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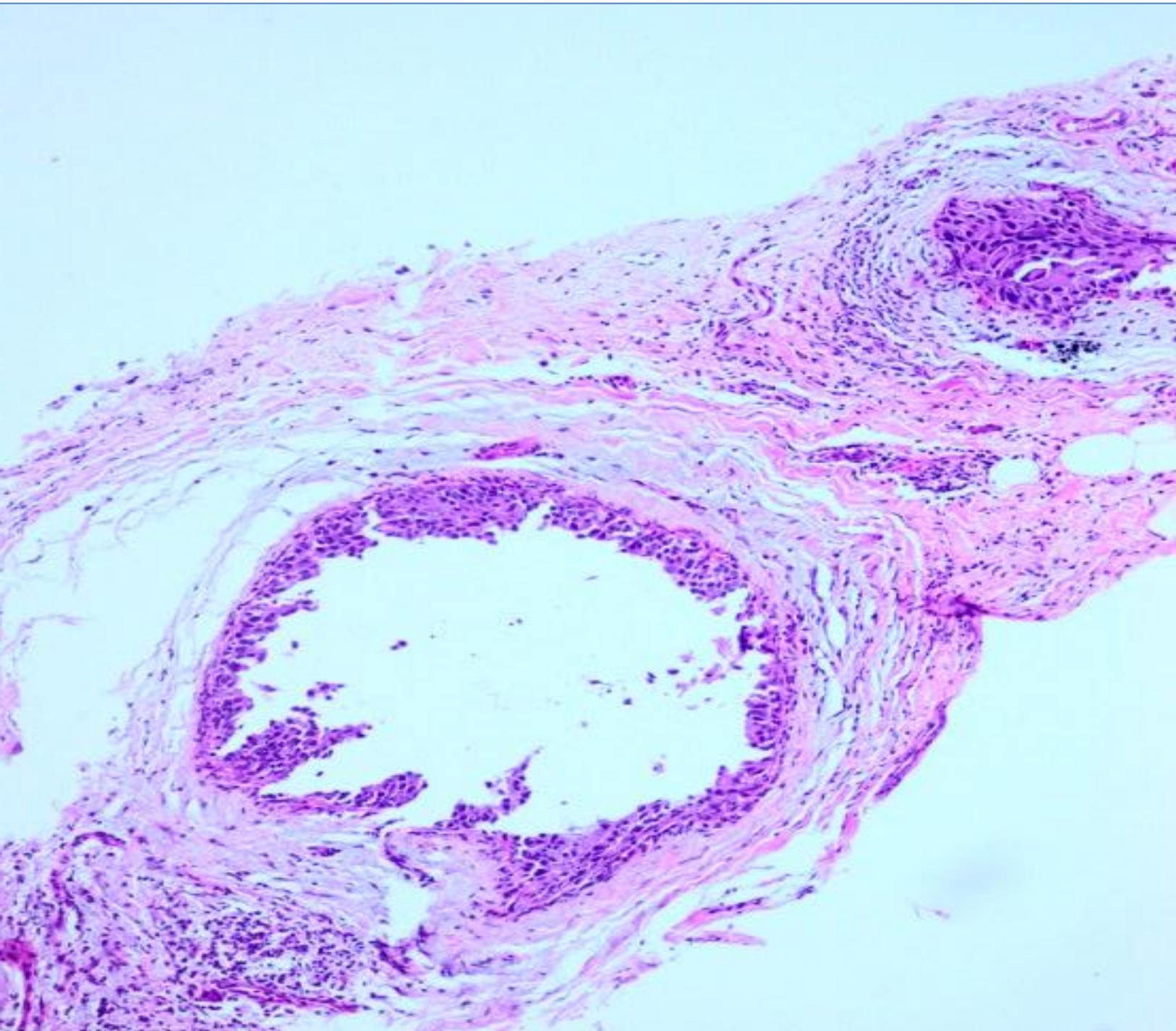

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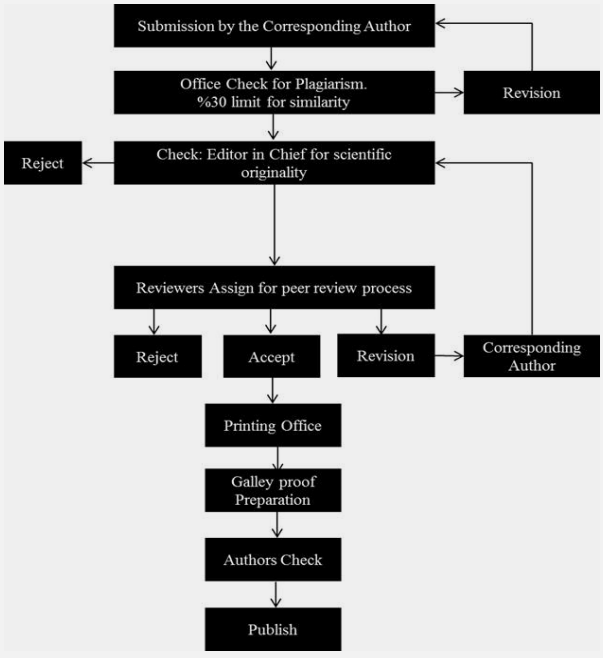

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Serum AMH levels are not associated with adverse perinatal outcomes in women undergoing IVF treatment due to diminished ovarian reserve

Coşkun Şimşir[1*], Tolga Ecemiş[1], Aynur Adeviye Erşahin[2], Gürhan Güney[3], Buğra Coşkun[1], Bora Çoşkun[1], Sevtap Handemir Kılıç[4]

Abstract

Objective: Anti-Mullerian hormone (AMH) is used as a biomarker for the estimation of fertility related parameters such as quality and quantity of oocytes in in vitro fertilization (IVF) procedures. High oocyte quality may also be associated with healthy trophoblastic invasion and lower complication rates during pregnancy. The aim of this study is to evaluate the relationship between AMH values and perinatal complications in infertile women with poor ovarian reserve (POR).

Material and Methods: A total of 68 women undergoing IVF treatment were included in the study. Thirty six of them constituted the study group (POR) and 32 pregnant women constituted the control group (Tubal factor). All women in the study were chosen from patients who have undergone to their first IVF cycle. Serum AMH levels were analyzed with an ELISA kit in all patients.

Results: AMH level was 5.4 times higher in the control group compared to that of the study group ($p<0.05$). No significant differences were observed between the groups with regard to preterm birth rate, gestational age at birth, birth weight, 1st and 5th minute Apgar scores, and neonatal intensive care unit admission rates (all, $p>0.05$).

Conclusion: We found that AMH did not predict adverse perinatal outcomes in women with POR.

Keywords: Anti-Mullerian hormone, IVF, biological marker, diminished ovarian reserve, perinatal outcomes

Introduction

Given the high cost and possible complications of assisted reproduction technologies (ART), investigation of some parameters that can be used to predict the outcomes of ART pregnancies is of great importance. Therefore, such a marker should be able to predict both the response to in-vitro fertilization (IVF) therapy and correlate well with pregnancy rates and as well as perinatal outcomes. Although some evidence indicates that age is the main determinant of IVF success, it is known that the relationship between a woman's chronological age and reproductive capacity is highly variable (1, 2).

Anti-Mullerian hormone (AMH), a dimeric glycoprotein belong to transforming growth factor-β (TGF-β) family, is primarily produced by the fetal Sertoli cells at the time of testicular differentiation and allows the Müllerian channel to regress.

In women, it is secreted by the granulosa cells in the preantral and early antral follicles (3). Therefore, it has been suggested as a marker of ovarian reserve in women, which may predict the number of ovarian follicles and reproductive age (4, 5). In the vast majority of the studies, AMH has been shown to be a better marker than antral follicle count (AFC), baseline FSH, estradiol (E2) and inhibin B in estimating ovarian reserve and ovarian response to IVF treatment (6). Success of IVF procedure is related with different factors including serum AMH (7-9). On the other hand, pregnancy and live birth in women with low/extremely low AMH levels have also been reported over the 40 years of age (10-12). The contradictory observations may be due to the different analytical assays and population characteristics.

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The ovarian response to stimulation with medication in IVF is an important step of outcomes, especially live birth rates and adverse effects of the treatment (13-15). Therefore, there is a need for individualization of the gonadotropin-starting dose by using predictive markers to provide a better oocyte yield and minimize the side effects. Even though serum AMH levels may be a useful tool for the prediction of IVF outcomes in low ovarian response patients, it does not seem to be meaningful in IVF patients with normal ovarian response (16). On the other hand, there is still no conclusive data about the optimal cut-off level of blood AMH to use as a marker of IVF prognosis.

At the follicular level, granulosa cells surrounding the oocyte have been shown to express more AMH than mural granulosa cells suggesting that oocytes may play a role in regulation of AMH production (17). In accordance with this hypothesis, granulosa cells placed in the culture medium produced more AMH in the presence of oocytes (18). Moreover, it has been shown that patients with high preovulatory follicular fluid AMH levels, who underwent modified natural cycle IVF, produced reproductively more capable oocytes (19). Thus, based on this scientific basis, it is thought that there may be a relationship between oocyte activity and ovarian AMH production.

Accordingly, there may be a relationship between AMH and oocyte quality. Similarly, high oocyte quality may also be associated with healthy trophoblastic invasion and lower complication rates during pregnancy. However, the controversial results of studies that explain the relationship between AMH, conception and live birth rates have led us to investigate whether there is a relationship between AMH values and perinatal complications.

Material and Methods

The present study was approved by the ethics committee and informed written consent was obtained from each patient before the study was undertaken. The Helsinki Declaration was followed throughout the study. A total of 68 women undergoing IVF treatment were included in the study. Thirty six of them constituted the study group (POR) and 32 pregnant women constituted the control group (Tubal Factor). Patients in both groups were recruited from infertile women aged 22-38 years, who became pregnant with IVF treatment. All women in this study were chosen from patients who have undergone to their first fresh-embryo transfer cycles, while those with tubal factor infertility were included as the controls. Women with chronic systemic disease, drug users other than folic acid, patients who underwent any pelvic surgery, radiotherapy and chemotherapy were excluded from the study. All patients were followed regularly in our antenatal outpatient clinics throughout pregnancy and received multivitamin supplementation after 4 months and iron supplement after 6 months. Luteal phase support with vaginal progesterone was also given to all patients in the IVF group during the first trimester of pregnancy.

The birth weights, gestation weeks at birth, obstetric characteristics and perinatal outcomes including newborn APGAR scores and neonatal intensive care unit admission (NICU) rates were recorded for analysis. Cesarean decisions were based on obstetric indications and maternal demand. The number of birth and twins were also recorded for each group.

