raters/methods and to present practical tables about the minimum sample size required for researchers before they start the research in clinical studies.

Materials and Methods

Agreement Statistics

Assume that the result variable for a disease interested by two different raters like A and B is determined as patient (+) and healthy (-). This case is shown with a contingency table as in Table I.

In Table I, while the diagonal values show that the measurements belonging to both evaluators are in agreement, the measurement results except the diagonal values show disagreement. In other words, while d_{11} shows the number of classifications in the category "+" by both raters and d_{00} shows the number of classifications in the category of "–" by both raters, d_{01} and d_{10} shows the number of classifications in the situations where both raters disagree [4]. Agreement probability is calculated by utilizing the sum of the number

Table I: Cross table belonging to a state of two raters and two categories.

		Rater A		
		-	+	Total
Rater B	-	d_{00}	d_{01}	
	+	d_{10}	d_{11}	
	Total			N

of same classification (+/- categories) for both raters, also disagreement probability is calculated by utilizing the sum of the number of different classification (+/- categories) for raters. The probabilities of both raters being in agreement (π_A) and disagreement (π_D) is given in Equation 1.

$$\pi_A = \frac{d_{00} + d_{11}}{N} \text{ and } \pi_D = \frac{d_{01} + d_{10}}{N} \quad (1)$$

If the measurements belonging to both raters and the agreement coefficient between these raters is not known but the two raters' being in disagreement is possible, the sample size required for these cases can be calculated by using Equation 2. Here π_D shows the disagreement probability of two raters and W_D shows type II error (β). The power of the test is expressed as (1-β). In the case that the power of the test is 80 %, type II error value will be 0.20. The $Z^2_{1-\alpha/2}$ in Equation 2 gives the probability values in standard normal distribution table belonging to the significance levels (Type I error, alpha). The probability value belonging to the normal distribution table for 0.001 significance level is 3.2905 and 2.5758 for 0.01 significance level and 1.96 for 0.05 significance level [5].

$$n = \frac{4\pi_D(1-\pi_D)Z^2_{1-\alpha/2}}{W_D^2} \quad (2)$$

Cohen Kappa Statistics

Kappa statistics have been developed in 1960 by Cohen in order to evaluate the agreement between the two raters and have been formulized as in Equation 3 [6].

$$\kappa = \frac{\pi_A - \pi_E}{1 - \pi_E} \quad (3)$$

π_A shows the rate of both raters' being in perfect agreement and is calculated as $(d_{00}+d_{11})/N$. π_E shows the expected rate of chance agreement probability and is obtained from the sum of the negative and positive agreements. Negative agreement is calculated by utilizing the sum of row and column of negative results for two raters, positive agreement is calculated by utilizing the sum of row and column of positive results. Negative agreement is formulized by using Equation 4 and positive agreement is formulized by using Equality 5 and the proportion of chance agreement is formulized as in Equality 6 [5].

$$Negatif\ Agreement = \frac{(d_{00} + d_{01})(d_{00} + d_{10})}{N^2} \quad (4)$$

$$Pozitif\ Agreement = \frac{(d_{10} + d_{11})(d_{01} + d_{11})}{N^2} \quad (5)$$

$$\pi_E = \frac{(d_{00} + d_{01})(d_{00} + d_{10})}{N^2} + \frac{(d_{10} + d_{11})(d_{01} + d_{11})}{N^2} \quad (6)$$

Kappa coefficient takes a value between -1 and +1 but practically, a value between 0 and 1 is interested. So, the interpretable interval of Kappa coefficient is 0 and +1, and its being smaller than 0 (negative) does not have a meaning in terms of reliability. While Kappa coefficient's taking the value of 1 is said to be a perfect agreement, its taking the value 0 gives the result that there is no agreement and the decisions of the two raters are completely different [1]. In some sources, there are different classifications about the strength of the Kappa coefficient (agreement degree). These classifications may change on the topic that is studied. Generally, in the studies, as the limit value for Kappa coefficient, 0.20 is said to be poor, 0.21-0.40 is below the moderate, 0.41-0.60 is moderate, 0.61-0.80 is good and 0.81-1.00 is said to be in perfect agreement [7].

The quite common agreement statistic in the literature is Cohen Kappa statistics. However, it has been put forward that this agreement statistic is affected from sensitivity, specificity and prevalence and that it has to be more carefully handled while being used in reliability and agreement studies [8].

The agreement between the raters can be calculated with the help of Cohen Kappa statistic. In this case, before beginning the study, when the agreement coefficient between the raters is known, minimum necessary sample size (m_k) can be calculated as in Equation 7. In the equation, π_{Dis} shows the probability of disagreement and W_D shows the type II error. $Z^2_{1-\alpha/2}$ gives the probability values in standard distribution table belonging to significance levels (Type I error) [5].

$$m_\kappa = 4\frac{(1-\kappa)}{W_D^2}\left((1-\kappa)(1-2\kappa) + \frac{\kappa(2-\kappa)}{2\pi_D(1-\pi_D)}\right)Z^2_{1-\alpha/2} \quad (7)$$

***Intra-class Correlation Coefficient* (*ICC*)**

If our result variable is not categorical but in a continuous structure, Intra-class correlation coefficient is used for the agreement between the raters and when there are 2 or more raters, ICC value is formulized as in Equation 8. In the equation, σ_B represents the standard deviation between the raters and σ_W indicates the standard deviation within the raters [9].

(4)

$$\rho = \frac{\sigma_B^2}{\sigma_W^2 + \sigma_B^2} \quad (8)$$

The acceptable level of ICC shows difference according to the characteristics of data and the subject studied and the aim. When the agreement between the raters is considering, ICC is expected to be minimum 0.70. It can be said that the agreement between the raters is "perfect" if the ICC is taking a value between 0.95 and 1.00, "high" if it takes a value between 0.85 and 0.94, "moderate" level if it takes a value between 0.70 and 0.84 and no agreement at all if it takes a value below 0.70 [10, 11].

According to the confidence interval approach, in a case where there are k number of raters independent from each other, before starting the study, the minimum necessary sample size (m_{ICC}) is calculated as in Equation 9 when the agreement correlation between the raters is known. In the equation, W_D shows type II error, $Z^2_{1-\alpha/2}$ shows the probability values in the standard normal distribution table belonging to the significance levels (Type I error), ρ_{Plan} shows the ICC [5]. And it is obtained from a prior research or an expert opinion. The sample size that is determined for ICC is valid for three ANOVA models of ICC and for measurement reliability (Consistency, absolute agreement) [12].

$$m_{ICC} = 1 + \frac{8\, Z^2_{1-\alpha/2} \left(1-\rho_{Plan}\right)^2 \left[1+(k-1)\rho_{Plan}\right]^2}{k(k-1)W_D^2} \quad (9)$$

Alternative Sample Size Formulation for Gwet's Agreement Statistics

Gwet's agreement coefficient (AC1), has been put forward in 2001 by Gwet and is calculated as (Equation 10) [6,13].

$$AC1 = \gamma = \frac{\pi_A - \pi_E(\gamma)}{1 - \pi_E(\gamma)} \quad (10)$$

In the equation, the proportion of chance agreement is calculated as $\pi_E(\gamma) = 2P_1(1-P_1)$. The P_1 probability in the formula is calculated as in Equation 11 [6,13].

$$P_1 = \frac{(d_{00} + d_{10} + d_{00} + d_{01})/2}{N} \quad (11)$$

The AC1 statistic put forward by Gwet is said to be not affected from sensitivity, specificity and prevalence values compared to Cohen's Kappa statistic and to show a better performance [8]. Besides, if the prevalence value is known and matters for the study, the use of Gwet's AC1 statistics is advised to the researchers [14].

While the reliability coefficient between the real raters is obtained based on the entire population, the estimated reliability coefficient between the raters is obtained from the sample. According to Gwet, for the reliability coefficients between the raters to be valid, it has to be more than 20 % of the true value (the value obtained based on the population). Here, the value 20 % is taken arbitrary and can be changed by the researchers. Gwet argues that the sample size is affected from this arbitrary value in the reliability studies. Based on that, he suggests the formulation in Equation 12 for the calculation of necessary minimum sample size in agreement studies. In this formulation, N shows the sample size belonging to the population, r shows the relative error (the difference between the true value obtained from the population and the estimated value obtained from the sample, effect size), π_A shows the overall agreement probability and π_E shows the chance-agreement probability [15, 16].

$$n = \frac{n^*}{1 + \frac{n^*}{N}}, \quad n^* = \frac{1}{r^2\left(\pi_A - \pi_E\right)^2} \quad (12)$$

Results

Determination of sample size is one of the most important and even a difficult step in the planning and designing of clinical studies. This is why the suggested sample size formulations in the determination of minimum sample size enough to the researcher at the beginning of a study in agreement studies are calculated under different conditions and presented in tables. With this aim, a macro has been written in Excel for each formulation and the results obtained have been put into tables. Besides, with the help of the Demo version of SPSS 21 statistic packet program, the graphics for Tables 2-5 have been obtained.

In this study, the calculation steps for sample sizes have been given when there is no information about the population and the agreement between the raters is known. In this study, the tables have been prepared only for Cohen Kappa and ICC. Besides, sample size calculation steps have been given with the help of a common formulation that can

be used for all agreement statistics by Gwet and practical tables have been presented.

To calculate enough sample size according to the situation where the measurements belonging to the two raters and the agreement coefficient between these raters is known but it is possible that these two raters are in disagreement, with the help of Equation 2, the minimum necessary sample size has been calculated in 0.001, 0.01 and 0.05 significance levels, various power levels (95 %, 90 % and 80 %) and for 16 different disagreement rates (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.60, 0.70, 0.80, 0.90 and 1) and has been given in Table II. The graphic belonging to Table II is as in Figure 1. When Table II and Figure 1 are considered, while the rate of disagreement between the two raters shows an increase up to 0.50, size sample also increases and shows a symmetrical decrease after 0.50. When the disagreement probability is 100 %, the sample size is calculated as 0. As the test power increases, for the agreement between the raters to be significance, it is necessary that more samples are studied.

With the help of the Equation belonging to Kappa statistic suggested by Cohen to calculate the agreement between the raters, the minimum necessary sample size has been calculated for three different type 1 errors (0.001, 0.01, and 0.05), three different test powers (95 %, 90 % and 80 %), 6 different disagreement rates (0.05, 0.10, 0.20, 0.30, 0.40, 0.50) and 9 different Kappa statistic values (0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 and 0.90) and has been given in Table III. Besides, calculations for disagreement rates 0.60, 0.70, 0.80, 0.90 and 0.95 have also been done but 0.05 has given the same results with 0.95, 0.10 with 0.90, 0.20 with 0.80, 0.30 with 0.70 and 0.40 with 0.60. This is why the results belonging to only 6 different disagreement rates have been written in the table. Besides, it has been observed that, in all possible disagreement rates, all type I and type II errors get the value 0 when the Kappa statistic is "1" and that it always gives the same results when Kappa statistic is "0". The graphic belonging to Table III is given in Figure 2. When Table III and Figure 2 are considered, no matter what the disagreement rates between the raters, type I error and test power are, enough

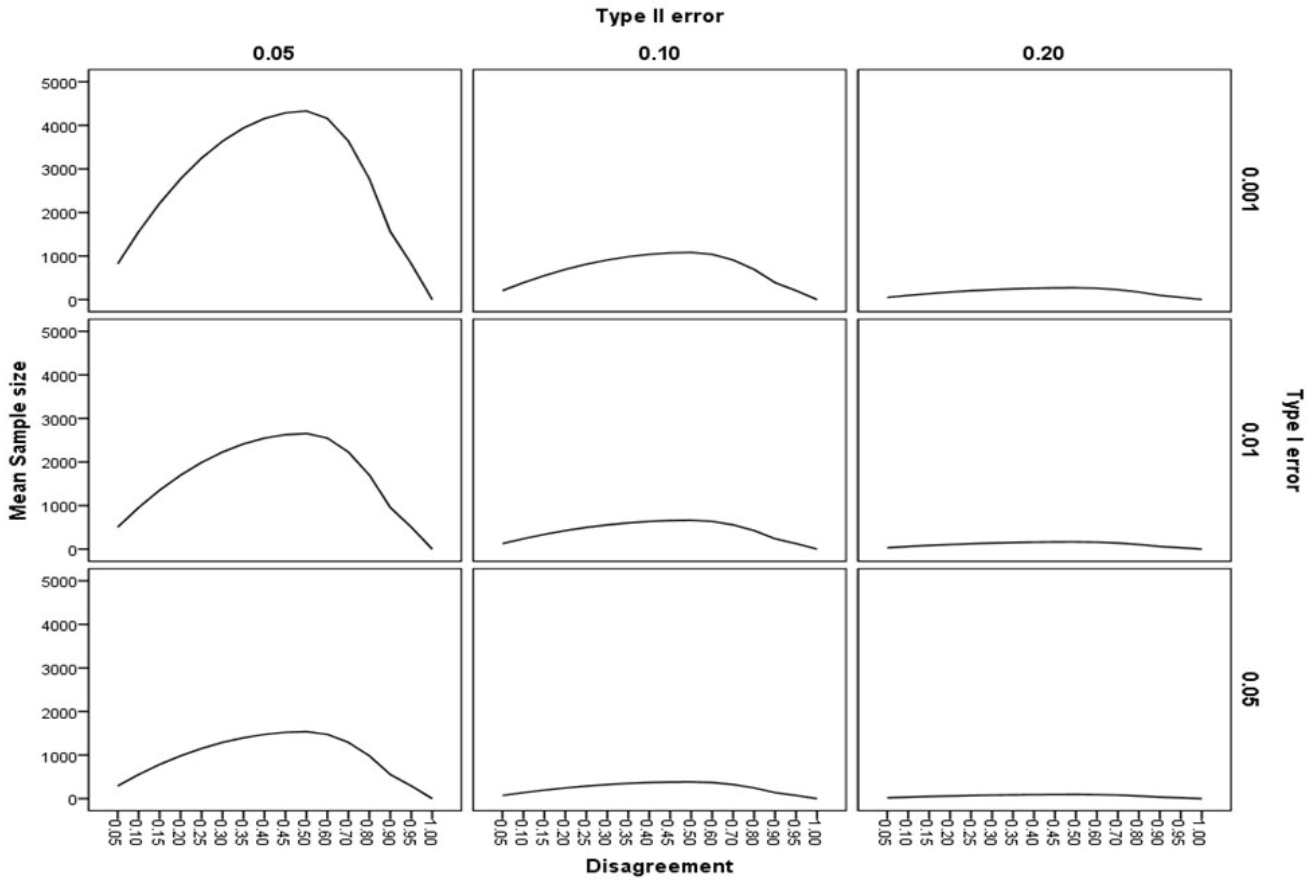

Figure 1: The necessary sample sizes according to the disagreement rates between the two raters, Type I and Type II errors.