Statistical analyses

The statistical analysis was done with statistical computer software (IBM SPSS Statistics version 22 software, IBM Corporation, Armonk, NY, USA). The distributions of data were analyzed by Kolmogorov Smirnov test. Normally distributed data were analyzed with parametric test (student's t test) for the comparison of two independent groups. Mann–Whitney U test was used to analyze the comparison of non-normally distributed findings. Continuous variables were shown as mean ± standard deviation (SD) whereas categorical variables were expressed as number (percentage). Differences between categorical data were evaluated using the Chi-square test or Fisher's exact test. Statistical significance was considered for $P<0.05$.

Results

The characteristics of 36 females in the study group and 32 females in the control group are summarized in Table 1. There was no statistically significant age difference between two groups. The gestational weeks of the study and control groups were not significantly different. The mean birth weight of control group was slightly but not statistically significantly higher compared to that of the study group. No significant differences were observed between the groups in terms of route of birth, infant gender, 1st and 5th minute APGAR scores and NICU admission rates. On the other hand, number of infants for each birth in the POR group was 1.19±0.40 (n=7) and significantly higher than the control group ($p<0.05$). Although preterm birth rate was more frequent in the POR group, the difference did not reach a statistically significant level (16.7% vs. 9.4%, $p>0.05$). Other obstetric complications such as preeclampsia, gestational diabetes and intrauterine growth restriction were only detected in one patient in both groups which was statistically insignificant when compared between the groups.

The mean serum AMH concentrations were 3.0±1.4 (range 1.1-6.0) and 0.6±0.3 (0.1-1.0) ng/ml in the study and control groups, respectively ($p<0.001$). The AMH level of the control group was 5.4 times higher than the POR group (Figure 1). There was no correlation between AMH levels and other parameters including age, gestation week, birth weight and number of infants at birth in the groups. Table 2 summarizes the characteristics of POR group according to number of infants in each birth. When IVF patients were divided into low and high oocytes yield groups, the number of oocytes collected and fertilized was statistically significantly lower in the former group ($p<0.001$) (Table 3).

In POR group, there were negative correlations between gestational weeks and age (r=-0.352, p: 0.035), and between gestational weeks and number of infants (r=-0.401, p: 0.015). There was a strong positive correlation between gestational weeks and infant weight at birth (r=0.856, $p<0.001$) within the POR group. There was a positive correlation between the duration of stimulation and number

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of oocytes collected (r=0.497, p: 0.003) and, number of fertilized oocytes (r=0.522, p: : 0.002) in the POR group. Number of oocytes collected and number of oocytes fertilized were strongly positively correlated (r=0.897, p<0.001). However, there was a negative correlation in the POR group between number of oocytes collected and fertilization rate (r=-0.489, p: 0.004).

Table 1: Comparison of obstetric and perinatal outcomes between the POR and control groups

	Control	POR	P value
Number of subjects	32	36	NS
Age (year)	30.4±4.3	30.6±4.5	NS
Gestational age (weeks)	38.1±1.8	37.3±2.1	NS
Preterm birth n(%)	3/32(%9.4)	6/36(16.7)	NS
Route of birth n(%)			NS
Abddominal	12(37.5)	11(30.6)	
Vaginal	20(62.5)	25(69.4)	
Infant weight at birth (gr)	3180.2±448.2	2960.6±522.4	NS
Infant gender n(%)			NS
Male	17/33(48.5)	28/43(65.1)	
Female	16/33(51.5)	15/43(34.9)	
Apgar score			NS
1st minute	7(6-7)	7(5-7)	
5th minute	9(8-9)	9(8-10)	
Number of infants	1.03±0.18	1.19±0.40	<0.05
Twin pregnancies n(%)	1/32(3.1)	7/36(19.4)	NS
NICU admission rate n(%)	2/33(6.1)	6/43(14.0)	NS

NICU : neonatal intensive care unit admission. P<0.05 is considered as statistically significant.

Table 2: Means (±SD) of AMH levels, ages, gestational weeks and birth weight at birth according to number of twins in the POR group

IVF group	Single	Twin	P value
Number of subjects	29	7	NS
AMH (ng/ml)	0.552±0.334	0.600±0.374	NS
Age (year)	30.03±4.65	33.14±2.8	NS
Gestational week	37.7±2.0	35.6±1.6	<0.05
Infant weight at birth (gr)	3087.6±500.4	2434.3±148.1	<0.05

IVF; in vitro fertilization, AMH; anti mullerian hormone. P<0.05 is considered as statistically significant.

Table 3. Clinical data of the POR patients by oocyte yield. The POR group was divided into two groups according to the fertilized oocytes after the oocyte collection: "low" represented a yield of 1–3 oocytes and "high" a yield of 4 or more oocytes

Clinical characteristics	1-3 Oocytes (n=20)	≥ 4 oocytes (n=12)	*P* value
Serum AMH level, ng/ml	0.470±0.285	0.642±0.355	NS
Age, years	30.0±4.4	32.2±4.6	NS
Gestational week	37.4±2.0	36.8±2.5	NS
Infant weight at birth, gr	2941.5±508.7	2905.0±585.5	NS
Number of infants	1.20±0.41	1.25±0.45	NS
Duration of stimulation, days	11.3±1.0	12.17±1.3	NS
Number of oocytes collected	3.30±2.16	9.25±3.36	<0.001
Number of oocytes fertilized	1.95±0.89	5.83±2.73	<0.001
Low oocyte yield	1.35±1.69	3.42±1.73	0.002
Fertilization rate	0.74±0.28	0.62±0.12	NS

P<0.05 is considered as statistically significant.

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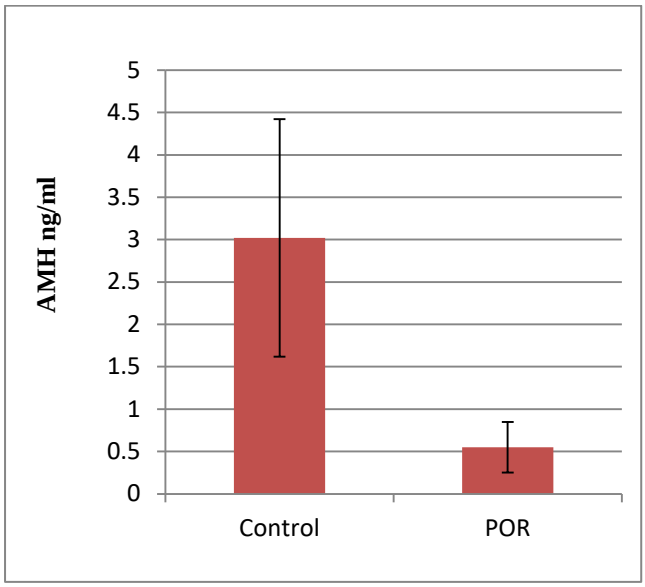

Figure 1: The serum levels of Anti-Mullerian hormone (AMH) in the control (n=32) and perinatal outcome (POR) (n=36) groups. (p value: NS)

Discussion

There are some reports that infertility caused by female factor may be associated with increased perinatal risks (20). The quality of the oocyte determines embryo quality, and embryo quality may also affect pregnancy outcomes and result in increased perinatal risks. In this study, we hypothesized that pre-pregnancy serum AMH concentrations may predict adverse perinatal complications. However, we observed that pre-pregnancy low AMH values which are indicative of ovarian reserve and oocyte quality did not worsen perinatal outcomes. Therefore, poor ovarian reserve is not associated with increased risk for negative perinatal results. We also investigated the cycle characteristics and multiple pregnancy rates of POR patients conceived by IVF in relation to preconceptional serum AMH levels. Our secondary outcome is that AMH has some value for the prediction of fertility parameters, ovarian reserve, IVF procedure, etc., but there is still a need for more information in different populations.

It is very well known that AMH is closely related to ovarian reserve. The decrease in serum AMH due to aging in the ovaries is accompanied by a decrease in the size of the primordial follicle pool, as well as increased apoptosis in the granulosa cells per follicle, which indicates reduced oocyte quality. Although low AMH levels in IVF cycles indicate that oocyte counts to be collected may be low and oocyte quality may be poor, studies have shown that even a poor quality embryo, which can form from such an oocyte, may result in a live birth (21).

Low oocyte quality and hence quality of embryo may be associated with impaired energy production in oocyte cytoplasm, although they result in a live birth (22). This energy impairment may have different effects in a wide range from implantation to nutrition, from birth to postnatal period, and can also determine the degree of quick response and adaptation of the newborn after birth. The number of studies examining the relationship between ovarian reserve, oocyte quality and perinatal complications is not very high. Even as far as we know, there is no study comparing IVF pregnancies with non-infertile and spontaneously conceived women in terms of serum AMH levels. The researchers generally investigated the relationship with preeclampsia and found conflicting results (23).

Woldringh et al. (24) pointed out that if the pregnancy develops, the risk of developing preeclampsia will be higher in patients with decreased ovarian reserve which is characterized by decreased response to FSH in the IVF cycle. It is thought that the inadequate vascular reserve in patients with low ovarian reserve may lead to pregnancy-related vascular complications. In another study by Van Disseldorp et al. (25), the incidence of hypertensive disorders of pregnancy did not differ between poor responders and normal responders for ovarian stimulation.

In their study, Levron et al. (26) have shown that women who become pregnant by receiving oocyte from younger donors have a higher risk of developing preeclampsia and that this may be due to immunologic interactions rather than quality of oocytes. The perinatal period also includes the neonatal period, which is defined as the first 7 postnatal day. Low Apgar score, NICU admission rates for newborns, necrotizing enterocolitis and low birth weight, preterm delivery, respiratory or gastrointestinal complications and poor neonatal complications are all perinatal complications. The only study in the literature that also encompassing neonatal period was done by Oron et al (27). In this study, pregnancies resulting from single fresh poor-quality embryo transfer did not constitute any risk of adverse obstetric or perinatal outcome when compared with transfer of good quality single fresh embryos.

As compared with fresh embryo transfer, having less perinatal complication rates of frozen embryo transfer have been explained by less asynchronization between the endometrium and the embryo (28, 29). We have seen in our own patient group that we cannot support the idea of asynchronization, because of none of the patients with low AMH values conceived by the freeze-thaw IVF cycles. There is also some evidence that laboratory or medical procedures may be responsible for controversial perinatal outcomes in IVF pregnancies (30). Specific laboratory procedures, such as embryo culture media, culture duration, intracytoplasmic sperm injection (ICSI), and cryopreservation method can disturb implantation by creating stress in the developing embryo despite high AMH levels and subsequently increase complication rates by affecting following intrapartum and perinatal processes.

Recently, Nelson et al. report AMH-based approach for a controlled ovarian stimulation in IVF cycles (11). AMH level was associated with oocyte yield following to the ovarian stimulation. The levels between 1 and 5 pmol/l have been associated with a reduced clinical pregnancy rate. The previous studies evaluating that relationship mostly report a positive correlation between AMH levels and better IVF outcomes, however few of them note a poor association as well (31-33).

The result of our study supports the literature data presented in most of the studies and demonstrates AMH as a good predictor of IVF outcomes.

In our study, we did not observe a significant difference in AMH levels of the IVF patients when they were classified to the number of infants delivered as single or twin. In the literate there is limited data on number of infants and AMH levels (34, 35). Tal et al. report an age dependent association between AMH levels and twin births. AMH level seems to predict twin pregnancy over 34 years old of age but not in the patients with lower age group (34). This finding is consistent with our result because the mean age of the IVF patients in our study was 30.6 years.

Different analytical methods have been described for the determination of AMH levels in serum (36-38). In the literature, it has been stated that type of the analytical method may be one of the factors as a possible reason for the contradictory results, especially in the patients with low AMH levels. In the present study, we used a conventional ELISA kit which provided the analysis of all the samples accurately within the detection range. All the values measured in the groups were within the detection limit. Therefore, we do not expect analytical procedure-dependent error as a confounder in the present study.

The major drawback of this study was small number of samples in the groups and evaluation of only AMH as a predictive marker. Therefore, perinatal complication rates may be low in our study. In addition, we could not perform logistic regression analysis which would be more meaningful to reveal real effect of serum AMH levels on adverse perinatal outcomes due to the limited number of patients.

Conclusion

As a result, we found that AMH did not predict adverse perinatal outcomes. The present study also supports the literature data that suggest a positive association between AMH and ovarian response in IVF cycles. Since the number of studies evaluating the relationship between AMH and perinatal outcome is limited, further extensive studies including multicenters with more patients should be designed in order to make the results more meaningful.

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The impact of serum c-peptide levels on bone mineral density in postmenopausal type 2 diabetic women

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Abstract

Objective: Recent studies suggested that c-peptide had biological activity on bone metabolism. We aimed to evaluate the impact of c-peptide level on bone mineral density (BMD) in postmenopausal type 2 diabetic women.

Material and Methods: Thirty-five insulin naïve type 2 diabetic postmenopausal women were included in our prospective cohort study. Fasting and random c-peptide, parathyroid hormone (PTH), vitamin D, insulin, alkaline phosphatase level (ALP) levels and BMD were evaluated.

Results: The mean age of patients was 61.8±8.56 years. The mean fasting c-peptide, random c-peptide and insulin levels were 3.0±1.24, 7.7±3.7 and 13.9±11.2 µIU/ml, respectively. The mean femoral neck (FN-BMD) and total lumbar spine bone mineral density (L-BMD) were 0.787±0.127 and 0.919±0.122 g/cm^2, respectively. C-peptide level was associated with total hip BMD ($p<0.05$) but this relation disappeared after regression analysis adjusted for confounders. A negative correlation between PTH level and FN-BMD was found (p: 0.01). Total hip BMD and L-BMD were negatively correlated with age (p: 0.01 and p: 0.02, respectively). A positive association between DPP4 inhibitor treatment and total hip BMD was observed (p: 0.03).

Conclusions: We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared in multiple linear regression analysis. Further studies are necessary to evaluate the impact of c-peptide level on the risk of osteoporosis in T2DM.

Keywords: C-peptide, osteoporosis, type 2 diabetes mellitus, bone mineral density

Introduction

Osteoporosis and osteoporotic fractures are associated with increased risk of morbidity and all-cause mortality in elderly population (1-4). Type 2 diabetes mellitus is another health concern which becomes more frequent with ageing and often coexists with osteoporosis in elderly population.

The relationship between type 1 diabetes mellitus (T1DM) and decreased bone mineral density (BMD) is well-known (5). Both T1DM and T2DM are associated with hip fracture risk, whereas the risk is greater in T1DM when compared to T2DM (5-7). However, changes in BMD in T2DM remains controversial (7, 8). A significant increase of BMD in type 2 diabetics was observed in some studies and this finding was attributable to causal relationships between T2DM, hyperinsulinemia, obesity, and protective effect of obesity from osteoporosis (7, 9, 10). Increased insulin levels and insulin resistance were reported to be positively associated with BMD in several studies (11, 12).

These findings were consistent with anabolic action of insulin in bone formation (13). Increased risk of fracture in T2DM despite increased BMD, was suggested to be caused by worsened bone quality (14). Previous studies proposed decreased cortical bone density and low bone material strength index in type 2 diabetic patients (15, 16).

C-peptide level is a reliable index of insulin secretion (17, 18). Although it was previously known as an inactive peptide, recent studies proposed that c-peptide may be involved in physiological pathways (19, 20). A possible role of c-peptide in bone metabolism was suggested in diabetic and non-diabetic patients (21-24). The mechanism in which the c-peptide affects bone mineral density has not been explained clearly. A previous study demonstrated that c-peptide activated ERK1/2 signaling in osteoblast-like cell lines and decreased receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA in vivo (25).

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We aimed to evaluate the impact of serum c-peptide level on bone mineral density in patients with T2DM.

Material and Methods

Patients: Thirty-five postmenopausal women with insulin-naïve Type 2 DM were included in this prospective cross-sectional study. The exclusion criteria of the study were; the use of agents or substances that affect Ca+2 homeostasis or metabolism, uncontrolled diabetes (HbA1C level ≥8.5%), serum creatinine levels ≥1.5 mg/dl for men and ≥1.4 for women , patients with any kind of malignancy, other conditions that may affect vitamin D/calcium/bone metabolism (Cushing' s syndrome, active/chronic liver disease, primary hyperparathyroidism, chronic obstructive pulmonary disease, malabsorption syndromes etc.), fracture history, premature ovarian failure, current/ex-smoking, alcohol consumption and drug abuse. Body mass index was calculated by dividing the weight (in kg) by the square meters of the height. Written informed consents were obtained from all participants. This study was approved by local ethical committee of Ankara University (08-614-19).

Biochemistry: Venous blood samples were obtained after 8 hours of fasting. Fasting blood glucose was measured by using enzymatic method with Roche P800 device. Insulin and c-peptide were measured using the Cobas e411 (Roche Diagnostics, Switzerland). Serum creatinine, calcium, and phosphorus measurements were made with a Beckman Coulter DXI 800 device (Brea, California, USA). Parathyroid hormone (PTH) was measured by the chemiluminescence method with a Beckman Coulter AU5800 device (Brea, California, USA). The 25 hydroxy vitamin D (25OHD) was measured with high performance liquid chromatography method. The corrected calcium (cCa) was calculated by the equation: cCa = [(4 - albumin) × 0.8] + Ca.

Bone mineral density: Bone mineral density (g/cm2) of femoral neck, total hip and lumbal spine were measured by dual energy X-ray absorptiometry (The Hologic Discovery QDR™ series DXA systems, USA). Bone mineral density was expressed as the amount of mineral (g)/area scanned (cm2). Osteopenia was defined as a BMD between 1.0 and 2.5 SD below that of a "young normal" adult and osteoporosis was defined as femoral or lumbar density of 2.5 standard deviations below that of a young adult according to WHO criteria (26).

Statistical Analysis: All statistical analysis were performed using SPSS version 11.5 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to assess the assumption of normality. Normally distributed continuous variables were presented as mean ± standard deviation. Non-normally distributed continuous variables were presented as median (min-max). Categorical data were summarized as counts and percentages. Nominal variables were assessed with chi-square analysis/Fisher's exact tests. The associations between continuous variables were determined by Pearson/Spearman correlation analysis. For continous variables, Student's t test/Mann-Whitney U test was used to evaluate the difference between two groups (who had osteoporosis and who had not). A two-sided p-value < 0.05 was considered as statistically significant. The variables which had a significance level of p< 0.20 from the univariate analysis were identified as candidate variables for the multiple linear regression models. The multiple linear regression models was created with Backward Method. A priori power analysis was conducted and showed that at least 29 women should be included to evaluate a relationship between c-peptide level and BMD with 0.5 correlation, using a two-sided hypothesis test with a significance level of 0.05 and 0.8 power.

Results

The mean (±s.d) age of patients was 61.8 (±8.56) years. The patients were postmenopausal for median (min.-max.) 13 (2-28) years. Baseline characteristics and biochemical measurements are summarized in Table 1. The mean total lumbar spine (L-BMD), femoral neck (FN-FMD) and total hip BMD were 0.919±0.122, 0.787±0.127, 0.921±0.147 g/cm2, respectively.

In the univariate analysis, there was no significant association between osteoporosis and any of the evaluated parameters (Table 2). In adjusted model for age, diabetes duration, menopause duration, nephropathy, PTH and Ca levels; only age was positively associated with presence of osteoporosis (p: 0.01, 95% CI=1.05-1.51).

Insulin level was positively correlated with fasting and c-peptide level (r=0.42, p: 0.01 and r=0.58, p: 0.001, respectively). Random c-peptide level was negatively correlated with HbA1c level, duration of diabetes, FPG and positively correlated with insulin level (r=-0.08, p<0.001; r=-0.22, p<0.001; r=-0.29, p: 0.01, r=0.42, p<0.001, respectively) (Table 3).

Lumbar spine total BMD was higher in patients without nephropathy when compared to with nephropathy (0.930±0.117 vs 0.737±0.048, p: 0.02). Associations between L-BMD and biochemical parameters are summarized in Table 4.

Total hip BMD was positively associated with age and fasting c-peptide level (p: 0.01 and p: 0.04, respectively) (Table 4). Total hip BMD was higher in patients under DPP4 inhibitor treatment (1.021±0.120 vs 0.892±0.143, p: 0.02). Other clinical and biochemical parameters were not associated with total hip BMD. Associations between total hip BMD and biochemical parameters are summarized in Table 4.

Femoral neck BMD was positively associated with age (p: 0.006) and negatively associated with PTH level (p: 0.01). Associations between FN-BMD and biochemical parameters are summarized in Table 4.

Age was the only parameter associated with L-BMD in multiple linear regression analysis p: 0.02). Parathyroid hormone level was slightly associated with FN-BMD but this relationship was not statistically significant (p: 0.06). Total hip BMD was negatively associated with age and positively associated with DPP-4 inhibitor usage (p: 0.01 and p: 0.03, respectively). Multiple linear regression analyses are summarized in table 5.