Table II: The necessary sample sizes according to the disagreement rates between the two raters, Type I and Type II errors.

	alfa=0.001			alfa=0.01			alfa=0.05		
π_D	β= 0.05	β= 0.10	β= 0.20	β= 0.05	β= 0.10	β= 0.20	β= 0.05	β= 0.10	β= 0.20
0.05	823	206	51	504	126	32	292	73	18
0.10	1559	390	97	955	239	60	553	138	35
0.15	2209	552	138	1353	338	85	784	196	50
0.20	2772	693	173	1698	425	106	983	246	61
0.25	3248	812	203	1990	498	124	1152	288	72
0.30	3638	910	227	2229	557	139	1291	323	81
0.35	3941	985	246	2415	604	151	1398	350	87
0.40	4158	1039	260	2548	637	159	1475	369	92
0.45	4288	1072	268	2627	657	164	1521	380	95
0.50	4331	1083	271	2654	663	166	1537	384	96
0.60	4158	1039	260	2548	637	159	1475	369	92
0.70	3638	910	227	2229	557	139	1291	323	81
0.80	2772	693	173	1698	425	106	983	246	61
0.90	1559	390	97	955	239	60	553	138	35
0.95	823	206	51	504	126	32	292	73	18
1.00	0	0	0	0	0	0	0	0	0

π_D:Disagreement rates; alfa: Type I error; β:Type II error

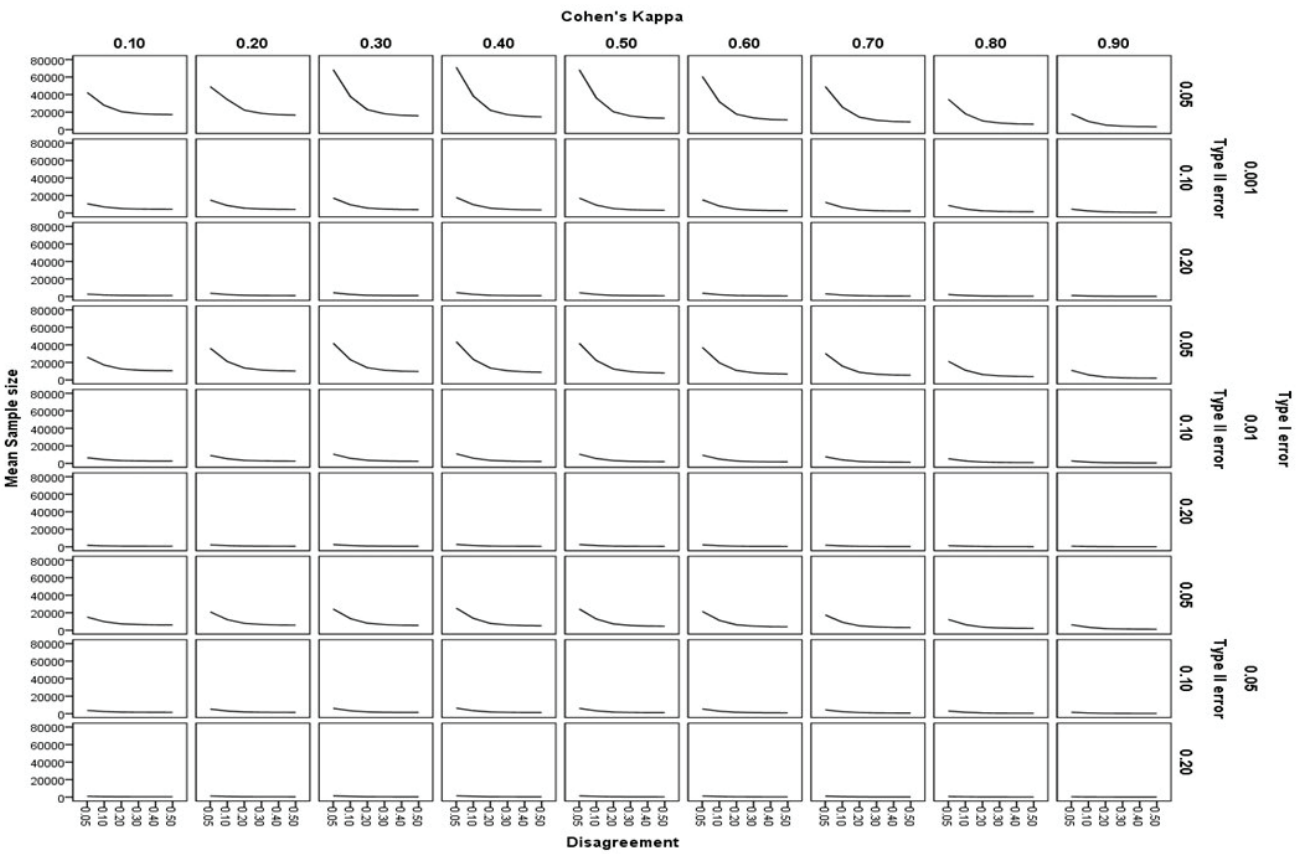

Figure 2: The necessary sample sizes according to Type I and Type II errors given by using Cohen Kappa.

sample size increases while Kappa statistic is rising up to 0.50 and the enough sample size decreases when the Kappa statistic shows an increase from 0.50 to 1. Besides, while the disagreement rate between the raters shows an increase up to 0.50, the enough sample size also decreases no matter what the Kappa statistic value, type I error and test power are; the disagreement rate between the raters shows an increase from 0.50 to 0.95, the enough sample size shows a symmetrical increase no matter what the Kappa statistic value, type I error and test power are.

Table III: The necessary sample sizes according to Type I and Type II errors given by using Cohen Kappa.

		alfa=0.001			alfa=0.01			alfa=0.05		
π_D	Kappa	β= 0.05	β= 0.10	β= 0.20	β= 0.05	β= 0.10	β= 0.20	β= 0.05	β= 0.10	β= 0.20
0.05	0.10	42409	10602	2651	25987	6497	1624	15047	3762	940
	0.20	49171	14793	3698	36258	9065	2266	20994	5249	1312
	0.30	68497	17124	4281	41973	10493	2623	24303	6076	1519
	0.40	71272	17818	4455	43674	10918	2730	25288	6322	1580
	0.50	68384	17096	4274	41904	10476	2619	24263	6066	1516
	0.60	60717	15179	3795	37206	9301	2325	21543	5386	1346
	0.70	49160	12290	3072	30124	7531	1882	17442	4360	1090
	0.80	34597	8649	2162	21200	5300	1325	12275	3069	767
	0.90	17915	4479	1120	10978	2744	686	6356	1589	397
0.10	0.10	27683	6921	1730	16964	4241	1060	9822	2456	614
	0.20	34370	8593	2148	21061	5265	1316	12195	3049	762
	0.30	37754	9439	2360	23135	5784	1446	13395	3349	837
	0.40	38205	9551	2388	23411	5853	1463	13555	3389	847
	0.50	36091	9023	2256	22116	5529	1382	12805	3201	800
	0.60	31783	7946	1986	19476	4869	1217	11277	2819	705
	0.70	25651	6413	1603	15718	3930	982	9101	2275	569
	0.80	18063	4516	1129	11069	2767	692	6409	1602	401
	0.90	9390	2347	587	5754	1438	360	3331	833	208
0.20	0.10	20483	5121	1280	12552	3138	784	7268	1817	454
	0.20	22244	5561	1390	13630	3408	852	7892	1973	493
	0.30	22722	5681	1420	13924	3481	870	8062	2015	504
	0.40	22036	5509	1377	13503	3376	844	7818	1954	489
	0.50	20301	5075	1269	12440	3110	778	7203	1801	451
	0.60	17636	4409	1102	10806	2702	675	6257	1564	391
	0.70	14156	3539	885	8674	2169	542	5023	1256	314
	0.80	9979	2495	624	6115	1529	382	3540	885	221
	0.90	5221	1305	326	3199	800	200	1852	463	116
0.30	0.10	18279	4570	1142	11201	2800	700	6485	1621	405
	0.20	18532	4633	1158	11356	2839	710	6575	1644	411
	0.30	18121	4530	1133	11104	2776	694	6429	1607	402
	0.40	17086	4272	1068	10470	2618	654	6062	1516	379
	0.50	15468	3867	967	9478	2370	592	5488	1372	343
	0.60	13305	3326	832	8153	2038	509	4721	1180	295
	0.70	10637	2659	665	6518	1629	407	3774	943	236
	0.80	7504	1876	469	4598	1150	287	2662	666	166
	0.90	3945	986	247	2417	604	151	1400	350	87
0.40	0.10	17397	4349	1087	10661	2665	666	6173	1543	386
	0.20	17047	4262	1065	10446	2611	653	6048	1512	378
	0.30	16280	4070	1018	9976	2494	623	5776	1444	361
	0.40	15106	3777	944	9257	2314	578	5360	1340	335
	0.50	13534	3384	846	8293	2073	518	4802	1201	300
	0.60	11572	2893	723	7091	1773	443	4106	1026	256
	0.70	9229	2307	577	5655	1414	353	3275	819	205
	0.80	6514	1628	407	3991	998	249	2311	578	144
	0.90	3434	859	215	2105	526	132	1219	305	76
0.50	0.10	17151	4288	1072	10509	2627	657	6085	1521	380
	0.20	16631	4158	1039	10191	2548	637	5901	1475	369
	0.30	15765	3941	985	9660	2415	604	5593	1398	350
	0.40	14552	3638	910	8917	2229	557	5163	1291	323
	0.50	12993	3248	812	7962	1990	498	4609	1152	288
	0.60	11087	2772	693	6794	1698	425	3934	983	246
	0.70	8835	2209	552	5414	1353	338	3135	784	196
	0.80	6237	1559	390	3822	955	239	2213	553	138
	0.90	3292	823	206	2017	504	126	1168	292	73

π_D:Disagreement probability; alfa: Type I error; β:Type II error

With the help of the Equation belonging to ICC, the necessary minimum sample sizes have been calculated for three different type I error (0.001, 0.01 and 0.05), three different test power (95 %, 90 % and 80 %), 8 different ICC values (0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90 and 0.95) and 3 different number of raters (2, 3 and 4) and have been given in Table IV. The graphic belonging to the Table IV is as in Figure 3. The numbers belonging to the samples sizes given in tables express the sample size necessary for only one rater. When Table IV and Figure 3 are examined, the sample number to be included in the study decreases directly proportionally regardless of the test power and significance level. When the number of raters is 2, more sample is needed to be studied compared to 3 or 4 raters.

With the sample size formulation for all possible agreement statistics by Gwet, the minimum sample sizes to be pulled from the population have been calculated in five different relative errors (effect size) (0.10, 0.20, 0.30, 0.40 and 0.50), the value differences between 10 different agreement probabilities (0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90 and 1) and in five different sample sizes belonging to the population (30, 100, 500, 1000 and 10000) and have been given in Table V. The graphical display of Table V is as in Figure 4. When Table V and Figure 4 are examined, there is an inverse proportion between the relative error and sample size. As effect size increases, the enough sample size decreases.

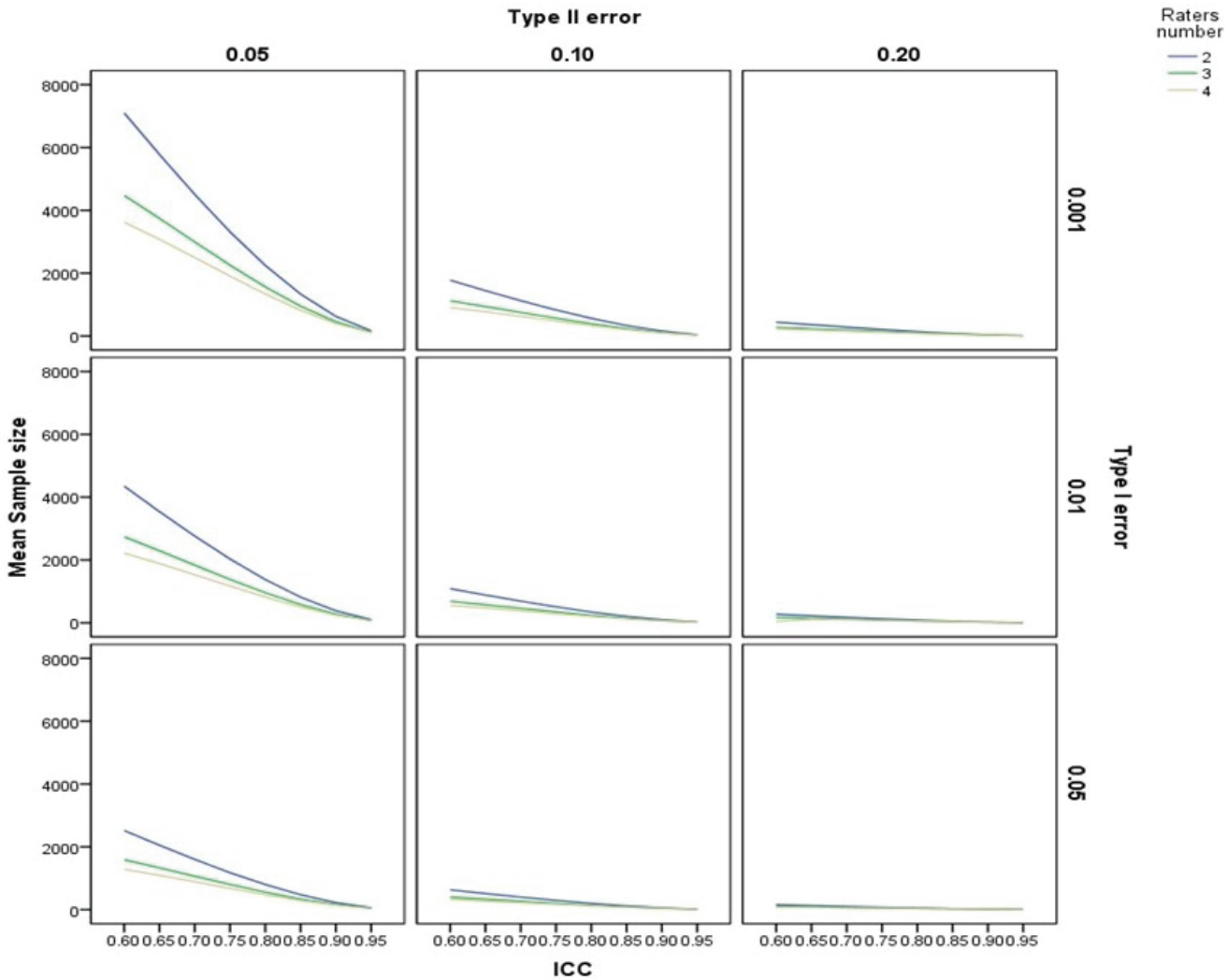

Figure 3: The necessary sample sizes according to Type I and Type II errors given for the agreement between the raters by using intra-class correlation coefficient (ICC).