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Table 1. Baseline characteristics of study participants

Parameters	Mean (±s.d) / Median (min.-max.)
Age (years) *	61.8±8.5
Age of menopause (years) **	46 (43-55)
Menopause duration (years) **	13 (2-28)
Duration of diabetes (years) *	10.5±7.9
BMI (kg/m^2) *	31.5±4.4
Waist circumference (cm) *	107.1±12.9
Hypertension, n(%) (A/P)	25(71.4)/10(28.6)
HbA1C (%) *	7.1±0.87
FBG (70-100 mg/dl) **	122 (73-216)
Insulin (4-16 uIU/ml) **	12.1 (3.8-71)
Fasting c-peptide (RR:1.1-4.4 ng/ml) *	3.0±1.2
Random c-peptide *	7.7±3.8
cCalcium (RR:8.5-10.5 mg/dL) *	9.6±0.52
Vitamin D (RR:20-100 ng/mL) **	12.1 (5.1-35)
PTH (RR:14-72 pg/mL) **	56 (26.8-160)
TSH (RR:0.5-5.5 mIU/L) **	1.88 (1-6)
ALP (RR: 30-130 U/L)	75.4±24.5
Medications;	
Metformin, n(%) (A/P)	0(0.0)/35(100.0)
DPP4 inhibitors, n(%) (A/P)	27(77.1)/8(22.9)
Sulphonylurea, n(%) (A/P)	22(62.9)/13(37.1)
Diabetic retinopathy , n(%) (A/P)	32(91.4)/3(8.6)
Diabetic neuropathy, n(%) (A/P)	29(82.9)/6(17.1)
Diabetic nephropathy, n(%) (A/P)	33(94.3)/2(5.7)
Coronary artery disease n(%)	28(80.0)/7(20.0)

*: mean±standard deviation, **: median (min.-max.) ,A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 2. Impact of clinical and biochemical parameters on osteoporosis

Parameters	Osteoporosis		
	Absent (n=25)	**Present (n=10)**	**p-value**
	Mean (±s.d) / Median (min.-max.)		
Age (years)	60.2±8.4	65.8±7.9	0.08
Age of menopause (years) **	46 (43-55)	48 (45-53)	0.46
Menopause duration (years)**	10 (2-25)	15.5 (5-28)	0.19
Duration of diabetes (years)*	11.1±8.6	8.6±6.1	0.17
BMI (kg/m^2)*	31.76±4.45	30.75±4.43	0.54
Waist circumference (cm)*	106.4±13.4	109.1±12.1	0.56
HbA1C (%)*	7.2±0.9	6.7±0.73	0.25
FBG (mg/dl) **	123 (73-216)	121.5 (76-206)	0.92
Insulin (µIU/mL)*	15.2±12.8	10.7±3.97	0.37
C-peptide (fasting) (ng/ml)*	2.8±1.2	3.4±1.4	0.18
C-peptide (random)(ng/ml)**	7.17 (2.1-14.9)	6.4 (3.5-20.9)	0.89
cCalcium (mg/dl)*	9.48±0.51	9.79±0.52	0.12
Vitamin D (ng/ml)**	9.9 (5.1-35)	13.7 (5.1-31)	0.36
Parathormon (pg/mL)**	55.4 (26-113)	62.7 (42-160)	0.15
ALP (U/L)*	73.8±22.8	78.9±29.1	0.63
TSH (mIU/L)**	2 (1-6)	2 (1-5)	0.92
Hypertension, n(%) (A/P)	7(28.0)/18(72.0)	3 (30.0)/7 (70.0)	0.61
Hyperlipidemia, n(%) (A/P)	4(16.0)/21(84.0)	4 (40.0)/6 (60.0)	0.14
Medications;			
Metformin, n(%) (A/P)	0(0.0)/25(100.0)	0 (0.0)/10 (100.0)	0.28
DPP4 inhibitors, n(%) (A/P)	19(76.0)/6(24.0)	8 (80.0)/2 (20.0)	0.58
Sulphonylurea, n(%) (A/P)	14(56.0)/11(44.0)	8(80.0)/2(20.0)	0.21
Diabetic retinopathy, n(%) (A/P)	23(92.0)/2(8.00)	9(90.0)/1(10.0)	0.28
Diabetic neuropathy, n(%) (A/P)	22(88.0)/3(12.0)	7(70.0)/3(30.0)	0.32
Diabetic nephropathy, n(%) (A/P)	25(100.0)/0(0.0)	8(80.0)/2(20.0)	0.07

**: median (min.-max.), A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea,

Table 3. Associations between fasting/ random c-peptide levels, clinical and biochemical parameters.

Parameters	Fasting c-peptide		Random c-peptide	
	Correlation Coefficient (r)	**p-value**	**Correlation Coefficient (r)**	**p-value**
Age	0.18	0.31	0.11	0.54
Age at menopause	-0.21	0.25	-0.11	0.53
Duration of menopause	0.12	0.51	0.01	0.95
Duration of diabetes	-0.22	0.21	-0.52	**0.001**
BMI	0.27	0.11	0.24	0.16
Waist circumference	0.11	0.54	0.078	0.65
calcium	0.30	0.08	-0.04	0.79
phosphorus	-0.21	0.22	-0.52	0.02
ALP	0.22	0.22	0.17	0.43
PTH	0.08	0.64	0.29	0.11
Vitamin D	-0.14	0.43	-0.06	0.72
FPG	-0.29	0.09	-0.41	**0.01**
Insulin	0.42	**0.01**	0.58	**0.001**
HbA1c	-0.08	0.64	-0.59	**0.001**
TSH	-0.22	0.21	-0.28	0.2
Hypertension	0.04	0.79	0.19	0.26
Metformin	-0.08	0.63	0.02	0.91
DPP4 inhibitors	-0.31	0.07	-0.38	0.02
SU	0.03	0.86	-0.31	0.07
Retinopathy	-0.24	0.17	-0.19	0.27
Neuropathy	-0.10	0.54	0.06	0.72
Nephropathy	-0.11	0.55	-0.15	0.38

ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected Calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 4. Associations between femoral neck, total hip, lumbar spine BMD, clinical and biochemical parameters.

Parameters	FN-BMD (g/cm2)		Total hip BMD (g/cm^2)		L-BMD (g/cm^2)	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**
Age	-0.45	0.006	-0.42	0.01	0.28	0.08
Age at menopause	-0.07	0.6	0.06	0.72	0.22	0.90
Duration of menopause	-0.28	0.11	-0.21	0.23	0.19	0.20
Duration of diabetes	0.13	0.43	0.25	0.13	0.17	0.32
BMI	0.02	0.88	0.12	0.48	0.14	0.40
Waist circumference	-0.01	0.91	0.16	0.33	0.02	0.87
cCalcium	0.09	0.58	-0.21	0.27	-0.01	0.91
Phosphorus	0.17	0.31	0.03	0.84	0.19	0.25
ALP	0.18	0.30	0.10	0.56	0.05	0.76
PTH	-.0.44	0.01	-0.11	0.53	-0.19	0.28
Vitamin D	-0.05	0.74	0.06	0.71	0.004	0.98
FPG	-0.05	0.74	-0.04	0.79	-0.14	0.39
Insulin	-0.20	0.26	0.20	0.25	0.10	0.56
HbA1c	0.17	0.33	0.04	0.78	0.21	0.24
c-peptide (fasting)	-0.13	0.44	-0.34	0.04	-0.33	0.85
c-peptide (random)	-0.27	0.12	-0.06	0.72	-0.14	0.40
TSH	0.10	0.54	0.03	0.85	-0.07	0.56

-r: Correlation Coefficient

Table 5. Multiple linear regression analysis of parameters related to lumbar spine BMD, femur shaft BMD and total hip BMD

Variables		Unstandardized coefficients		Standardized coefficiens	t	p-value
		B	Std. error	beta		
L-BMD*	Age	-0.007	0.002	-0.51	-3.3	0.02
FN-BMD**	PTH	-0.001	0.001	-0.30	-1.8	0.06
Total hip BMD***	Age	-0.007	0.003	-0.41	-2.6	0.01
	DPP4 inh.	0.12	0.056	0.34	2.2	0.03

*excluded variables: duration of menopause, nephropathy, sulphonylurea treatments. ** excluded variables: Age, random c-peptide level, duration of menopause, sulphonylurea treatment. *** excluded variables: Fasting c-peptide level.

Discussion

In the present study, serum c-peptide level was positively related with total hip BMD in postmenopausal women with diabetes. However, in multivariate analysis we did not observe a significant relationship between serum c-peptide levels and BMD at any sites. Total hip BMD and lumbar spine BMD (L-BMD) were both negatively associated with age. Femoral neck BMD (FN-BMD) was negatively associated with parathyroid hormone (PTH) level. Dipeptidyl peptidase-4 inhibitor treatment was associated with higher total hip BMD.

Previous studies suggested that c-peptide had multiple effects in physiological pathways. Its role in prevention of diabetic vascular complications was demonstrated in several studies (27-29). Regarding to bone metabolism, c-peptide was shown to cause stimulation of Na-K- ATPase and activate Ca2+ dependent signaling pathways which are essential for osteoblast activities (20, 30, 31). Stimulation of ERK1/2 by c-peptide via phosphoinositide 3-kinase pathway (PI3K) was demonstrated (32). In osteoblast-like cell lines, activation of ERK1/2 was shown to decrease RANKL mRNA levels (33). In the study of Russo et al., c-peptide prevented the reduction of type1 collagen mRNA/protein, in addition to activating of ERK1/2 signaling and reducing RANKL mRNA/protein levels in Saos-2 cells which represent a model for osteoblastic differentiation in human cells (25).

The association between BMD and c-peptide level was investigated in a limited number of clinical studies (21, 23, 24, 34). Results were conflicting in both diabetic and non-diabetic populations (21, 23, 34). A cross-sectional survey was conducted with 6625 participants of National Health and Nutrition Examination Survey. The study showed that there was a significant negative association between serum c-peptide level, total and most regional BMDs in both genders independent of confounding factors (24). However, this relationship was not observed among subjects older than 60 years, whose c-peptide levels were higher when compared to youngers (24). A study by Montalcini et al. reported that serum c-peptide was strongly associated with L-BMD in postmenopausal non-diabetic women independent of confounders like insulin, 25-OH-D, PTH, FGF-23 levels and BMI values (23). A recent study reported that serum c-peptide level was inversely associated with fracture risk and positively with BMD, in postmenopausal women without diabetes (35). An alternative hypothesis of this study was that c-peptide may be a marker of osteoporosis rather than a cause (35). In type 1 diabetic patients, glucagon stimulated c-peptide level was shown to be related with lumbar and femoral BMD (21). However, a recent study investigated BMD in type 1 diabetic and non-diabetic premenopausal women, and did not demonstrate its association with urinary c-peptide level (34).

In postmenopausal patients with T2DM, c-peptide level was positively associated with FN-BMD in both genders and inversely with fracture risk in women (22). Authors attributed these findings to anabolic effects of insulin; stimulation of bone formation and osteoblast proliferation. However, the impact of urinary c-peptide on fracture risk was independent of BMD in women. Authors pointed out a possible favorable effect of c-peptide on bone quality which was not reflected by BMD. In our cohort, FN-BMD was not associated with BMD. In univariate analysis, BMD was positively associated with c –peptide level, but this relationship disappeared after adjustments for confounding factors.

Substrates of DPP4 and their receptors are expressed in bone (36, 37). Glucose dependent insulinotropic peptide (GIP) administration was shown to increase bone density in rats. Glucagon-like peptide 1 receptor agonists were shown to decrease bone loss and increase bone formation in vitro and in vivo (38, 39). A previous meta-analysis suggested that DPP-4 inhibitors was related with reduced fracture risk (40). In our study, DPP4 inhibitor treatment was positively associated with the total hip BMD.

A major strength of our study was the exclusion of many factors that promote to osteoporosis, just as alcohol consumption, current-ex smoking, medications which effect calcium and bone metabolism, comorbidities related to osteoporosis. The major limitation of study was relatively small sample size.

Conclusion

We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared after adjustment for confounders. A possible link between BMD of total hip may be masked by limited number of participants. Direct effect of c-peptide levels on BMD and fracture risk independent of BMD should be evaluated.

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Analysis of breast true-cut biopsies by applying immunohistochemical study of myoepithelial markers

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Abstract

Objective: Breast pathologies are very common in women. Breast cancer is the most common and most frequent cause of death in women. The most common type of breast cancer is invasive ductal carcinoma. Histopathological examination of the tissue taken with a fine needle aspiration biopsy or true-cut biopsy is the main diagnostic method when clinical examination of breast and/ or radiological mass is detected. The definitive diagnosis of benign and malignant lesions of the breast is important in the form of treatment. The most important features in the diagnosis of breast cancer are atypical cellular features such as invasion, desmoplasia, pleomorphism, hyperchromasia, nuclear irregularity, prominent nucleoli, high mitosis count. Loss of myoepithelial layer in the malignant cases is a very important feature in the diagnosis. Aim of this study is to evaluate the benign and malignant breast pathologies with the immunohistochemical panel.

Material and Methods: The 52 breast true-cut biopsy materials in the archives of Department of Pathology at Faculty of Medicine, Erzincan Binali Yıldırım University between 2015-2017 were re-examined with the immunohistochemical panel. The immunohistochemical staining markers such as estrogen, progesterone, cerb-B2, E-cadherin, P63, CD10, calponin, CK5/6 have been applied in all cases.

Results: Cases including a benign disease such as fibroadenoma, adenosis, fibrosis, fibrocystic changes, and intraductal papilloma were 23. Cases including a malignant epithelial tumor were 29.

Conclusion: In the diagnosis of breast cancers, mainly cellular properties are determinative. The evaluation with the immunohistochemical panel will reduce the risk of diagnostic error when the cases that difficultly diagnosed with cellular properties.

Key words: Breast, cancer, immunohistochemistry

Introduction

The breast is composed of two main components of tissues. These are glandular tissues and stromal tissues. Glandular tissues consist of the milk-producing glands called lobule and the ducts that allow the passage of milk. Stromal tissues include fatty and fibrous connective tissues. Glandular structure is including epithelial and myoepithelial layer (1).

Benign breast disease is very common in women (2). Benign breast diseases include mainly fibroadenoma, adenosis, fibrosis, fibrocystic changes, intraductal papilloma and inflammatory diseases (3).

Fibroadenoma is the most common benign tumor of the breast. It consist of increased stromal component and epithelial component trapped within the stromal component (4).

Adenosis is a benign proliferative breast condition. It includes a milk-producing glands called lobule. The lobules are enlarged in adenosis. There are more glands than usual. (5). Adenosis is often found in biopsies of women who have fibrosis or cysts in their breasts (6). Fibrocystic change is characterized by the development of fluid-containing cysts surrounded by fibrous tissue (7). Histological cystic lesion prevelance about 50-60 % among the women (8). Fibrosis is the formation of scar-like connective tissue. It is a common finding in the breast (7). Intraductal papilloma is a benign epithelial tumor. It constitutes less than 10% of benign breast lesions (9). Microglandular adenosis is a very rare benign lesion. There is a loss of staining in the myoepithelial layer, unlike other benign lesions. In microglandular adenosis, histologically, there is a haphazard infiltration of small and uniformly round glands in fibrous tissue.

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Its differential diagnosis was made from cancer with the absence of cellular atypia and the absence of the staining with estrogen and progesterone receptors (10-12). It is often confused with a tubular carcinoma (13).

The most important features in the diagnosis of breast cancer are atypical cellular features such as invasion, desmoplasia, pleomorphism, hyperchromasia, nuclear irregularity, prominent nucleoli, and high mitosis count. Loss of myoepithelial layer in the malignant cases is very important feature in the diagnosis (14).

Breast cancer comprises approximately 10% of all cancer in women. It is the second most common cancer after lung cancer except skin cancer in all population. Breast cancer is the most common cause of death in women. It is the fifth most common death cause depending on cancer in all population. It is about 100 times more common in women than in men. Males have poorer outcomes due to delays in diagnosis (1).

Most breast malignancies are histologically adenocarcinoma. It constitutes more than 95% of breast cancers (15).

The main types of breast cancer are ductal carcinoma in situ, invasive ductal carcinoma (invasive carcinoma of no special type (NST) or invasive ductal carcinoma not otherwise specified (NOS)), lobular carcinoma in situ, invasive lobular carcinoma (1).

Ductal carcinoma in situ is the most common histologic type of non-invasive breast cancer. It is confined to the ducts of the breast (1). It is the precursor of invasive ductal carcinoma (16). The incidence of ductal carcinoma in situ associated with invasive ductal carcinoma is high (17).

Invasive ductal carcinoma (invasive carcinoma of no special type (NST) or invasive ductal carcinoma not otherwise specified (NOS)) is the most common histologic type with a rate of 70-80% of all invasive breast cancers (18-20). It includes subtypes such as tubular, medullary, papillary, mucinous, and cribriform carcinoma. Invasive tubular carcinoma, medullary carcinoma, cribriform carcinoma, and invasive mucinous carcinoma have a better prognosis, while invasive papillary carcinoma has a poor prognosis (21,22). It has a 5-year relative survival of 79% (23). Estrogen and progesterone expression of tumor cells is associated with good prognosis (24).

Lobular carcinoma in situ is the second common histologic type of non-invasive breast cancer. In lobular carcinoma in situ, there is a sharp increase in the number of cells within the milk glands called lobule in the breast (1). It is the precursor of invasive lobular carcinoma. The incidence of lobular carcinoma in situ associated with invasive lobular carcinoma is high, as is the case with ductal carcinoma in situ associated with invasive ductal carcinoma (17). It is multicentric in approximately 70% of cases. It is bilateral in 30–40% of cases (25).

Invasive lobular carcinoma is the second common histologic type with a rate of 5-15 % of all invasive breast cancers. (19,20,26,27) It has a 5-year relative survival of 84% (23).

Paget's disease is a cancer of nipple and comprises 1% of breast cancers (1).

Breast sarcoma, excluding phyllodes tumor, is an extremely rare and heterogeneous group of malignancies. It constitutes less than 1% of all breast malignancies (28).

Benign lesions such as fibroadenoma are more common in the early decades with a peak in the third decade while the incidence of malignancy is higher in advanced decades (29).

When there are diagnostic difficulties with cellular features, evaluation with an extensive immunohistochemical panel is helpful in the diagnosis.

This study aimed to share the result of our breast true-cut biopsy materials with the literature and to emphasize the importance of evaluation with an immunohistochemical panel in cases with difficulty in the differential diagnosis.

Material and Methods

Ethics committee approval was received on June 25, 2019 with numbered 07/03. In the archives of Department of Pathology at Faculty of Medicine, Erzincan Binali Yildirim University between 2015-2017, 52 breast true-cut biopsy materials were re-examined with the immunohistochemical panel. Most of the patients were diagnosed with breast mass and true cut biopsy. The cases reported as malignant epithelial tumors were 29. The cases reported as a benign disease such as fibroadenoma, adenosis, fibrosis, fibrocystic changes were 23. Paraffin blocks of breast true-cut biopsy specimens were supplied from the pathology archive and 4-micron-thick sections were taken from these blocks. After deparaffinization, the sections were stained with Hematoxylin-Eosin stain. The immunohistochemical panel was performed in all cases. 4-micron-thick sections were taken from the blocks of tumor suspected preparations on positively charged slides. The immunohistochemical staining markers such as estrogen (2019/10, ER1/20, Leica, Lot: 61037), progesterone (2020/03, ER2/20, Leica, Lot: 63037), cerb-B2, (2021/09, ER1/20, Dako, Lot: 20062529) E-cadherin (2020/08, ER1/10, Dako, Lot: 10148034), P63 (2020/07, ER2/10, Dako, Cod: IR662), CD10 (2020/08, ER2/20, Dako, Lot: 56C6), calponin (2020/12, ER1/5, Biogenex, Lot: AM3330817), and CK5/6 (2021/03, ER2/20, Thermo, Lot: 1803A) have been applied in all cases. The sections were stained using a fully automated immunohistochemistry device (Leica BOND-MAX®; Leica Biosystems, Melbourne, Australia). Immunohistochemical studies of calponin, P63, CD10, and CK5/6 showed that the myoepithelial layer disappeared in the malignant cases.

Data were evaluated by simple statistical method. The results were expressed as percentages.

Results

The age range was 19-64 year in benign cases and 33-81 in malignant cases. The mean age was 33.6 in benign cases and 57.5 in malignant cases. The most common age group was between 40-50 years in benign cases and between 50-60 years in malignant cases. (Table 1).

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The distribution of cases according to benign disease types was as follows: Of the 23 cases, 10 were a fibroadenoma, 8 were fibrocystic changes, 3 were adenosis, 1 was fibrosis, and 1 was intraductal papilloma (Graphic 1). There were no atypical histopathological features in benign diseases, the myoepithelial layer was observed with the myoepithelial marker in benign diseases like sclerosing adenosis (Figure 1).