Table IV: The necessary sample sizes according to Type I and Type II errors given for the agreement between the raters by using intra-class correlation coefficient (ICC).

		alfa=0.001			alfa=0.01			alfa=0.05		
k	**ICC**	**β=0.05**	**β=0.10**	**β=0.20**	**β=0.05**	**β=0.10**	**β=0.20**	**β=0.05**	**β=0.10**	**β=0.20**
2	**0.60**	7097	1775	444	4349	1088	273	2519	630	158
	0.65	5779	1445	362	3541	886	222	2051	513	129
	0.70	4507	1127	283	2762	691	174	1600	401	101
	0.75	3317	830	208	2033	509	128	1177	295	75
	0.80	2246	562	141	1377	345	87	798	200	51
	0.85	1335	335	84	818	205	52	474	119	31
	0.90	626	157	40	384	97	25	223	56	15
	0.95	166	42	11	102	26	7	59	16	5
3	**0.60**	4473	1119	280	2741	686	172	1588	398	100
	0.65	3743	937	235	2294	574	144	1329	333	84
	0.70	2995	749	188	1835	460	116	1063	267	67
	0.75	2257	565	142	1383	347	87	801	201	51
	0.80	1562	391	99	958	240	61	555	140	36
	0.85	948	238	60	581	146	37	337	85	22
	0.90	454	114	29	278	70	18	162	41	11
	0.95	122	31	9	75	20	6	44	12	4
4	**0.60**	3623	906	227	2220	556	40	1286	322	81
	0.65	3079	771	193	1887	473	119	1093	274	69
	0.70	2498	625	157	1531	384	97	887	223	56
	0.75	1907	478	120	1169	293	74	677	170	43
	0.80	1336	335	84	819	206	52	475	119	31
	0.85	820	206	52	503	126	32	291	74	19
	0.90	396	100	26	243	62	16	141	36	10
	0.95	108	28	8	67	17	5	39	10	3

k: Number of raters; ICC: Intra-class correlation coefficient; alfa: Type I error; β:Type II error

Effect size is a very important concept in the determination of sample size. Effect size shows difference according to the studies. Effect size is sometimes determined with literature scan with the help of the studies done before, and sometimes it can be determined with a pilot study in the absence of a study done before. Sometimes, none of them happen, in such a case, according to the advice of Cohen, the effect size is determined with the help of predetermined 0.20 low effect, 0.50 middle level impact and 0.80 high impact or other pieces of advice [6, 12]. Based on these, the sample size for different agreement probabilities from different size populations have been calculated and as is Table V.

Strengths and Limitations of the Study

Strengths

The most important step in a study is to find answer to the question of how many cases should be studied with in the planning, design and application phases. In this study, practical sample size tables have been formed in the rates of three different type I error and three different tests and different disagreement rates. Through these tables, it would be possible to study with suitable and enough number of cases for the design of the study and the outcome variable. Besides, in this study, the agreement statistics used in the calculation of the agreement between the raters/methods in clinical studies and the superiorities of these agreement statistics to each other and the bias of these statistics and the mistakes made have been focused on.

Limitations

In this study, only one measurement of two raters belonging to the agreement statistics commonly used in literature; and sample size tables for the agreement statistics between these measurements have been formed.

Table V: The necessary sample size for the agreement coefficients given developed for the agreement coefficients by Kilem Gwet

$(\pi_A-\pi_E)$	Relative error (EB)	N=30	N=100	N=500	N=1000	N=10000
0.10	0.10	30	99	477	909	5000
	0.20	30	96	417	714	2000
	0.30	29	92	345	526	1000
	0.40	29	86	278	385	588
	0.50	28	80	222	286	385
0.20	0.10	30	96	417	714	2000
	0.20	29	86	278	385	588
	0.30	27	74	179	217	270
	0.40	25	61	119	135	154
	0.50	23	50	83	91	99
0.30	0.10	29	92	345	526	1000
	0.20	27	74	179	217	270
	0.30	24	55	99	110	122
	0.40	21	41	61	65	69
	0.50	18	31	41	43	44
0.40	0.10	28	86	278	385	588
	0.20	25	61	119	135	154
	0.30	21	41	61	65	69
	0.40	17	28	36	38	39
	0.50	14	20	24	24	25
0.50	0.10	28	80	222	286	385
	0.20	23	50	83	91	99
	0.30	18	31	41	43	44
	0.40	14	20	24	24	25
	0.50	10	14	16	16	16
0.60	0.10	27	74	179	217	270
	0.20	21	41	61	65	69
	0.30	15	24	29	30	31
	0.40	11	15	17	17	17
	0.50	8	10	11	11	12
0.70	0.10	26	67	145	169	200
	0.20	19	34	46	49	51
	0.30	13	18	22	22	23
	0.40	9	11	12	13	13
	0.50	6	8	8	8	8
0.80	0.10	25	61	119	135	154
	0.20	17	28	36	38	39
	0.30	11	15	17	17	17
	0.40	7	9	10	10	10
	0.50	5	6	6	6	6
0.90	0.10	24	55	99	110	122
	0.20	15	24	29	30	31
	0.30	9	12	13	14	14
	0.40	6	7	8	8	8
	0.50	4	5	5	5	5
1.00	0.10	23	50	83	91	99
	0.20	14	20	24	24	25
	0.30	8	10	11	11	11
	0.40	5	6	6	6	6
	0.50	4	4	4	4	4

$(\pi_A-\pi_E)$: The difference between of the overall agreement probability and the chance-agreement probability; Relative error (EB); Effect size

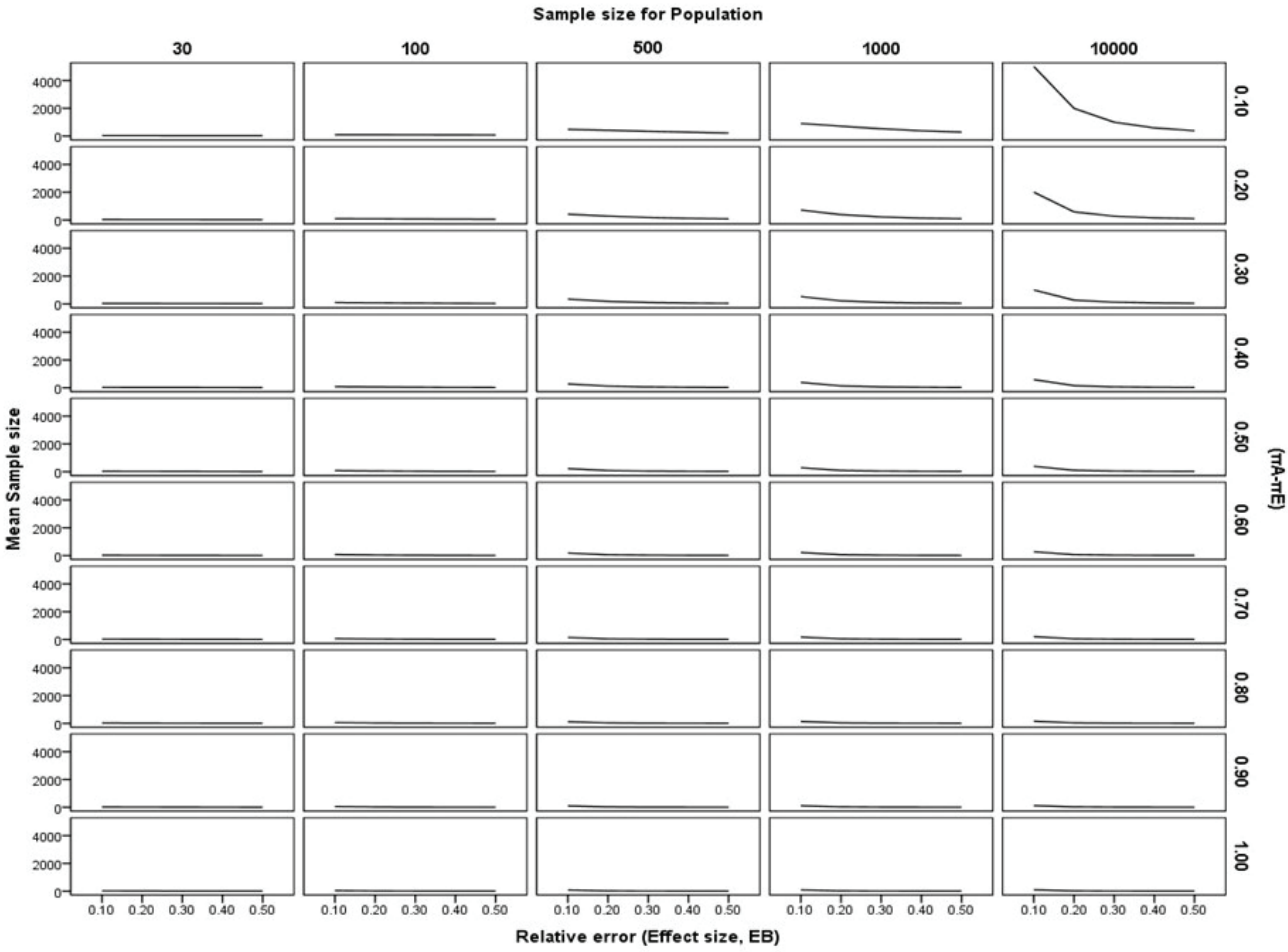

Figure 4: The necessary sample size for the agreement coefficients given developed for the agreement coefficients by Kilem Gwet

Discussion

Determination of sample size is one of the most important and even the most difficult steps in the planning of clinical studies. Studying with enough samples is quite important because of scientific, economical and ethical reasons. Studying on a sample large enough is the most important factor guaranteeing the findings to be obtained from the research, its scientific validity and reliability. While studying with less than enough samples in number in scientific studies would decrease the power of the study, studying with more than enough samples in number would lead to a futile effort and resource waste. Besides, in the determination of sample size with power analysis in clinical studies, type I error, power and effect size have to be known. The importance value's being small and power's being large are the reasons increasing the sample size. Besides, it is necessary to study with more samples in small effect sizes [2,3].

One of the most common mistakes made in the agreement studies between the raters is the confusion of the concepts of agreement and relationship. When our outcome variable is in a continuous state, instead of getting help from ICC, Pearson or Spearman correlation coefficients giving the relationship between the two continuous variables are used to test the agreement between the raters. Generally, when the measurement agreement is high, it is possible to obtain information that the agreement with these two tests is also good. However, it is a mistake to use these two tests in the use of measurement agreement analysis [11].

In the case when our outcome variable is in a categorical structure, instead of Kappa statistic value, Mc-Nemar test used in the testing of whether there is a difference between the results of the two raters is preferred to test the agreement. However, it is a mistake to use this test in agreement analysis, too. Besides, it is also suggested by the researchers

that Kappa statistic is affected from the prevalence and that very intensive importance have to be given when it is used in agreement studies as agreement coefficient [8]. Thus, while calculating the sample size, the design of the study has to be known very well, too.

As a result, with the accurate determination of enough minimum sample size suitable for the design of a study and the state of the result variable at the beginning of the test, besides providing the reliability of the study results, the waste of samples would also be avoided.

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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The role of magnetic resonance diffusion tensor imaging in differentiation of benign from malignant focal renal lesions

Fokal renal lezyonlarının benign ve malign ayırımında manyetik rezonans difüzyon tensör görüntülemenin rolü

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ABSTRACT

Objectives: To determine the diagnostic performance of apparent diffusion coefficient (ADC) and fractional anisotropy (Fa) measurements in differentiation of malignant from benign focal renal lesions.

Material and Methods: Twenty-one patients (9 women and 12 men ; mean age 54,1 years) with 25 focal renal lesions diagnosed by different imaging modalities such as computed tomography (CT) and ultrasound (US) and examined with magnetic resonance diffusion tensor imaging (DTI) were included in the study. There were 6 solid renal cell carcinomas (RCC), 1 cystic RCC, 1 angiomyolipoma (AML) and 17 cystic lesions (16 Bosniak type I cysts, 1 Bosniak type II cyst). ADC and Fa measurements were performed by two radiologists, in consensus. The differences in ADC and Fa values of malignant and benign renal lesions were compared using ANOVA and Tukey tests. A ROC analysis was applied; sensitivities and specificities were calculated.

Results: The mean ADC and Fa values of malignant lesions were 1.29±0.4 mm2/sec and 0.34±0.1, respectively. The mean ADC and Fa values of benign lesions were 2.6±0.7 mm2/sec and 0.19±0.12, respectively. The differences were significant (P<0.05). The Az values for discriminating malignant lesions from benign ones for ADC and Fa value were 0.95 and 0.83, respectively. The sensitivity-specificity of ADC and Fa were 100%-83.3%, and 100%-77.8%, respectively.

Conclusion: There are statistically significant differences between ADC and Fa values of malignant and benign focal renal lesions. Malignant renal lesions have low ADCs and high Fa values, whereas benign renal lesions have high ADCs and low Fa values.

Keywords: Magnetic resonance, Diffusion tensor, Renal lesion

ÖZ

Amaç: Fokal böbrek lezyonlarının benign, malign ayırımında görünüşteki difüzyon katsayısı (apparent diffusion coefficient - ADC) ve fraksiyonel anizotropi (Fa) ölçümlerinin diagnostik performanslarının belirlenmesi.

Gereç ve Yöntem: Çalışmamıza, bilgisayarlı tomografi (BT) ve ultrason (US) gibi değişik görüntüleme modaliteleri ile tanı konulan ve manyetik rezonans diffüzyon tensör görüntülemesi (DTI) yapılmış 21 hastada (9 kadın and 12 erkek ; ortalama yaş 54,1), 25 fokal renal lezyon dahil edildi. Lezyonlar 6 solid renal hücreli karsinom (RCC), 1 kistik RCC, 1 anjiomyolipom (AML) ve 17 kistik lezyondan (16 Bosniak tip I kist, 1 Bosniak tip II kist) oluşmaktaydı. ADC ve Fa ölçümleri iki radyolog tarafından, konsensusa varılarak yapıldı. Malign ve benign lezyonlara ait ADC ve Fa değerleri ANOVA ve Tukey testleri kullanılarak karşılaştırıldı. ROC analizi kullanıldı; sensitivite ve spesifite hesaplandı.