The distribution of cases according to malignant tumor types was as follows: Of the 29 cases, 27 were invasive ductal carcinoma, 1 was invasive lobular carcinoma, and 1 was ductal carcinoma *in situ* (Graphic 1).

There were atypical histopathological features such as pleomorphism, hyperchromasia, nuclear irregularity in the ductal carcinoma *in situ*, the myoepithelial layer was observed with the myoepithelial marker in more areas. There was no stromal invasion (Figure 2).

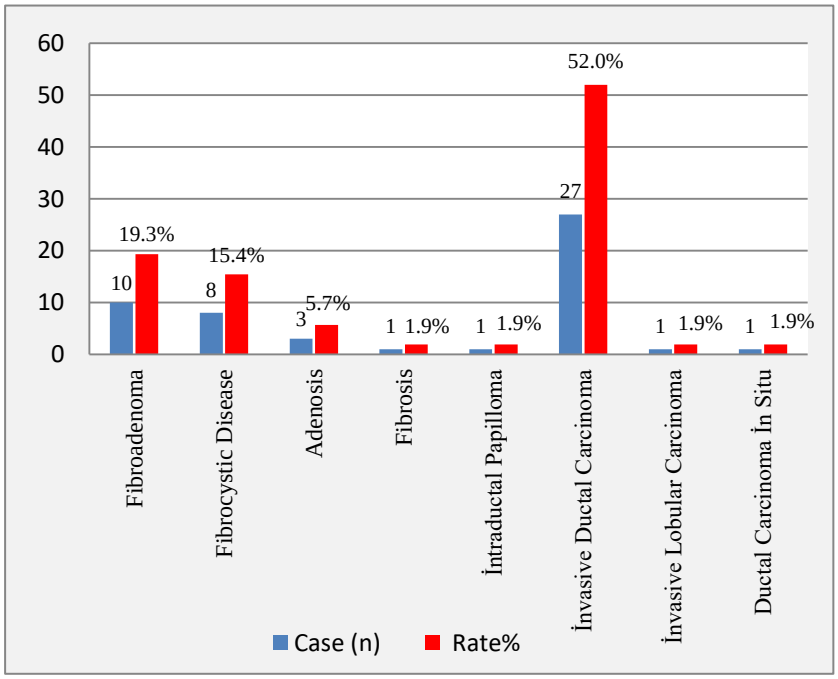
There were atypical histopathological features such as pleomorphism, hyperchromasia, nuclear irregularity in the invasive ductal carcinoma, loss of the myoepithelial layer was observed with myoepithelial marker, unlike benign breast diseases and ductal carcinoma *in situ*. E-cadherin positivity was applied in the differential diagnosis of lobular carcinoma of the breast (Figure 3).

The showing of myoepithelial layer with CD10, P63, CK5/6, and calponin in a case with cellular atypia was usefull in the differential diagnosis of ductal carcinoma *in situ* from invasive carcinoma. The absence of staining with estrogen and progesterone receptors was useful in the differential diagnosis of microglandular adenosis than a carcinoma, such as tubular carcinoma, with low cellular atypia.

Demonstration of myoepithelial layer loss by CD10, P63, CK5/6, and calponin in the invasive carcinoma cases, and the detection of myoepithelial layer with these markers in the benign cases was helpful in the diagnosis.

Table 1: The distribution of 52 breast true-cut biopsies.

Diagnosis	Case (n)	Rate%	Mean Age	
Benign	Fibroadenoma	10	19,3	33.6
	Fibrocystic Disease	8	15,4	
	Adenosis	3	5,7	
	Fibrosis	1	1,9	
	İntraductal Papilloma	1	1,9	
Malignant	Invasive Ductal Carcinoma	27	52,0	57.5
	Invasive Lobular Carcinoma	1	1,9	
	Ductal Carcinoma İn Situ	1	1,9	



Graphic 1. The distribution of 52 breast true-cut biopsies.

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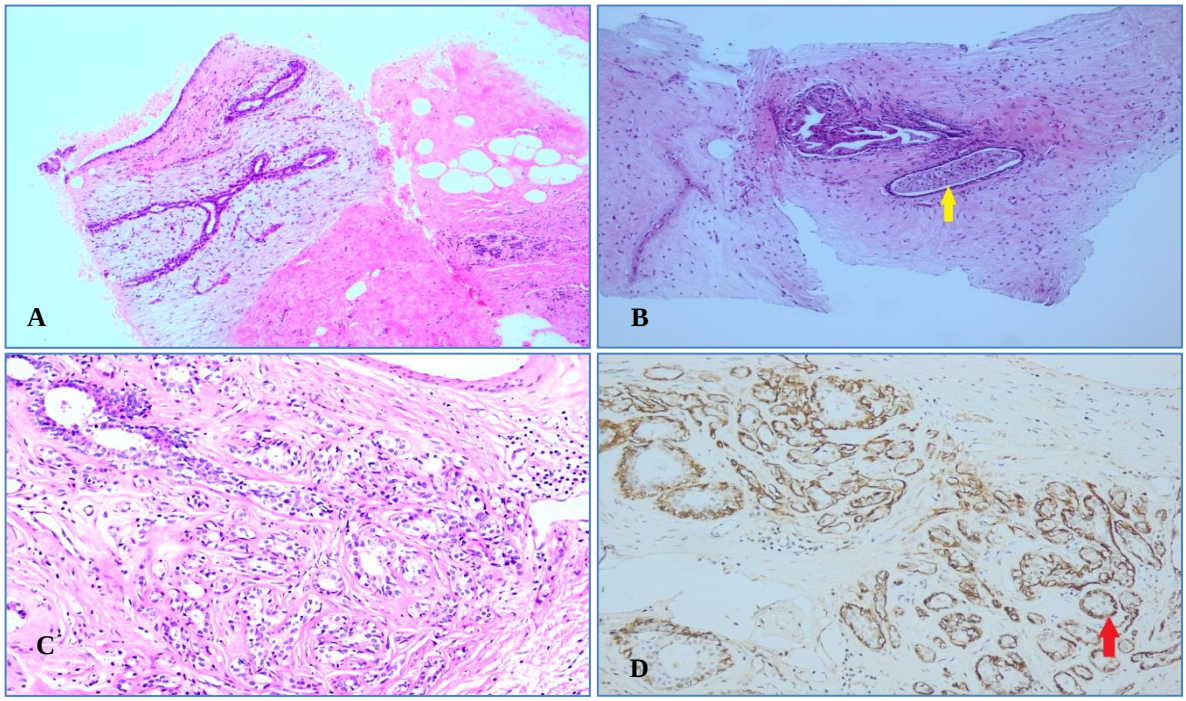

Figure 1: The histopathologic view of benign breast diseases. **A-** Fibroadenoma. (HEX100) **B-** Fibrocystic change including cyst with histiocytes (yellow arrow).(HEX100) **C-** Sclerosing adenosis. (HEX200) **D-** The showing of the myoepithelial layer with CD10 in sclerosing adenosis (red arrow). (X200)

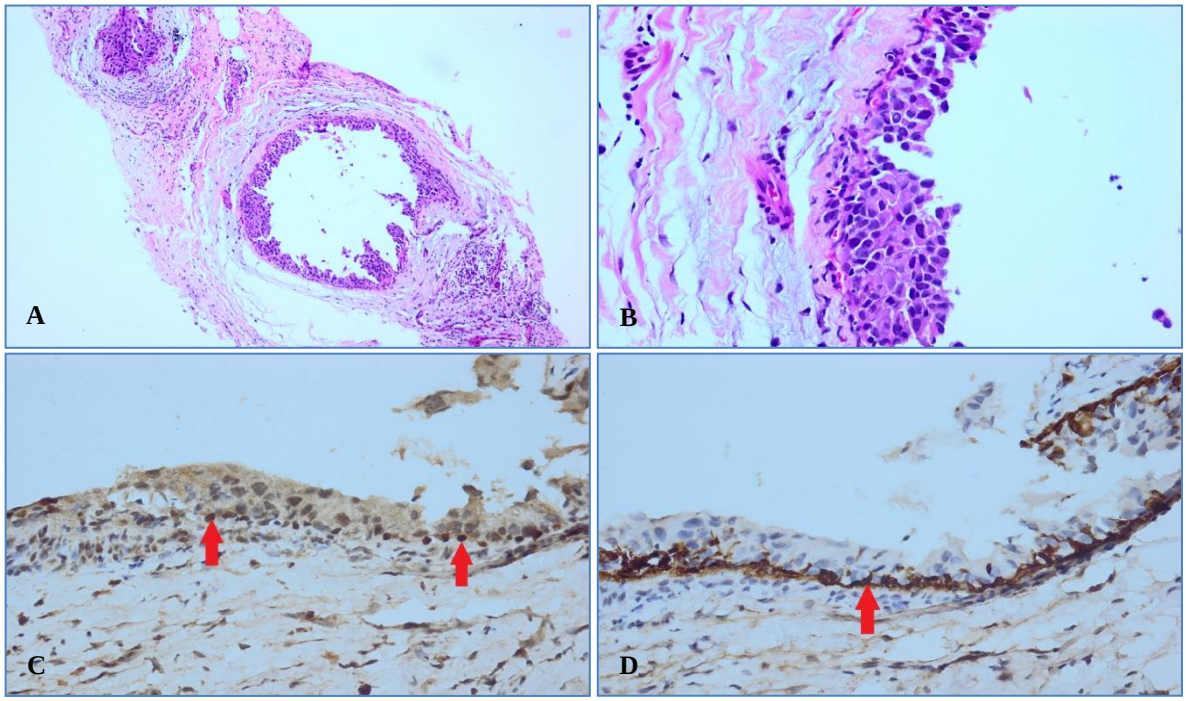

Figure 2: The histopathologic view of ductal carcinoma *in situ* in the breast. **A-** (HEX100) **B-** (HEX400) **C-** The nuclear staining of the myoepithelial layer with P63 (red arrows). (X400) **D-** The showing of the myoepithelial layer with CD10 (red arrow). (X400)

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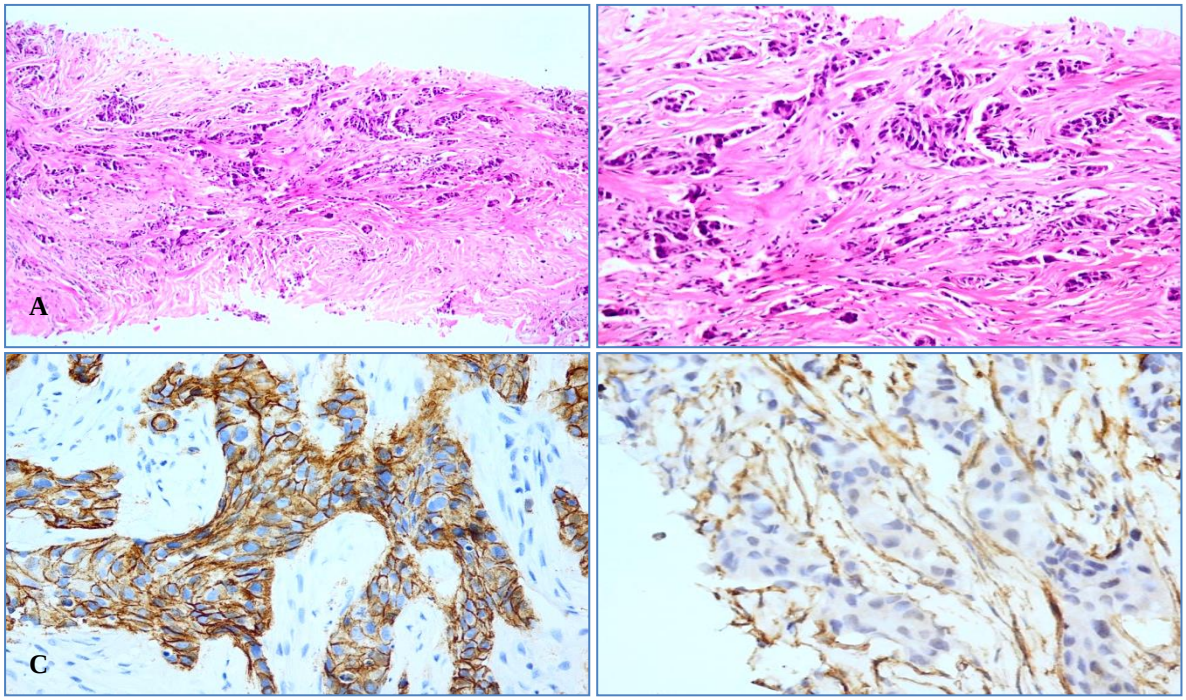

Figure-3. The histopathologic view of invasive ductal carcinoma in the breast. **A-** HEX100. **B-** HEX200. **C-** E-cadherin positivity in invasive ductal carcinoma of the breast. **D-** The loss of myoepithelial layer. Staining is observed only on the ground. (CD10X400)

Discussion

Breast pathologies are very common in women. Benign lesions are more common in the early decades. Benign breast diseases include mainly fibroadenoma, adenosis, fibrosis, fibrocystic changes, intraductal papilloma and inflammatory diseases (3).

The main types of breast cancer are ductal carcinoma in situ, invasive ductal carcinoma (invasive carcinoma of no special type (NST) or invasive ductal carcinoma not otherwise specified (NOS)), lobular carcinoma in situ, invasive lobular carcinoma (1). While benign lesions are more common in the early decades with a peak in the third decade, the incidence of malignant lesions is higher in advanced decades (29).

The most important features in the diagnosis of breast cancer are atypical cellular features such as invasion, desmoplasia, pleomorphism, hyperchromasia, nuclear irregularity, prominent nucleoli, high mitosis count. Loss of myoepithelial layer in the malignant cases is a very important feature in the diagnosis (14). Immunohistochemical stains such as calponin, P63, CD10, and CK5/6 are important in the identity of myoepithelial layer (30,31).

In this study, there were 10 fibroadenoma cases and 8 fibrocystic change cases among 23 benign breast disease cases. The rate of fibroadenoma cases was 43% and the rate of fibrocystic change cases was 35%. In the study including 352 benign breast disease cases of Sagiroglu et al., it was revealed that the rate of fibroadenoma cases was 53%, the rate of fibrocystic changes was 21% (32).

In both studies, the percentage of fibroadenoma was greater than fibrocystic change. In this study, the rate of fibroadenoma cases was slightly lower than the rate in the study of Sagiroglu et al. The rate of cases with fibrocystyc change was slightly higher than the rate in the study of Sagiroglu et al. However, in both studies, the percentages of fibroadenoma and fibrocystic change were relatively consistent.

In this study, the mean age was 33.6 in benign cases and 57.5 in malignant cases. In the study including 2118 ductal carcinoma cases of Wang et al, the mean age was 57.3 (33). In the study including 174 breast cancer cases of Balekouzou et al, the mean age was 45.8 (34). In the study including 76 breast cancer cases of Mansouri et al, the mean age was 51.3 (35). In this study, this result was compatible with the literature. In this study, the mean age was closer to the result in the study of Wang et al. than others.

In this study, the age range was 33-81 in malignant cases. In the study of Balekouzou et al, the age range was 16-90 year (34). In this study, the age range is narrower according to the result of Balekouzou et al. The limitation of this study, the number of cases were less. If the number of our cases were greater, this range could be wider.

In this study, the most common age group was between 40-50 years in benign cases and between 50-60 years in malignant cases. In the study of Balekouzou et al, the most common age group was between 45-54 years (34). In this study, this result was compatible with the literature. In this

study, the most common age group in benign cases was close to the result in the study of Balekouzou et al.

Microglandular adenosis is a benign lesion with loss of myoepielial layer and therefore may be difficult to diagnose with cancer. Lack of staining with estrogen and progesterone receptors is a supportive finding (10-12). In this study, one of the cases was microglandular adenosis. There was a loss of staining in the myoepithelial layer. Histologically, there was a haphazard infiltration of small and uniformly round glands in fibrous tissue. Its differential diagnosis was made from carcinoma with the absence of cellular atypia and the absence of the staining with estrogen and progesterone receptors. The histopathological diagnosis was confirmed by demonstrating the myoepithelial layer in microglandular adenosis using immunohistochemical markers such as CD10, P63, CK5 / 6, and calponin.

Ductal carcinoma in situ is the most common histologic type of non-invasive breast cancer (1). The showing myoepithelial layer with immunohistochemical markers is important in differential diagnosis than invasive carcinoma. In addition, the absence of stromal desmoplasia and invasion are important in the differential diagnosis. In this study, the showing of myoepithelial layer with CD10, P63, CK5/6, and calponin in a case with cellular atypia was usefull in diagnosis.

The results of our own laboratory were shared with the literature and it was emphasized that the evaluation with a large immunohistochemical panel in breast tru cut biopsy materials would reduce the risk of diagnostic errors in cases difficult to diagnose with cellular features.

Conclusion

In the diagnosis of breast cancers, mainly cellular properties are determinative. The evaluation with the immunohistochemical panel will reduce the risk of diagnostic error when the cases that difficultly diagnosed with cellular properties.

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Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **MGB, MT**; Research concept and design, Research the literature, histopathological examinations, preparation of the article**.. MGB;** Revision of the article.

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An evaluation of type 2 diabetes mellitus patients on different oral antidiabetic medications with regard to glycemic control and diabetic complications

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Abstract

Objective: Diabetes Mellitus is a chronic and progressive disease that significantly impairs the workforce and economy due to its complications. This study aims to evaluate patients diagnosed with type 2 diabetes mellitus who use different oral antidiabetic medications with regard to glycemic control and diabetic complications.

Materials and Methods: This study included 200 patients who were being followed-up for a diagnosis of Type 2 DM.

Results: Of the 200 patients included in the study, 131 were on metformin monotherapy and 69 were on metformin and gliclazide combination therapy. HbA1c value of Metformin monotherapy prescribed patients was7,6%±1,5, metformin+gliclazide prescribed patients was 8,2%±1,9. There was a statistically significant difference between the two groups in terms of blood glucose levels ($p<0.05$). There was no significant difference between the two groups with regard to microvascular complications and body mass index.

Conclusion: Our study determined that the level of glycemic control manifested by Type 2 DM patients was suboptimal despite using different types of oral antidiabetics and that their body mass indices were high. We reached the conclusion that the present situation is linked to factors such as incorrect dietary habits, inadequate exercise and walking, failure to comply with the medical treatment suggested by the physician, and lack of awareness about the severity of the disease.

Keywords: diabetes mellitus, metformin, gliclazide

Introduction

The International Diabetes Foundation (IDF) estimates that at least 425 million individuals around the world suffer from diabetes (1). From 1980 to 2014, the standardized global prevalence of adult diabetes has doubled among males and increased by almost 60% in females. If these trends persist, the World Health Organization (WHO) target of halting the rise in the prevalence of diabetes by 2025 will not be achieved (2). The increase in the prevalence of diabetes challenges individuals, families, and health systems globally. Besides being associated with a significant mortality rate, type 2 diabetes mellitus (T2DM) is a chronic disease that can lead to serious comorbid conditions (3).

Type 1 diabetes is an autoimmune disease and depends on the destruction of insulin producer pancreatic beta cells. Type 1 DM generally observes at young..

Type 2 diabetes, insulin resistance and pancreatic beta cells It occurs as a result of the coexistence of the disorder seen in insulin secretion (4).

Insulin offers high effectiveness in the treatment of diabetes, however, the fact that it cannot be used orally poses a significant problem and reduces patient compliance with treatment. For this reason, numerous studies have been conducted to discover oral antidiabetic medications; and the first of these, the plant-based alkaloid decamethylenediguanide, was discovered in the 1920s. Although it succeeded in reducing blood glucose levels, its use was banned due to its marked hepatotoxic effect. In 1955, a sulfonylurea compound named carbutamide was introduced and it was followed by less toxic variants (5, 6). Patients who benefit the most from oral antidiabetic medications are those with a diabetes onset age above 40 years and a diabetes duration less than 5 years.