Bulgular: Malign lezyonların ortalama ADC ve Fa değerleri sırasıyla 1.29±0.4 mm2/sn ve 0.34±0.1 ölçüldü. Benign lezyonların ortalama ADC ve Fa değerleri sırasıyla 2.6±0.7 mm2/sn ve 0.19±0.12 ölçüldü. Aralarındaki fark istatistiksel olarak anlamlı idi (P<0.05). Malign lezyonları, benign lezyonlardan ayırmada ADC ve Fa ölçümlerinin, Az değerleri sırasıyla 0.95 ve 0.83 bulundu. ADC ve Fa ölçümlerinin sensitivite ve spesifite değerleri sırasıyla 100%-83.3% ve 100%-77.8% bulundu.

Sonuç: Malign ve benign fokal renal lezyonların ADC ve Fa ölçümleri değerleri arasında istatistiksel anlamlı farklar mevcuttur. Malign renal lezyonlarda düşük ADC ve yüksek Fa değerleri izlenirken, benign renal lezyonlarda yüksek ADC ve düşük Fa değerleri izlenir.

Anahtar kelimeler: Manyetik rezonans, Difüzyon tensör, Renal lezyon

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Introduction

Diffusion-weighted magnetic resonance imaging (DW-MRI) is a noninvasive technique that visualizes molecular diffusion by measuring the Brownian motion of water molecules in the tissues. This mobility shows the integrity of cell membrans and the cellularity of the underlying tissue [1]. The water within the intracellular and extracellular spaces have changes in the micro diffusion which have an effect on DW-MRI outcomes. Apparent diffusion coefficient (ADC) is the indicator of movement of water molecules in a tissue [2]. Generally, in a malignant lesion the cells are densely packed and due to existence of more organelles, membranes and fibers within the malignant cells, diffusion is restricted and ADC values are lower [3].

Because diffusion is a multidimensional process the use of DWI alone may result in a loss of important data within highly organized (i.e., anisotropic) tissues. Using additional gradients magnetic resonance diffusion tensor imaging (DTI) can evaluate the anisotropic features of tissues and allow the analysis of diffusion in multiple dimensions, thus provide more detailed data regarding the diffusion of water in various tissues [4-6]. Fractional anisotropy (Fa) shows the fraction of anisotropic diffusion to total diffusion. Furthermore, in a study, the authors showed that Fa has a good and strong correlation with cell density [7].

Our hypothesis is thus that the diffusion in malignant lesions should be more restricted and more anisotropic when compared with benign renal lesions because of higher cell density in malignant ones. The purpose of this preliminary study is to test this hypothesis.

Materials and Methods

Patients

Institutional approval for this retrospective study and written informed consents from all patients had been obtained. During six months 21 patients (9 women and 12 men ; mean age 54,1 years) with renal lesions diagnosed by different imaging modalities such as computed tomography (CT) and ultrasound (US) and examined with DTI were included in this study.

ADC and Fa values of 25 lesions of these 21 patients were evaluated. There were 6 solid renal cell carcinomas (RCC), 1 cystic RCC, 1 angiomyolipoma (AML) and 17 cystic lesions (16 Bosniak type I cysts, 1 Bosniak type II cyst). The diagnosis of RCC's were proven by means of biopsy. Angiomyolipoma was diagnosed according to typical MRI appearance. Cysts enrolled in the study were diagnosed according to their typical US and MRI findings. All benign cysts were followed up for at least 12 months.

MR Imaging

A 1.5T Scanner (Signa Excite HD; Healthcare, Milwaukee, WI, USA) was used for all MR examinations. Axial breath-hold, single-shot gradient echo planar DTI covering both kidneys were acquired using the following parameters: Matrix: 256x160, Acquisition time: 1:25 (min:sec), TE: 89.4 msec, TR: 6125.0 msec, Bandwith: 31.25 kHz, number of excitations: 4.0, field of view: 48, Slide thickness: 8.0 mm, Spacing: 1.0 mm, b-value: 1000 sec/mm2, number of diffusion directions: 6 Spectro Spatial RF pulse was used to reduce chemical shift artifacts.

Image Analysis

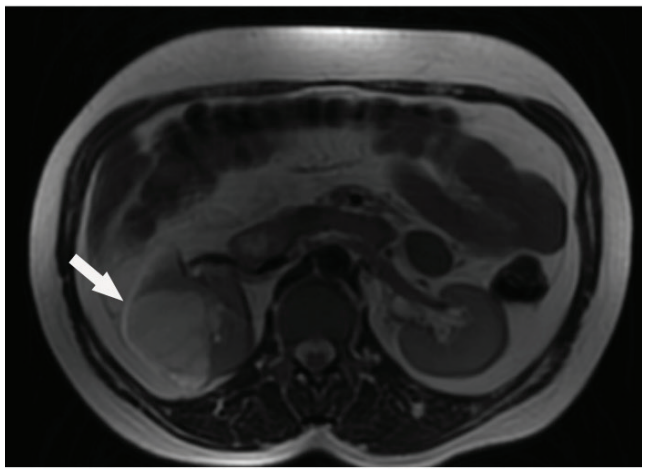
All images were reviewed by two radiologists (study coordinators), who were aware of the final diagnoses of the lesions, on postprocessing workstations and lesions were determined to be measured. When a patient had different types of lesions, all of them were included, otherwise the largest was chosen.

To analyse ADC and Fa values, two readers, who were blinded to the lesions, established regions of interest in each lesion on ADC and Fa maps, in consensus. All regions of interest were placed within the confines of the lesions; for heterogeneous lesions, regions of interest included the entire lesion.

Statistical Evaluation

The differences in ADC and Fa values of RCC's, angiomyolipoma and cysts were evaluated using ANOVA and post-hoc Tukey test. ROC analysis was applied to evaluate the use of ADC and Fa values in distinguishing malignant lesions from benign ones. The areas under the curve were calculated. Cut-off values for ADCs and Fa values were determined for discriminating malignant lesions from benign ones. Sensitivities and specificities with 95% confidence intervals (CI) were calculated accordingly.

a



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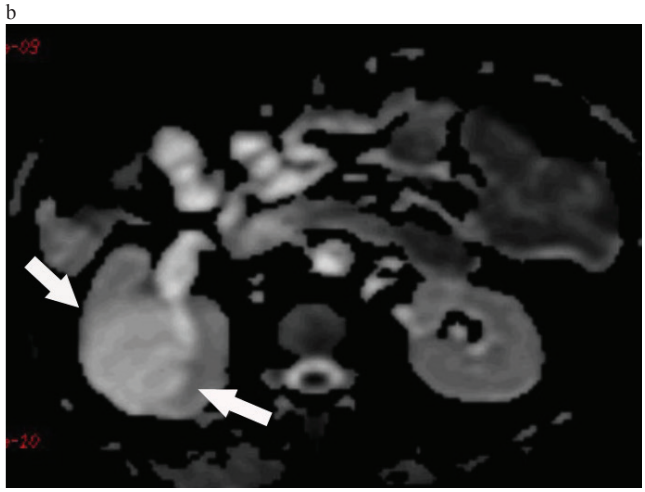

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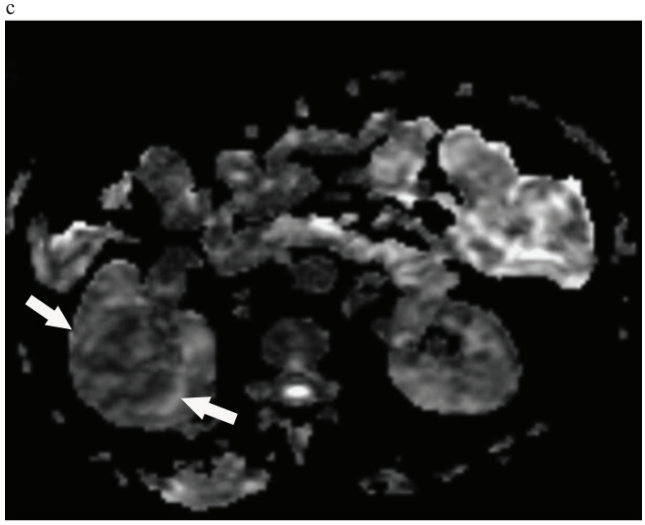
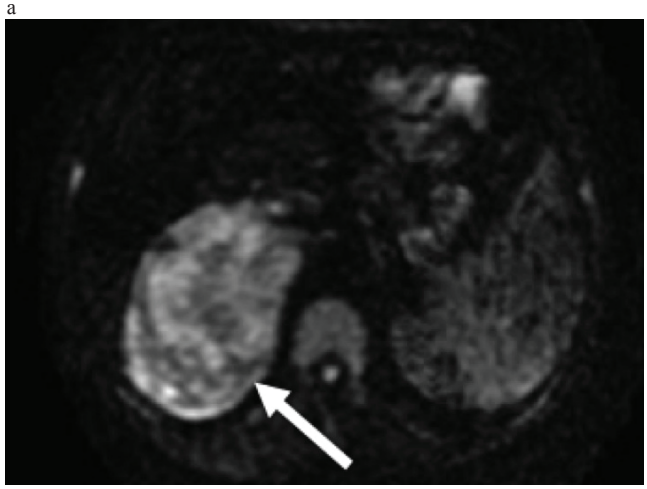


Figure 1: A renal cyst (white arrow) in the right kidney appears hyperintense on T2-weighted image (figure 1a). The cyst has an ADC value of 2.72x 10-3 mm²/s and an Fa value of 0.16. Figure parts 1b and 1c are ADC and Fa maps, respectively.

a



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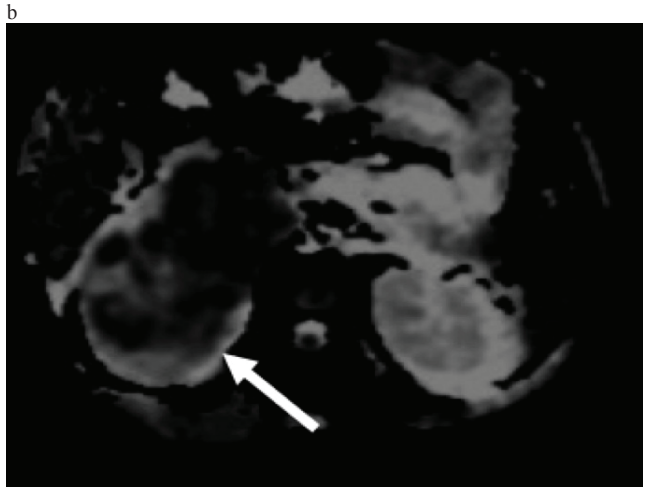

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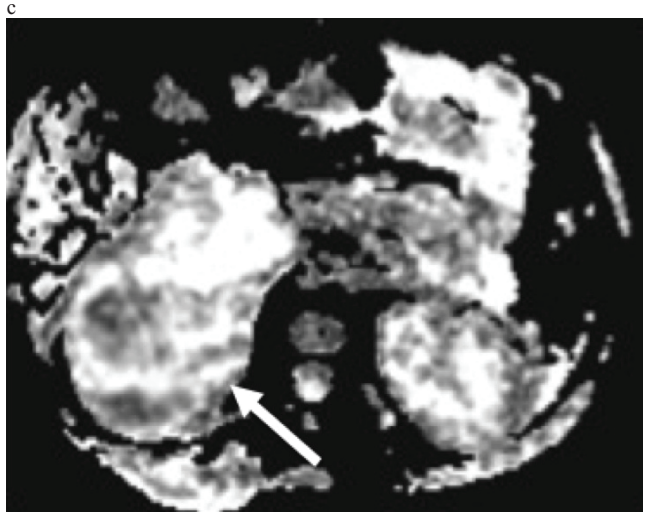
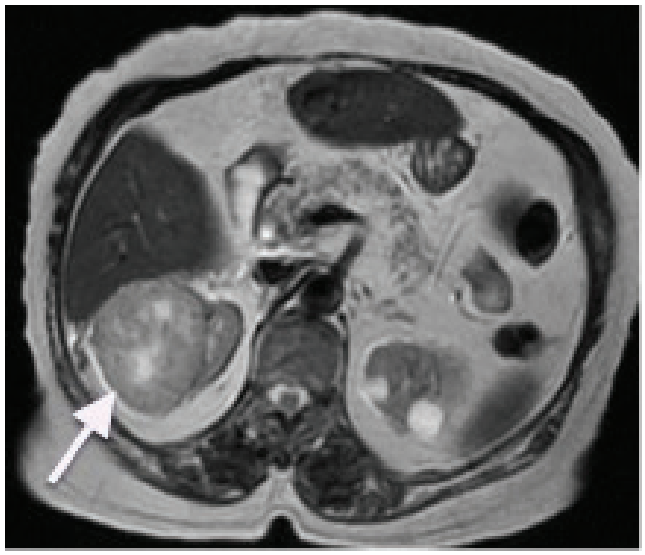


Figure 2: A cystic RCC (white arrow) in the right kidney appears hyperintense on diffusion-weighted axial image (figure 2a). After intravenous injection of gadolinium, the lesion shows weak enhancement because of its cystic nature (figure 2b). The RCC had an ADC value of 1.07 x 10^{-3} mm²/s and an Fa value of 0.31. Figure parts 2c and 2d are ADC and Fa maps, respectively.

a



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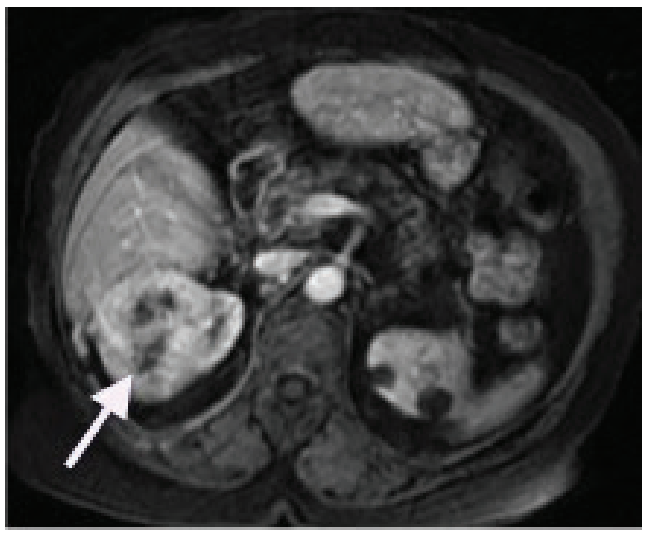

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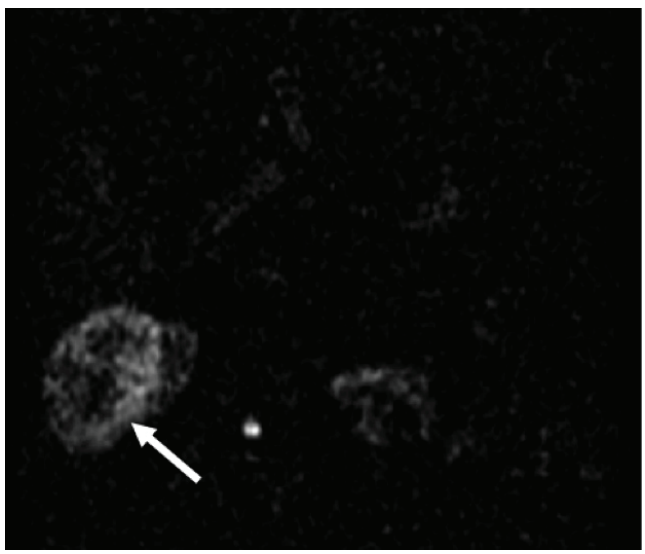

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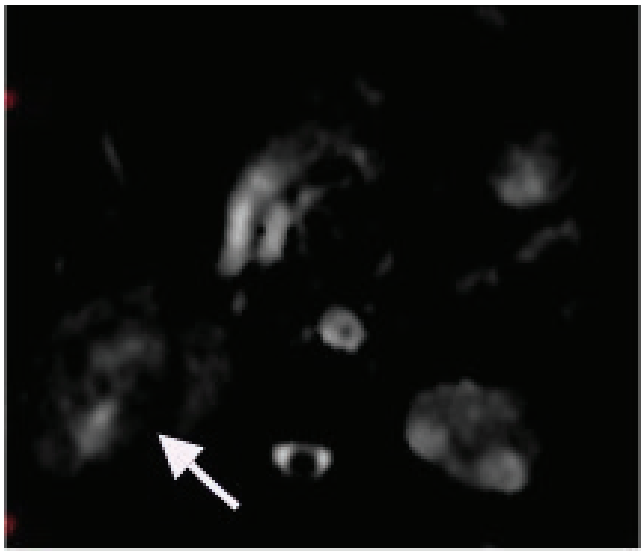

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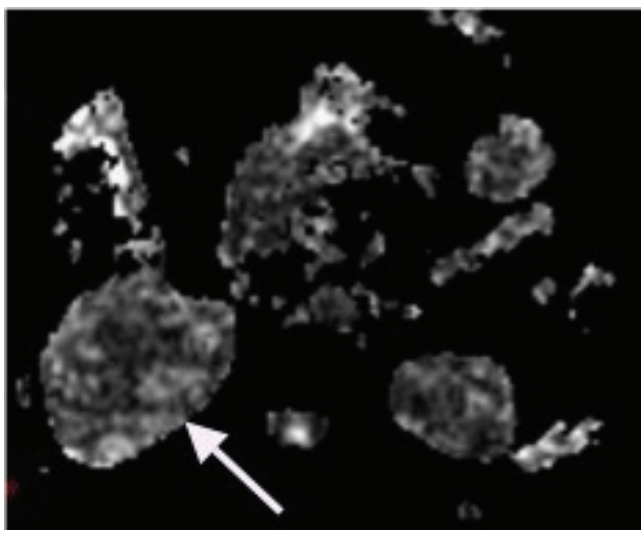

Figure 3: A RCC (white arrow) in the right kidney appears heterogeneously isointense when compared with renal parenchyma with internal hyperintense areas of cystic degeneration on T2-weighted image; two simple cysts in the left kidney appear hyperintense (figure 3a). After gadolinium administration, the RCC shows strong enhancement, the cysts appear hypointense (figure 3b). The RCC shows diffusion restriction and appears hyperintense on diffusion weighted MR image; the cysts appear hypointense (figure 3c); figures 3d and 3e are ADC and Fa maps, respectively. The RCC had an ADC value of 1.67 x 10^{-3} mm²/s and an Fa value of 0.29, with necrotic areas of higher ADC and lower Fa values. The larger simple cyst had an ADC value of 2.61 x 10^{-3} mm²/s and 0.15, respectively.

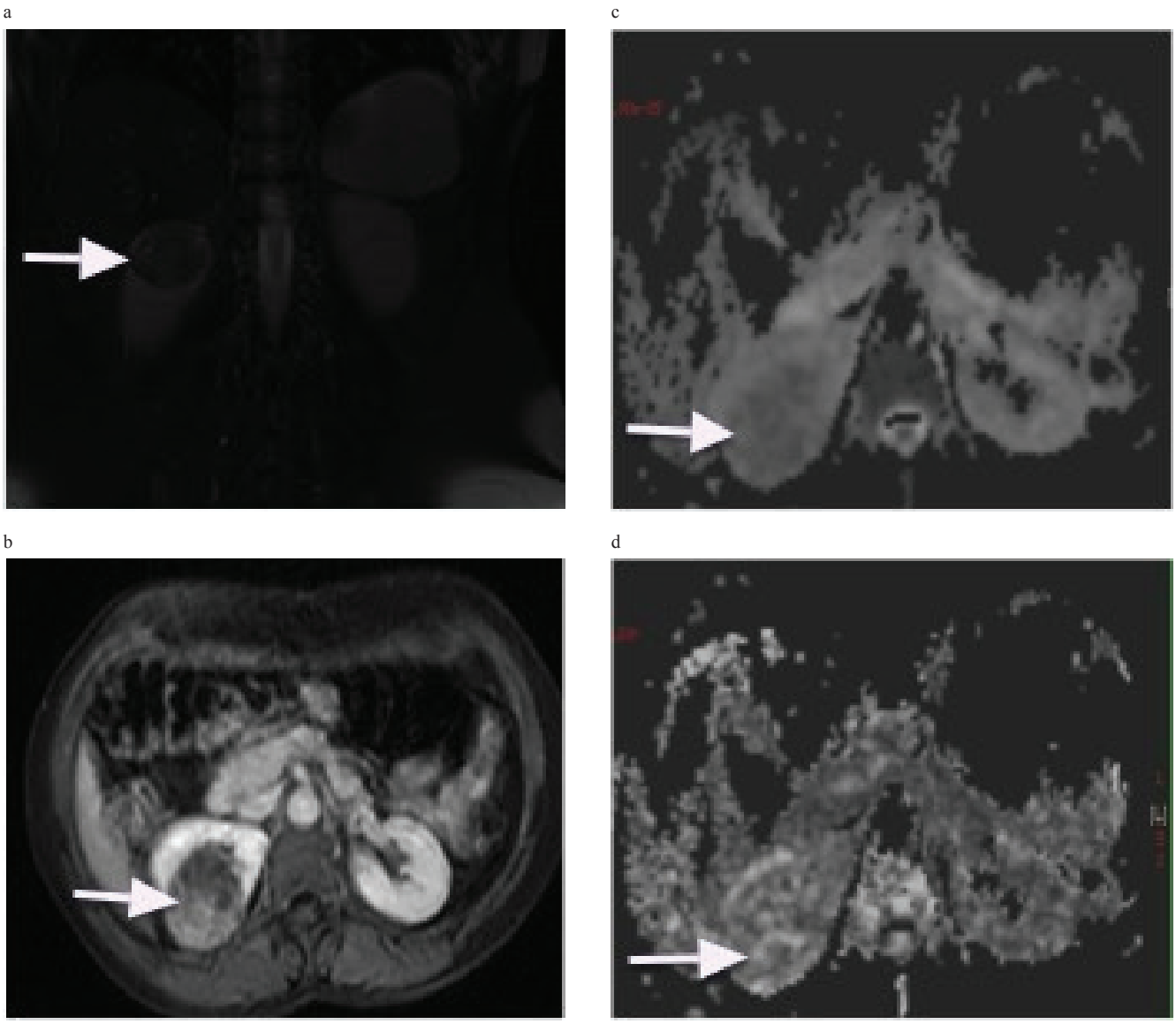

Figure 4: An angiomyolipoma (white arrow) in the right kidney appears as a fat containing lesion on coronal fatsupressed T2-weighted images (figure 4a). Lesion shows enhancement after gadolinium administration (figure 4b). The angiomyolipoma had an ADC value of 1.38 x 10^{-3} mm²/s and an Fa value of 0.34. Figures 4c and 4d are ADC and Fa maps, respectively.

Results

The mean ADC values of RCCs and benign renal lesions were 1.29±0.4x10^{-3} mm²/s, 2.6±0.7x10^{-3} mm²/s, respectively. All differences were significant (P <0.05). The mean Fa values of malignant and benign lesions were 0.34±0.1 and 0.19±0.12, respectively. The differences in Fa values were statistically significant (P <0.05). Figures 1, 2, 3 and 4 are representative cases.

In distinguishing between malignant and benign lesions, ADC value had an area under the curve of 0.95. Using 1.87x10^{-3} mm²/s as the cut-off value the sensitivity was 100% (7/7; 95% CI= 64.6-100 %) and the specificity was 83.3% (15/18; 95% CI= 60.8-94.2%). In distinguishing malignant lesions from benign ones, Fa value had an area under the curve of 0.83. Using 0.25 as the cut-off value the sensitivity was 100% (7/7; 95% CI= 64.6-100%) and the specificity was 77.8% (14/18; 95% CI= 54.8-91.0%).

Discussion

The ADC values depend largely on the presence of barriers to diffusion within the macromolecules and cell organels. Restriction to the molecular diffusion of water can be related to the density of tissues. With the increasing density according to the compartment within different cellular structures and macromolecules, ADC values changes, thus helps us to differentiate the types of tissues. Incresed cell density causes an increase in the anisotropy, as well [7]. Since it has been already shown that the cells in malignant lesions are densely packed [3], the diffusion in malignant lesions should be more restricted and more anisotropic when compared to benign lesions resulting in outcomes as lower ADC values and higher Fa values for malignant lesions.

In the literature there are studies that investigate the accuracy of DWI to assess parenchymal structure of kidneys, characterization of renal masses and cystic renal lesions [8-10]. Kim et al., found the lowest ADC values in angiomyolipoma and renal cell carcinoma in their study [11]. Notohamiprodjo et al., used DTI in patients with renal pathologies and in healthy volunteers. The results of their study showed that solid tumors had significantly higher Fa values compared to simple cysts, but a wide range of Fa values were detected for renal cell carcinoma [12]. In a study by Taouli et al., RCCs had significantly lower mean ADC values compared to benign renal lesions at DWI. The mean ADC was 1.41±0.61 $x10^{-3}$ mm^2/s for RCCs and 2.23±0.87 $x10^{-3}$ mm^2/s for all benign lesions in their study [13]. In our study, the mean ADC values we obtained for RCCs and benign renal lesions are in concordance with the literature. And as expected, due to their higher cell density, RCCs had higher Fa values when compared with the benign lesions.

This preliminary study has some limitations. First, the patient population was small. Nevertheless, we were still able to find statisticaly significant differences between ADC and Fa values of malignant and benign focal renal lesions. Malignant lesions had low ADCs and high Fa values, whereas benign lesions had high ADCs and low Fa values. Second we were not able to include different type of solid renal lesions other than one angiomyolipoma. As we mentioned earlier, our aim in this preliminary study, was to evaluate the feasibility of diffusion tensor imaging. To reach robust conclusions, studies with larger patient populations, and a variety of renal lesions are definitely needed.

Conflict of Interest: The authors declare that they have no conflict of interest.

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CASE REPORT / OLGU SUNUMU

Salt wasting in two neonates with posterior urethral valves: secondary pseudohypoaldosteronism

Posterior üretral valvlı iki yenidoğanda tuz kaybı: sekonder psödohipoaldosteronizm

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ABSTRACT

Secondary pseudohypoaldosteronism (PHA) is a rare condition that presents with hyperkalemia, hyponatremia, and metabolic acidosis, which develops due to renal tubular unresponsiveness to aldosterone. It may be seen in infants with obstructive uropathy and acute pyelonephritis. Three important reasons of the aldosterone unresponsiveness of the tubules are early infancy kidney, obstructive uropathy, and urinary tract infection. Besides other findings, diagnosis is established with highly increased plasma aldosterone and renin levels. In infants who present with hyperkalemia, hyponatremia, and metabolic acidosis, PHA should be considered in the differential diagnosis of congenital adrenal hyperplasia. Urinary ultrasonography and urine analysis should be performed in these patients. Herein, we present two patients with secondary PHA caused by posterior urethral valves.

Keywords: Obstructive uropathy, Secondary pseudohypoaldosteronism, Infant

ÖZ

Sekonder psödohipoaldosteronizm (PHA), böbrek tübüllerinin aldosterona duyarsızlığı ile karakterize, hiperkalemi, hiponatremi ve metabolik asidoz ile kendini gösteren nadir bir durumdur. Obstrüktif üropatisi olan bebeklerde ve piyelonefrit sırasında görülebilir. Gelişimi devam eden erken bebeklik dönemi böbreği, üriner sistemin obstrüktif anomalisi ve enfeksiyonu sekonder PHA hastalarında tübüluslarda aldosteron direncine neden olan üç önemli faktördür. Genellikle hiponatremi, hiperkalemi ve metabolik asidoz ilk bulgulardır, tetkiklerde belirgin olarak yükselmiş plazma renin ve aldosteron seviyelerinin saptanması ile tanı konur. Süt çoçuklarında biyokimyasal incelemede hiperpotasemi, hiponatremi ve metabolik asidoz saptandığında konjenital adrenal hiperplazi ayırıcı tanısında PHA göz önünde bulundurulmalı ve bu hastalarda üriner ultrasonografisi (USG) ve idrar analizi rutin olarak yapılmalıdır. Bu yazımızda sekonder PHA gelişen posterior üretral valv (PUV)'lı iki hasta sunuldu.

Anahtar kelimeler: Obstrüktif üropati, Sekonder psödohipoaldosteronizm, Süt çocuğu

Introduction

Pseudohypoaldosteronism (PHA) is a clinical condition characterized by hyponatremia, hyperkalemia, and metabolic acidosis, which develops due to unresponsiveness to aldosterone in renal tubular cells. It is classified in two forms as Type 1 (PHA1), and Type 2 (PHA2). Type 1 PHA may result from primary (genetic) or secondary (acquired) causes. In primary PHA1, dysfunction in kidneys and also in other epithelial sodium channel (ENaC)-involving organs (colon, salivary and sweat glands) occurs due to a congenital disorder in ENaC or mineralocorticoid receptors. PHA2, which is accompanied by hyperkalemia and hypertension, is also called Gordon syndrome or familial

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hyperkalemic hypertension (FHHt), and the disease has an autosomal dominant (AD) transition [1, 2]. Renal tubular cells have transient aldosterone resistance in secondary PHA1 and early infant period, urinary tract infections (UTI) and congenital anomalies of the kidney and urinary tract (CAKUT) are the main risk factors for secondary PHA1.

In the present study, we present two patients who underwent valve resection due to posterior urethral valve (PUV) and who developed secondary PHA1. We aimed to emphasize secondary PHA1 in differential diagnosis of hyponatremia and hyperkalemia detected in patients with obstructive uropathy.

Case-reports

Case-report 1

Oligohydramnios and bilateral hydronephrosis were detected in the antenatal ultrasonography (USG) of a 10-day-old infant who was delivered through cesarean section in week 35 of pregnancy with a birth weight of 2720 g. The physical development was normal, blood pressure was 80/50 mm Hg, heart rate was 120 /min, and genitourinary and other system examinations were normal; however, the infant had difficulty in urinating and interrupted urination. Laboratory examinations revealed the complete blood count and C-reactive protein level (CRP) as normal. Creatinine was 1.9 mg/dL, potassium 7 mEq/L, and sodium 144 mE/L. Urine analysis and microscopic urine examination were normal. Blood gas analysis was normal. Urinary USG revealed bilateral grade 4 hydroureteronephrosis (HUN), and trabeculation on the bladder wall. A cystoscopy examination revealed PUV, and the infant underwent valve resection on day 10. Voiding cystourethrography (VCUG) revealed grade 5 vesicoureteral reflux (VUR) in the right ureter. The patient was discharged with antipotassium treatment for hyperkalemia and amoxicillin treatment for UTI prophylaxis. The follow-up sodium level was 128 mEq/L, potassium was 5 mEq/L under antipotassium treatment, and creatinine was 0.96 mg/dL. Blood gas analysis revealed metabolic acidosis. Polyuria developed during follow-up. Fractional excretion of sodium (FeNa) was high, transtubular potassium gradient (TTKG) was low, and aldosterone and renin levels were detected high. Cortisol, 17 OH progesterone, and adrenocorticotropic hormone (ACTH) were within normal ranges (Table I). Congenital adrenal hyperplasia (CAH) was excluded with these findings, and the patient was diagnosed as secondary PHA1. Sodium bicarbonate treatment for metabolic acidosis was started.

The patient, who had recurrent UTI during follow-up, required antipotassium and sodium replacement therapy in decreasing amounts. The replacement therapy was stopped at 9 months; the renin level was detected as normal and the aldosterone level was at the upper limit of the normal level. Serum sodium and potassium levels were normal at the last visit when the patient was 3 years of age although the patient did not receive any replacement therapy for 27 months.

Case-report 2

A 7-day-old boy who was born via a normal vaginal delivery at 36 weeks, with a birth weight of 3040 g was admitted to pediatric nephrology clinic due to oligohydramnios and bilateral hydronephrosis which were detected antenatally. Physical examination of the baby was normal. Blood pressure was 80/50 mm Hg and heart rate was 132 / min. Genitourinary and other system examinations were normal. The patient underwent valve resection in day 10 after detection of PUV in cystoscopy. The biochemical examinations revealed creatinine as 4 mg/dL, sodium 144 mEq/L, and potassium 7.2 mEq/L. Blood gas and urine examinations were normal. FeNa was high, TTTK was low, aldosterone and renin levels were detected high (Table I). The patient was initiated antipotassium treatment and developed hyponatremia during follow-up. Cortisol, 17 OH progesterone, and ACTH levels were within the normal ranges in accordance with the patient's age (Table I). Congenital adrenal hyperplasia was excluded, and secondary PHA1 due to obstructive uropathy was diagnosed. Sodium replacement therapy was initiated. Valve residue was detected in repeat cystoscopy after the development of a UTI under amoxicillin prophylaxis, and the valve resection was repeated. Voiding cystourethrography revealed bilateral grade 5 VUR. Replacement therapy was stopped at 5 month, and a repeat examination demonstrated that aldosterone and renin levels were declining. Renin and aldosterone levels were detected as normal at the last visit when the patient was 2.5 years of age. Serum sodium and potassium levels were within the normal ranges although the patient did not receive replacement therapy for 25 months.

Table I: Biochemical and hormonal characteristics of the patients

	Case 1	Case 2	Reference ranges
Sodium (mEq/L)	128	135	135 - 145
Potassium (mEq/L)	5*	7.2	3.5 - 4.5
Bicarbonate (mmol/L)	19	21	22 - 26
Plasma renin activity (ng/L)	117	270	3 - 16
Plasma aldosterone activity (pg/mL)	1285	3300	29.4 - 161.5
17 OH progesterone (ng/mL)	1.47	8.1	0.7- 5
ACTH (pg/mL)	45.4	14.8	0 - 46
Cortisol (µg/dL)	17.04	14.5	6.2 - 19.4
FeNa (%)	3	9.7	< 1
TTKG (%)	3	4.8	> 8
Urinary anomaly	PUV + VUR	PUV + VUR	
Urinary tract infection	Yes	Yes	

* During antipotassium treatment, FeNa: Fractional excretion of sodium, TTKG: Transtubular potassium gradient, PUV: Posterior urethral valve, VUR: Vesicoureteral reflux

Discussion

Posterior urethral valve is the most frequent congenital anomaly that causes obstructive uropathy in males. The disorder develops in the early gestational period and causes significant bladder dysfunction and kidney damage due to high pressure exposure of the bladder and upper urinary system. Obstruction causes glomerular and tubular dysfunction, and concentration and acidification ability of the tubules are damaged, therefore polyuria, sodium wasting in urine and electrolite imbalance develop [3,4]. Asano et al., reported that the increased intrarenal pressure in obstructive uropathy increases cytokine synthesis and causes tubular dysfunction. In addition, the obstruction decreases kidney blood flow, and this activates the renin-angiotensin system and increases aldosterone levels [5].

Pseudohypoaldosteronism is a clinical condition characterized by salt wasting and hyperkalemia due to aldosterone resistance in tissues. Early infancy period, CAKUT and UTI are three significant factors that cause aldosterone resistance in tubules of patients with secondary PHA1. Secondary PHA1 may also be classified as Type 3 PHA (PHA3) and is known as transient PHA1. Various anomalies of the urinary system (ureterohydronephrosis, ureterocele, ureteropelvic obstruction) may cause secondary PHA1. Bogdanović et al., investigated 85 patients with secondary PHA1 and reported that 90% of total 93 patients with secondary PHA1 were aged younger than three months of age. Urinary sytem anomalies accompanied in 90%, and UTI was present in approximately 89% of patients. In addition, they reported that the rate of PUV was 19% in these anomalies [6]. Both patients in our study were aged younger than 3 months, they had obstructive uropathies secondary to PUV, and UTI accompanied during follow-ups.

Although many diseases cause hyponatremia, hyperkalemia, and metabolic acidosis during infancy, exclusion of CAH is important in these patients. Masculinization in girls, and rapid penis growth in boys may be observed in the salt wasting form of CAH. Vomiting and dehydratation are detected in both sexes a short while after birth. The differential diagnosis of PHA and CAH may sometimes be difficult. The literature indicates that these patients may frequently be misdiagnosed. Pai et al., initiated hydrocortisone and fludrocortisone in 3 infants with CAH who were hyponatremic, hyperkalemic, and with symptoms of vomiting and severe dehydration. However, the patients were diagnosed as PHA when laboratory examinations were done, and urinary system anomalies (ureteropelvic obstruction, megaureter, double system), and UTI were detected [7]. Authors have indicated that adrenal steroids were frequently given to the patients with secondary PHA1 with the initial diagnosis as CAH [7,8].

Contrary to that, Ağladıoğlu et al., first diagnosed their patients as having PHA, and they were then diagnosed as CAH after ACTH stimulation test and genetic analysis; renin aldosterone and basal ACTH levels were found to be elevated in two patients in their report [9]. The genital

examinations of our patients were normal. We thought that high levels of potassium during the first presentation developed secondary to kidney dysfunction. The patients had hyponatremia, metabolic acidosis, and salt wasting from urine during follow-up and were diagnosed as having secondary PHA1 after the detection of high levels of renin and aldosterone levels. Other adrenal hormones and ACTH levels were within the normal ranges in both patients. Repeat examinations showed that renin and aldosterone levels were declining, and no increase was detected in ACTH levels. We did not suspect CAH owing to the clinical and laboratory data. We did not require a genetic investigation or ACTH stimulation test.

Salt wasting and growth retardation are generally detected in the neonatal period of patients with primary (genetic) PHA1, and the disease has two types. Generalized or autosomal recessive (AR) PHA1 develops due to a mutation and function loss of EnaC, which is present in most organs. The clinical condition is more severe in these patients due to salt wasting from the kidneys, colon, salivary and sweat glands. However, clinical symptoms are mild, and limited to the kidneys in renal or AD PHA1, which stems from the NR3C2 gene mutation, which encodes the mineralocorticoid receptors. Clinical symptoms become milder with age in these patients. Salt replacement is generally not required after 2 years of age owing to the increase of sodium reabsorption maturation in renal tubules with advanced age. Clinical and laboratory findings of secondary PHA1 are similar to the findings those are present in AD PHA1. Urinary USG and history of UTI are important in the differentiation of secondary PHA1 from primary PHA1. Bowden et al., diagnosed their patients as having AD PHA1 after detection of NRC2 gene mutation, which they conducted because although the clinical condition improved, a high level of aldosterone was maintained in their patients who they first diagnosed as having secondary PHA1. The same gene mutation was detected in the patient's mother; however, she was asymptomatic, and hormonal examination revealed high level of aldosterone. The author emphasized that NR3C2 DNA analysis must be performed for patients who maintain high serum level of aldosterone after treatment of urinary system anomalies or UTI, and patients who present with clinical symptoms of secondary PHA1 must be suspected of having primary AD PHA1 [10]. In another similar case, an infant with acute pyelonephritis was first diagnosed as having secondary PHA1. In this baby due to continued high renin and aldosterone levels after clinical recovery, the patient's asymptomatic mother and two siblings were investigated and high levels of renin and aldosterone were detected. In this case, where genetic examination could not be performed, the authors reported that although AD PHA1 could be detected as asymptomatic in the clinic, the findings of disease could be clinically detected with accompanying secondary factors such as inflammation, which cause transient tubular dysfunction [11]. Replacement therapy was interrupted in month 9 in our first case; the renin level was normal and aldosterone level was at the upper limit of the normal values in repeat hormonal examinations. Replacement therapy was interrupted in month 5 in our second case, and high levels of renin and aldosterone were maintained in decreasing levels. However, renin and aldosteron levels were found normal in a repeat examination at 2.5 years of age. None of our patients required replacement therapy during their pediatric nephrology follow-up, which lasted more than 2 years. We did not perform a family investigation because primary PHA1 was excluded with clinical and laboratory findings.

In conclusion, secondary PHA1 is a rare clinical condition that develops in early infancy. Pediatricians must suspect PHA in addition to CAH in infants with salt wasting and hyperkalemia, and must perform urinary USG and simple urine analysis. Furthermore, serum electrolytes of infants who have urinary tract anomalies or infections must be closely monitored.

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CASE REPORT / OLGU SUNUMU

Carotid blowout syndrome after intensity-modulated radiation therapy: A case report

Yoğunluk ayarlı radyoterapi sonrasında gelişen carotid blowout sendromu: Olgu sunumu

Bilgehan SAHIN, Banu ATALAR, Abdullah YAKUPOGLU, Enis OZYAR

ABSTRACT

Carotid blowout syndrome (CBS) is a rarely seen complication of head-neck surgery and radiation therapy. It may present by massive ear bleeding or epistaxis with high morbidity and mortality rates. Success rate of endovascular therapy or emergency surgery is unfortunately low because of massive bleeding and weakened vessel architecture. Here, we present a 45-year-old male with nasopharyngeal carcinoma and treated with intensity-modulated radiation therapy (IMRT) and concurrent chemotherapy. The patient was diagnosed with stage III (T3N2M0) carcinoma of the nasopharynx. Eight months after radiotherapy, he was admitted to hospital with the complaint of epistaxis. Right internal carotid angiography revealed a 4 mm pseudoaneurysm at the superior-anterior wall of the petrous and his bleeding was controlled by stenting in the same session. In this report we aimed to draw attention to this rare clinical situation that might enhance the chance of early diagnosis enabling less morbid treatment opportunities.

Keywords: IMRT, Carotid blowout syndrome, CBS, Pseudoaneurysm

ÖZ

Carotid blowout sendromu (CBS) veya pseudoaneurysm, baş-boyun cerrahisi ve radyoterapi sonrası nadir görülen bir komplikasyondur. Yüksek morbidite ve mortalite oranları ile masif kulak kanaması veya epistaksis şeklinde görülür. Zayıflamış damar yapısı ve masif kanama sebebiyle acil cerrahi ya da endovasküler tedavilerin başarı şansı oldukça düşüktür. Bu yazıda, nazofarenks kanseri tanısı olan ve eş zamanlı kemoterapi ile beraber yoğunluk ayarlı radyoterapi (YART) C alan 3. evre (T3N2M0), 45 yaşında erkek hasta sunulmuştur. Radyoterapi sonrası 8. ayda epistaksis ile hastaneye başvuran hastanın sağ internal carotis arter anjiografisinde petros segmentin antero-superior duvarında 4mm pseudoanevrizma saptanıp aynı seansta stentle kanama kontrolu yapılmıştır. Bu yazıda, erken tanı konulabilmesi ve dolayısıyla daha az morbid tedavi seçeneklerinin sunulabilmesi için nadir görülen bu klinik duruma dikkat çekilmesi amaçlanmıştır.

Anahtar kelimeler: YART, Carotid blowout sendromu, CBS, Pseudoanevrizma

Introduction

Pseudoaneurysm, rupture of the carotid artery, or carotid blowout syndrome (CBS) is a rare complication of head and neck surgery and radiotherapy. Mostly after re-irradiation, a cascade of reactions occurs in the endothelial cells which causes disruption of the arterial wall leading to pseudoaneurysms. Herein, we report a nasopharyngeal cancer (NPC) patient who was diagnosed as CBS, 8 months after chemoradiotherapy and treated with stent implantation [1].

Case Report

A 45-year-old man referred to our department with the diagnosis of undifferentiated NPC in April 2013. His

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magnetic resonance imaging (MRI) scan revealed a nasopharyngeal mass lesion which invaded the base of the skull bones and posterior wall of the right maxillary sinus with bilateral pathologic level IIB lymph nodes. His positron emission tomography – computed tomography (PET-CT) scan revealed a nasopharyngeal primary tumor with bilateral level II metastatic lymph nodes and no distant metastasis. Thus, he was staged as T3N2M0 - Stage III. His pretreatment plasma Epstein-Barr virus (EBV) DNA level was 18.370 cp/ml before the treatment.

Between, 3 September 2013 and 22 October 2013, the patient was treated with concomitant chemoradiotherapy using 3-weekly cisplatinum. Radiotherapy was applied by using volumetric modulated arc treatment with 6 MV photons. Simultaneous integrated boost technique was used to give a total dose of 6990 cGy to the nasopharyngeal primary, retropharyngeal lymphadenopathy and all gross nodal disease, 6000 cGy to the entire nasopharynx (includes retropharyngeal lymph nodal regions, clivus, skull base, pterygoid fossae, parapharyngeal space, inferior sphenoid sinus and posterior third of the nasal cavity and maxillary sinuses), bilateral upper neck node regions and 5600 cGy to the supraclavicular lymph nodes in 33 fractions. Grade 2 mucositis, dermatitis, esophagitis, local alopecia, and neutropenia developed during treatment. One month after treatment his plasma EBV DNA level was found to be negative. His follow-up PET-CT revealed partial response at nasopharynx and complete response at nodal disease, however, skeletal metastasis developed at thoracal vertebras and pelvic bones 3 months after concomitant chemoradiotherapy. His plasma EBV DNA level at recurrence was 1.352 cp/ml. He received one cycle of combined Taxotere – Cisplatin – 5Fu (TPF) (Taxotere – Cisplatin – 5Fu) chemotherapy but he refused to get chemotherapy thereafter.

Eight months after the termination of radiotherapy he admitted to our hospital with the complaint of epistaxis. It was learnt that minimal epistaxis had been present for a few week before presentation. Otolaryngological examination revealed oozing type of epistaxis originating from nasopharyngeal region. Initially, simple transnasal tamponade was inserted and red blood cell transfusions were given. Under general anesthesia, bilateral external-internal carotid digital substraction angiography (DSA) was performed. Right internal carotid angiographies revealed a 4 mm pseudoaneurysm at the superior-anterior wall of the petrous segment (Figure 1). Because of poor cross-flow through the circle of Willis, parent artery protection

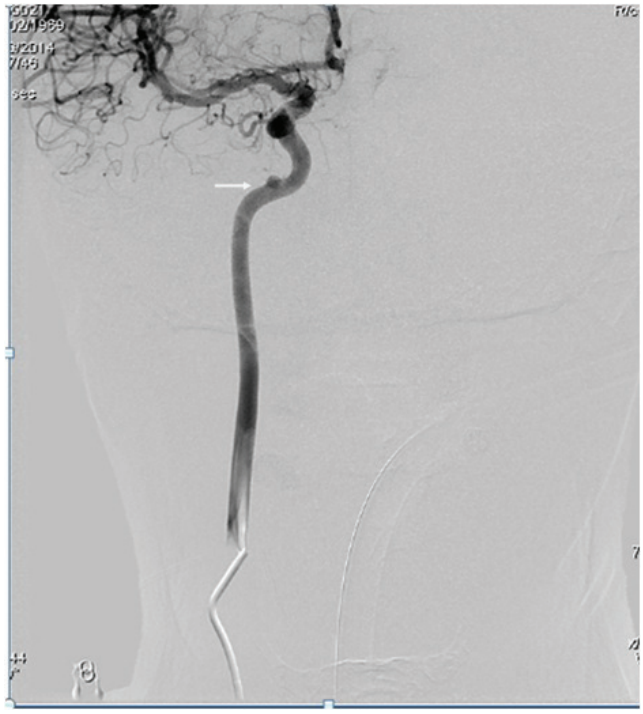

Figure 1: Right internal carotid artery DSA; white arrows are showing pseudoaneurysm in the petrous segment.

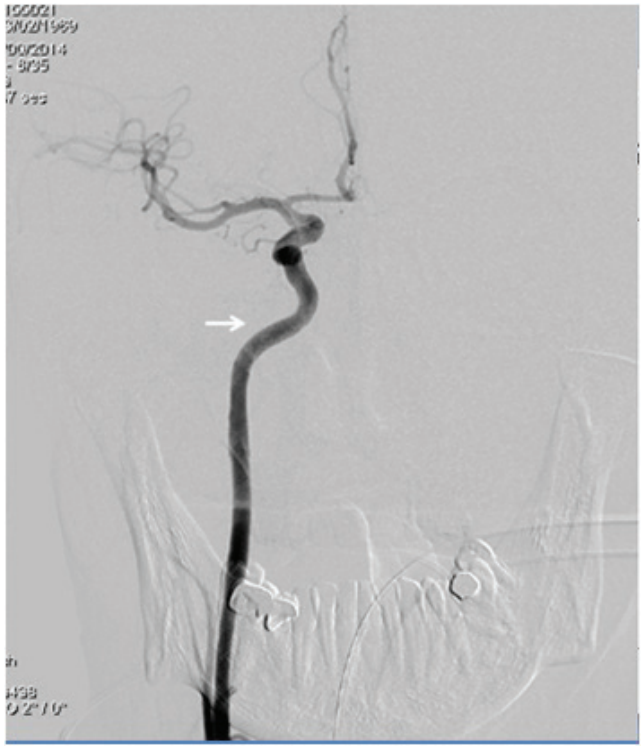

Figure 2: Right internal carotid artery DSA after stent-graft implantation; white arrows showing patency of the parent artery and lack of filling of the pseudoaneurysm with good reconstruction.

was needed. The patient received a loading dose of clopidogrel through the nasogastric tube in the operating room and implantation of a drug eluded stent (Be Graft 5x22mm) was performed in the same session. Control DSA demonstrated closure of the pseudoaneurysm and bleeding was controlled successfully (Figure 2). Following removal of nasal bandages intraoperatively, repeat DSA showed absence of filling of pseudoaneurysm and no nasal bleeding was observed. After stent implantation no neurological complication was observed, antithrombotic therapy was continued. The patient is still alive with disease.

Discussion

Pseudoaneurysm rupture of the carotid artery, or CBS is a rare but dreadful complication of head and neck surgery and/or radiotherapy. In a recent review by Powitzky et al., clinicopathologic features of CBS in patients with head and neck cancer was evaluated. They performed a retrospective review of all studies documenting 132 CBS cases with head and neck cancer from 21 studies. They found that patients with CBS typically have a history of radiotherapy (89%), nodal metastasis (69%), and neck dissection (63%). They indicated that morbidity and mortality rates of patients with CBS are significant; only 23% have survived. Almost 50% of the CBS patients presented with sentinel bleeding, but other half of patients developed a life-threatening hemorrhage requiring emergent intervention [2].

Acute carotid rupture occurs when a compromised arterial wall cannot maintain its integrity against relatively high blood pressure. Disruption of the integrity of the arterial wall may be caused by direct involvement of malignancy, by infection of fistula or an abscess, by loss of overlying soft tissue, or by desiccation effects of skin involvement, or by apoptotic and inflammatory reaction of radiotherapy causing vascular endothelial damage, or by combination of these factors [3]. Some of the predisposing factors that can lead to CBS include radiation, surgery, trauma, diabetes mellitus, poor nutrition, prolonged corticosteroid use, and uncontrolled hypertension [4]. As our presented case, was treated only with radiotherapy and chemotherapy, we believe that formation of this pseudoaneurysm was due to the treatment as there were no any other aforementioned predisposing factors which may lead to this complication.

Carotid blowout syndrome was a very rare event during the 3-D conformal radiotherapy era. Bleeding was also reported rarely after inensity-modulated radiotherapy (IMRT) treatment in the literature. Simultaneous integrated boost (SIB) technique is commonly used in patients treated with head and neck IMRT. This technique allows for improved dose differential by administering different fractional doses to different targets in the same treatment. SIB is a new accelerated fractionation schedule for the treatment of head-and-neck cancer with IMRT. Thus, besides physical dose escalation in the tumor, a biologic effect of accelerated fractionation can occur within tumor also. Lee et al., reported bleeding rate of 1.5% and death rate of 1.5% in median follow up of 2.6 years in patients treated with IMRT. Only one patient died of uncontrolled epistaxis approximately 6 months after completion of IMRT; however, the cause of death was not defined [5]. Kwong et al., reported bleeding rate of 4% and no death was reported in median follow up of 2.1 years. Two patients developed carotid pseudoaneurysm - one received both IMRT and subsequent stereotactic radiosurgery for persistent disease in nasopharynx and the other patient received IMRT and chemotherapy. Both patients presented with sudden onset of torrential epistaxis at about 7 months after completion of IMRT. Emergency surgical intervention successfully controlled the bleeding pseudoaneurysm in both cases [6]. Lin et al., reported bleeding rate of 0.3% and death rate of 0.6%. [7]. All three series who reported CBS treated their patients using SIB technique and their prescription dose to the primary tumor ranged between 2.12-2.25 Gy, 2.17 Gy and 2.2-2.25 Gy and total dose ranged between 65-70 Gy, 76 Gy and 66-70 Gy, respectively. It is well known that SIB technique may cause maximum doses in the target which exceeds >110% to 120% of the prescribed dose. According to Radiation Therapy Oncology Group (RTOG) 0225 nasopharyngeal cancer protocol, if greater than 5% of the PTV70 receives greater than 115% of prescribed dose, this situation is scored as major variation and these patients were considered noncompliant to the study [5].

In our patient, we tried to identify the maximum dose received for the superior-anterior wall of petrous segment of the right internal carotid artery (ICA). We made a fusion of treatment planning CT and post stent CT to document this high dose area (Figure 3). This dose ranges between 7350 cGy to 7969 cGy (105% to 113% of the prescribed dose). Mc Donald et al., reported that increasing the daily dose with hypofractionation would cause 3-fold increase incidence of CBS. In this case, high dose concentration with SIB technique could be the most enhancing causative factor for the development of the pseudoaneurysm [4].

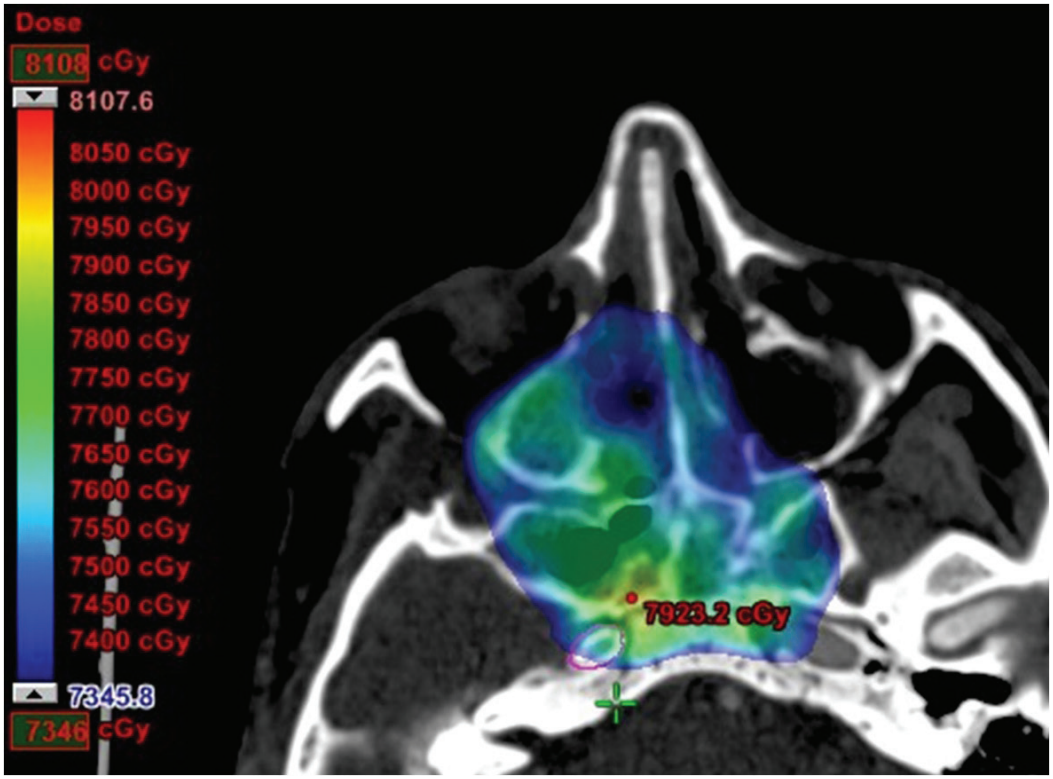

Figure 3: Axial view ; the relationship of the pseudoaneurysm with high dose area (Purple Line: Stent ; Colour wash illustration shows 105 % of the prescribed dose)

Although, IMRT and stereotactic body radiation therapy (SBRT) techniques are designed to minimize the dose of the radiation for the healthy tissue surrounding the tumor, we currently have little feedback on early and late toxicity of these techniques. This is mainly due to limited data including long term follow-up and to less precise knowledge of the radiobiology of high doses of radiation. The conventionally used model for predicting tumor response and normal tissue toxicity is the cell survival lineer quadratic model. This model is essentially based on repair and death mechanism related to radiation induced DNA damage [8]. However, other molecular mechanisms may be involved in the normal tissues other than cancer cell cultures. Among these pathways, the microvascular endothelial compartment has been shown to play a major role in radiation toxicity. Radiation levels above 10 Gy cause endothelial cells to enter apoptotic cell death ending with damage to the microvascular network. After apoptosis surviving cells secrete excessively pro-inflammatory cytokines causing activation of the coagulation system and increased vascular permeability. Late effects include microvascular collapse and thickening of the basement membrane and distruption of the normal architecture of the vessel wall. Dose escalation by using IMRT and SBRT techniques may lead to a different mechanism. Acid sphingomyelinase (ASMase)/ ceramide pathway requires more than conventional doses to be activated. It was shown that this pathway was not triggered in tissues exposed to conventional fractionated radiotherapy. These alternative pathways would be causative factors which lead to unexpected clinical results of the high doses by using IMRT and SBRT techniques [3].

While CBS incidence is very low after postoperative or definitive radiotherapy, this risk significantly increases in patients treated with re-irradiation or subsequent stereotactic radiosurgery boost for persistent disease after definitive radiotherapy. Local recurrence after definitive radiotherapy remains a major issue and nasopharynx is the most suitable site for re-irradiation. However, anatomic location and need for a high dose irradiation make this treatment extremely challenging. Conventional radiotherapy produced less optimal outcome and was associated with high rate of late toxicity. IMRT is a better strategy with better dose distribution. One year local control ranged between 44%-100% depending on T stage in the literature. However, Grade III toxicity rate ranged between 19% to 36%. [9,10,11] Tian

et al., reported their experience in patients with locally recurrent nasopharyngeal carcinoma treated with IMRT in a phase 2 randomized controlled trial. They reported that hypofractionation increasing dose per fraction from 200 cGy to 222 cGy causes significantly higher incidences of mucosal necrosis and hemorrhage (from 28.8% to 50.8%) and lower overall survival (44.2% vs 30.3%) [12]. Similarly Yamazaki et al., informed increased risk for CBS by enhanced dose concentration and high BED values [13]. In our case, time between radiotherapy and the CBS is 8 months. In the literature, this duration varies from during radiotherapy to 17 years, but CBS was mostly seen in a year after radiotherapy. This variation suggests the possibility of having different pathways of underlying pathologic processes [4].

Stereotactic radiosurgery with single high dose radiation or stereotactic radiotherapy using high dose per fraction are other strategies for re-irradiation. Cranial neuropathy, trismus, epistaxis, temporal lobe necrosis, osteoradionecrosis and fatal CBS are among the late complications. Recently, Cengiz et al., reported 46 recurrent, unresectable, and previously irradiated head-and-neck cancer patients treated using robotic hypofractionated radiotherapy. Their median tumor dose was 30 Gy in a median of five fractions. One-year progression-free survival and overall survival were 41% and 46%, respectively. They reported Grade II or greater long-term complications rate of 13.3%. However, they also reported that 8 (17.3%) patients developed CBS on follow-up, and 7 (15.2%) patients died because of bleeding of the carotid arteries. They discovered that CBS occurred only in patients with tumor surrounding carotid arteries and carotid arteries receiving all prescribed dose. Authors, changed their treatment protocol due to the high rate of CBS, treated following 32 patients every other day in a prospective protocol. Three out of 32 patients developed CBS with the new treatment strategy with relatively short follow up time. They also mentioned that CBS did not occur in any of the patients with a maximum carotid artery radiation dose of <34 Gy [15].

Carotid blowout syndrome was historically observed among patients who had recurrent disease after re-irradiation or salvage surgery. However, with more aggressive primary treatment, we may observe more of this complication in the future. Excessive doses in the planning target volume (PTV) should be avoided in the absence of gross tumor volume. Also, clinicians need to be aware of this potential complication in patients who present with severe epistaxis after treatment. Patients who are at high risk for CBS should be followed by MRI angiography beside routine MRI. Emergency resuscitation and interventions should be applied in these patients.

As a consequence; CBS is a rarely seen complication of head and neck surgery and radiation therapy. It may present by massive ear bleeding or epistaxis with high morbidity and mortality rates. Success rate of endovascular therapy or emergency surgery is unfortunately low because of massive bleeding and weakened vessel architecture. In this case-report we aimed to draw attention to clinical changes in patients after radiation therapy that may alert clinicians and elective angiography after local therapies of head and neck surgery might enhance the chance of early diagnosis that enables less morbid treatment opportunities.

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CASE REPORT / OLGU SUNUMU

Open safety pin ingestion: A pediatric case: Can it be spontaneously eliminated or not?

Açık uçlu çengelli iğne yutulması: Pediatrik bir vaka: Girişim gerektirmeden çıkar mı?

Guniz YASOZ, Suat BICER, Safiye ULKU OZER, Yakup SOGUTLU, Rabia ERGELEN, Defne COL

Dedicated to the memory of wonderful person and physician Dr. Suat Bicer

ABSTRACT

Foreign body ingestion is a common problem in childhood. Sharp objects such as needles, toothpicks or open safety pins can also be ingested.

A 13-month-old-boy was admitted to our pediatric emergency department with the suspicion of safety pin ingestion. The boy was taken to a private hospital and an abdominal X-ray was obtained. The open safety pin was seen in the pylorus and he was referred to a university hospital. When he arrived to our pediatric emergency department, an abdominal X-ray was retaken, and an open safety pin was seen in the first part of the duodenum. The patient was hospitalized for observation. After twenty hours, a control X-ray was taken; the open safety pin was seen in the ascending colon. The child was discharged from hospital, and instructions were given to the family for watching his stool closely. The day after, we called the family and learned that the open safety pin was eliminated spontaneously from stool.

Infants and children with safety pin ingestion can be closely followed clinically without complication and there will be no need for an endoscopy and/or surgery. An open safety pin ingested small child was reported with the aim to draw attention to safety pin ingestion.

Keywords: Foreign body ingestion, Open safety pin, Blue bead

ÖZ

Çocukluk çağında yabancı cisim yutulması oldukça sık görülen bir durumdur. Bazen toplu iğne, kürdan ya da ucu açık çengelli iğne gibi keskin yabancı cisimler de yutulmaktadır.

13 aylık erkek bebek, çengelli iğne yutma süphesiyle acil servisimize getirildi. Altı saat önce bebeğin sağ omzundaki iğnenin kaybolduğunun fark edilmesi üzerine götürüldüğü özel bir hastanede çekilen düz batın grafisinde, çengelli iğnenin ucu açık olarak pilorda olduğu görülerek bir üniversite hastanesine yönlendirildi. Pediatrik acil servisimize başvurduğunda tekrarlanan batın grafisinde ucu açık çengelli iğnenin duodenumun ilk kısmına ilerlediği gözlemlendi. Hasta gözlem amacıyla hastaneye yatırıldı. Yirmi saat sonra çekilen kontrol grafisinde çengelli iğnenin çıkan kolonda olduğu izlendi. Bebeğe oral beslenme başlandı ve ailesine dışkı kontrolü yapılması için eğitim verilerek poliklinik kontrolüne çağrıldı. Ertesi gün aileyle yapılan telefon görüşmesinde, ucu açık çengelli iğnenin spontan olarak, dışkıyla atıldığı öğrenildi.

Çengelli iğne yutan bebek ve çocuklar, endoskopi ve/veya cerrahi yapılmadan sadece klinik olarak komplikasyonsuz takip edilebilir. Yazımızda, çengelli iğne gibi keskin yabancı cisim yutulmasına dikkat çekmek amacıyla, spontan olarak dışkıyla atılan açık çengelli iğne yutmuş olan bebek rapor edilmiştir.

Anahtar kelimeler: Yabancı cisim yutulması, Ucu açık çengelli iğne, Nazar boncuğu

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Introduction

Foreign body ingestion is a common problem in childhood [1,2]. Most children who swallow a foreign body do not require specialized care since many of the foreign bodies pass through the gastrointestinal tract spontaneously without any intervention [3]. Although mortality or morbidity due to the foreign body is rare, some of the sharp objects can cause serious problems [3]. Sharp objects such as needles, open safety pins, toothpicks, screws, nails, tacks can cause obstruction, perforation, abscess, peritonitis, fistula

formation, appendicitis, penetration to adjacent organs (liver, bladder, heart, lung), incarcerated umbilical hernia, aortoesophageal fistula, rupture of common carotid artery, hemopericardium, and death [3-12]. Before the use of endoscopic techniques, morbidity rates for the ingestion of sharp objects were 35% and mortality rates were 26% [13]. Rapid diagnosis and suitable therapy such as endoscopy decreased the incidence of adverse events [14]. Sharp object ingestion rates are between 11% - 13% in Europe and Asia [15-17]. The type of ingested objects depends on the country and cultural factors. For example, blue bead ingestions are most frequently encountered in Turkey. These beads are attached to a safety pin for religious or cultural beliefs and used as a good luck charm [18]. Open safety pin ingestions are also common since the blue beads are attached to infants' and children's clothes with safety pins. When the safety pins are unfastened, they can be swallowed easily and can stick at any location in the gastrointestinal tract, especially in the narrowest parts. The location of the swallowed foreign body on the X-ray is important in determining the treatment options. If the sharp object is in the esophagus, removal is considered mandatory [3]. However, once they are beyond the esophagus, most sharp objects pass without any complication and there is no need for any intervention [19]. Yet, they must be closely observed.

In this case report, a 13-month-old-boy who ingested an open safety pin was presented.

Case Report

A 13-month-old-boy was admitted to our pediatric emergency department with the suspicion of safety pin ingestion. The family found the blue bead in his bed. It was attached the child's clothes with a safety pin. They could not find the safety pin and thought it was ingested. The boy was taken to a private hospital and an abdominal x-ray was obtained. The open safety pin was seen in the pylorus and he was referred to a university hospital. When he arrived to our pediatric emergency department, an abdominal x-ray was retaken and he was referred to pediatric gastroenterology. The open safety pin was seen in the first part of the duodenum (Figure 1). The pediatric gastroenterologist decided to hospitalize the patient to follow up closely and observe him with nil per oral. After twenty hours, a control x-ray was taken; the open safety pin was seen in the ascending colon (Figure 2). The child was discharged from hospital, and instructions were given to the family to

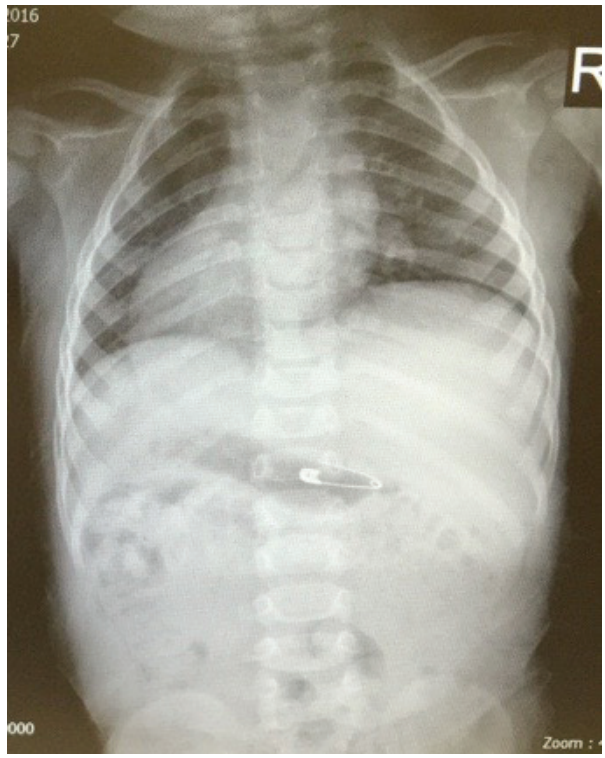

Figure 1: The open safety pin was seen in the first part of the duodenum

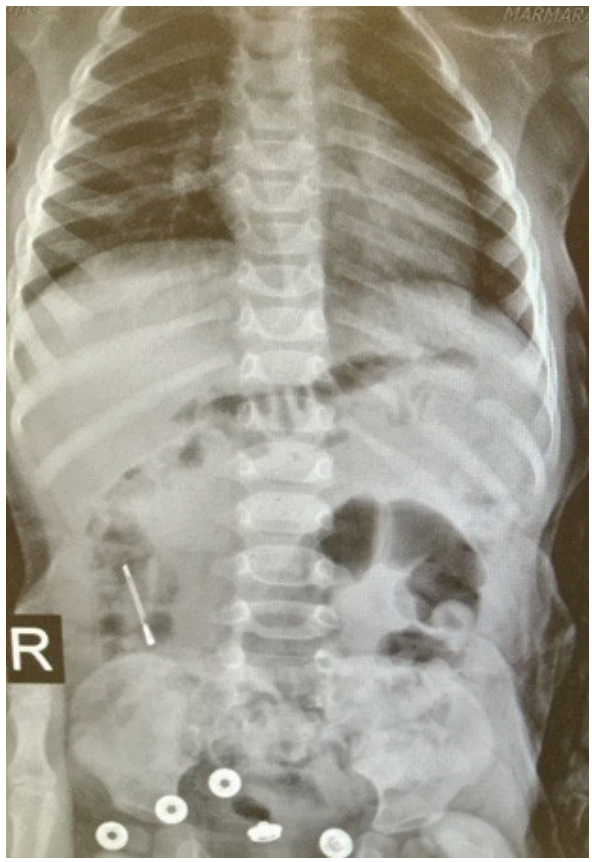

Figure 1: After twenty hours, the open safety pin was seen in the ascending colon

watch his stool closely. In addition, the parents were warned about the symptoms of the complications. The day after, we called the family and learned that the open safety pin was spontaneously eliminated from stool.

Discussion

Sharp object ingestion is a common problem in the pediatric population, especially in the first three years [18,19]. Safety pin is a commonly ingested/aspirated material in Turkey due to cultural beliefs [18,20]. Aydogdu et al., reported 176 children with foreign body aspirations from Turkey, where the most commonly ingested objects were blue beads/safety pins, coins, and turban pins, 38.6%, 27.8%, and 18.1%, respectively [18]. Similar to our case, they reported blue beads/safety pins as being the main objects ingested by infants [18].

Although, many sharp objects may pass the gastrointestinal tract without complication [3,17], any child, with a clear history or a suspicion by family or caregivers, needs an urgent radiographic evaluation to decrease possible adverse complications because of delayed diagnosis and management [10]. If the sharp object is in the esophagus, it must be removed immediately due to the risk of perforation [3,19]. If it is in the stomach, removing it by endoscopy can also be considered [3]. Unless the patient is symptomatic, he or she can be followed clinically with serial x-rays [3]. Otherwise, it should especially be removed if symptoms develop or > 3 days pass without passage [3]. In our case, since the open safety pin was in the first part of the duodenum and the patient was not symptomatic, we preferred to follow up the patient closely and took serial x-rays, which was a wait-and-observe attitude. The patient was followed closely, and we paid attention for the awareness of the family for probable complications. After 36 hours, the open safety pin was spontaneously eliminated from his stool without any intervention and complication.

In conclusion, infants and children with safety pin ingestion can be closely followed clinically without complication and there will be no need for any intervention like endoscopy and/or surgery. An open safety pin ingested small child was reported with the aim to draw attention to safety pin ingestion, which was eliminated spontaneously.

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