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Patients with longer diabetes duration may need to take insulin and oral antidiabetic medications in combination to keep blood glucose levels under control (7). Oral antidiabetic agents that regulate blood glucose are known to take effect by increasing insulin secretion, elevating insulin sensitivity, or decreasing carbohydrate absorption. An ideal antidiabetic agent should reduce plasma glucose values to the normal range, have minimal side effects, and also inhibit the development of microvascular complications. Glycemic targets can be reached by considering the advantages and disadvantages of these medications and administering them alone or in combination accordingly (8). Currently, metformin is the only biguanide medication in use. At the cellular level, metformin takes effect by indirectly activating AMPK (5'-adenosine monophosphate-activated protein kinase) and partially inhibiting mGPD (glycerophosphate dehydrogenase). Metformin inhibits the elevated glucogenesis in the liver seen in type 2 diabetes, and suppresses lipid and cholesterol biosynthesis through transient inhibition of the mitochondrial respiratory chain complex I. On the other hand, conventional information suggesting that it slightly increases muscle glucose uptake and fatty-acid oxidation is controversial. Metformin also reduces intestinal glucose absorption, increases insulin sensitivity, and partially suppresses appetite (probably due to its side effects on digestion and perhaps due to its effects that promote GLP-1). As it has been in use for a long time and has a low cost, there is extensive clinical experience on metformin therapy. Its advantages include a low hypoglycemia risk and being neutral for weight gain or having a slight weight-reduction effect (9).Gliclazide, which is a second generation sulfonylurea, increases insulin secretion independently from glucose by blocking the ATP-dependent K channels on the plasma membrane of beta cells respectively for long and short durations. As they have been in use for a long time and have a low cost, there is extensive clinical experience on sulfonylureas. Sulfonylureas were shown to decrease the risk of microvascular complications (9). Meanwhile, although it was proposed that they disrupt the ischaemic preconditioning mechanisms of myocardial cells, these concerns were not corroborated by clinical experience. The effects of sulfonylureas currently in use are relatively short-term and more stable. However, their effectiveness does not last very long (9).

If diabetes is not managed properly, it may create a risk for various complications such as diabetic nephropathy, diabetic neuropathy, coronary artery disease, strokes, leg amputations, and even early death (10). Delaying the progression of diabetes would also benefit national economy by increasing the wellbeing of the population and patients and decreasing the economic load on the health system (11). Optimal glycemic control may prevent potential diabetes-related complications. The importance of glycemic control in diabetic patients is known quite well. Various studies have reported a significant decrease in the incidence of diabetes-related complications, however, these targets are often not met (12, 13). Diabetes is also known as a self-managed disease because most of the care is provided by the patients themselves; therefore, patients are expected to show the required dedication to their self-care in their daily lives (14). Self-care activities of diabetic individuals include maintaining a healthy diet and physical activity, self-monitoring of blood glucose levels, and taking medications regularly. Daily self-care activities play a critical role in achieving positive health outcomes in diabetes. Many studies have reported a clinically significant relationship between glycemic control and self-care activities (15-20). As stated in the Da Qing Diabetes Prevention Study, diabetes can be prevented or delayed by making drastic changes in the lifestyles of individuals with a high diabetes risk. According to the data presented by the Da Qing Diabetes Prevention Study, following a 6-year lifestyle intervention, diet, exercise, and diet+exercise groups showed a decrease in the incidence of diabetes by 31%, 46%, and 42%, respectively (21). These benefits were shown to last for more than 20 years after the end of the lifestyle intervention (22).

Materials and Methods

Having the aim of evaluating patients diagnosed with type 2 DM who use different oral antidiabetic medications with regard to glycemic control and diabetic complications, this study was conducted after obtaining an ethics committee approval from the Firat University Scientific Research Projects Coordination Unit (Approval date: 19/07/2018, Approval number: 07).

The study group consisted of 200 individuals with Type 2 Diabetes, of which 131 were metformin and 69 were metformin+gliclazide prescribed patient, who presented to the Internal Medicine polyclinic and clinic at Firat University Medical Faculty Hospital between January 2018 and July 2018. Patient data were acquired by a retrospective scan of patient files. Diabetic patients with urinalysis results indicating + proteinuria and/or creatinine>1.2 mg/dl were considered to have diabetic nephropathy. Patients who had been diagnosed with diabetic retinopathy by the Ophthalmology Department after a consultation were included in the retinopathy group. Patients with positive polyneuropathy results in EMG or neuropathic complaints were clinically considered to have diabetic neuropathy. Patients diagnosed with hyperlipidemia and/or cardiovascular diseases after the diagnosis of diabetes were considered in the atherosclerotic cardiovascular diseases category. According to the Wagner grading system for diabetic foot ulcers; patients with superficial ulcers, deep and penetrating ulcers, osteomyelitis, local gangrene or diffuse gangrene were considered diabetic foot patients. Diabetic patients with a blood pressure>130/80 mmHg were considered hypertensive diabetics. Atherosclerosis and diabetic foot patients were included in the 'Type 2 Diabetes with macrovascular complications' group, while patients with nephropathy, retinopathy, and neuropathy were included in the microvascular complications group.

Demographic data of the entire study group (age, sex, waist and hip circumference measurements, body mass index values) were obtained from the scan of patient files. Body mass index measurements were in units of kg/height (m^2); obtained by the division of body weight in kilograms to body surface area in units of m^2. Waist and hip

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circumference measurements were taken using a measure (cm) in accordance with the WHO waist circumference measurement guidelines; making measurements at the midpoint between the costal margin and spina iliaca. Routine biochemistry samples of the patients were evaluated by the central biochemistry laboratory at our hospital and these were comprised of the routine tests requested during follow-up examinations (HbA1c, AST, ALT, Urea, Creatinine, Lipid levels). No additional blood samples were obtained for this study besides those collected routinely and only data recorded in the patient files were used.

Statistical Analysis

Obtained results were evaluated using the SPSS-22 computer software. Categorical data were analyzed with the Chi-square test, parametric data were analyzed with Student's t-test. $p<0.05$ was considered the threshold for statistical significance.

Results

This study included 200 patients, 131 of which were on metformin monotherapy and 69 on metformin and gliclazide combination therapy. Patients who used only metformin demonstrated an HbA1c value of 7,6%±1,5, while patients who used metformin+gliclazide demonstrated an HbA1c value of 8,2%±1,9. Fasting blood glucose (FBG) and postprandial blood glucose (PBG) levels of the metformin group were respectively determined as 172±50mg/dl and 253±68mg/dl. FBG and PBG levels of the group that underwent metformin and gliclazide therapy were found as 190±61mg/dl and 276±73 mg/dl, respectively. There was a statistically significant difference between the two groups with regard to these values ($p<0.05$). Body mass index (BMI) values were determined as 28,2±5.5 for the metformin group and as 28,6±5,4 for the metformin+gliclazide group (Table 1).

With regard to microvascular complications, the two groups did not demonstrate any statistically significant differences. However, we found that diabetic peripheral neuropathy was quantitatively more common among patients on metformin monotherapy compared to the metformin+gliclazide group (61 versus 41 patients) (Table 2).

Table1: Comparison of Laboratory Parameters of Metformin Therapy Group and Metformin + Glyclazide Therapy Group

Parameters	Metformin Group (n=131)	Metformin+gliklazid Group (n=69)	P Value
Age	55,26±8,717	57,01±8,702	0,17
HbA1c (%)	7,605±1,5258	8,296±1,9615	0,006*
FBG (mg/dl)	172,98±50,483	190,78±61,681	0,029*
PBG (mg/dl)	253,36±68,452	276,29±73,872	0,030*
BMI	28,28±5,539	28,61±5,443	0,691
LDL-C (mg/dl)	125,20±46,758	126,46±42,838	0,85
Creatinin (mg/dl)	0,7356±0,13635	0,7325±0,17039	0,889

* Statistically significant differences. BG; Blood Glocose FBG (Fasting blood glucose); PBG (Postprandial blood glucose); BMI (Body mass index)

Table2: Comparison of the group receiving metformin treatment and the group receiving metformin + glyclazide treatment in terms of microvascular complications

		Nephropathy		
		yes	**no**	**Total/p value**
Group	metformin	1	130	131/0.2
	metformin+gliclazid	2	67	69/0.5
Total		3	197	200
		Neuropathy		
		yes	**no**	**Total/p value**
Group	metformin	61	70	131/0.84
	metformin+gliclazid	41	28	69/0.11
Total		102	98	200
		Retinopathy		
		yes	**no**	**Total/p value**
Group	metformin	26	105	131/0.14
	metformin+gliclazid	20	49	69/0.19
Total		46	154	200

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Discussion

Type 2 diabetes mellitus (T2DM) is a chronic disease that can result in serious comorbid conditions, as well as a high rate of mortality (4). If metabolic parameters (fasting blood glucose, postprandial blood glucose, hemoglobin, A1c, blood pressure, lipids) are not monitored effectively in diabetes, various complications such as diabetic nephropathy, diabetic neuropathy, coronary artery disease, stroke, leg amputations due to diabetic foot infections, and even an early death may become a risk (10). The prevention of these complications would also benefit national economy by increasing the patients' quality of life and decreasing the associated economic load on the health system (11).

Optimal glycemic control may prevent potential diabetes-related complications. Although the importance of glycemic control in diabetic patients is clear, various studies have reported a significant decrease in the incidence of diabetes-related complications that is often not achieved in clinical practice (12,13). Diabetes is also known as a self-managed disease because most of the care is provided by the patients themselves; therefore, patients are expected to show the required dedication to their self-care in their daily lives (15).

Personal care activities of diabetic individuals include maintaining a healthy diet and physical activity, self-monitoring of blood glucose levels, and regular use of medication. Daily self-care activities play a critical role in achieving positive health outcomes in diabetes. Many studies have reported a clinically significant relationship between glycemic control and self-care activities (15-20).

In this study, we evaluated and compared patients diagnosed with Type 2 DM who were on different oral antidiabetic agents in terms of glycemic control and diabetic complications. This study included 200 patients, 131 of which were on metformin monotherapy and 69 on metformin and gliclazide combined therapy. Patients who used only metformin demonstrated an HbA1c value of 7,6%±1,5, while patients who used metformin+gliclazide demonstrated an HbA1c value of 8,2%±1,9. Here, the reason for the lower HbA1c values seen in patients on metformin monotherapy may be that baseline glycemic values were lower in this group and that patient adherence to monotherapy was higher. Fasting blood glucose (FBG) and postprandial blood glucose (PBG) levels of the metformin group were respectively determined as 172±50mg/dl and 253±68mg/dl. FBG and PBG levels of the group that underwent metformin and gliclazide therapy were found as 190±61mg/dl and 276±73 mg/dl, respectively. There was a statistically significant difference between the two groups with regard to the present values ($p<0.05$).

Here, it can be stated that the group on metformin+gliclazide is quite far from the glycemic goals in terms of fasting and postprandial blood glucose levels and that a different oral antidiabetic medication or insulin should be considered as alternatives. With regard to microvascular complications, the two groups did not demonstrate any statistically significant differences. The most important conditions for the appearance of microvascular complications are diabetes duration and fluctuating glycemic values throughout this duration, thus, our inability to acquire diabetes duration data from patient files due to the retrospective nature of our study constitutes one of the important limitations of this study. Body mass index (BMI) values were determined as 28,2±5.5 for the metformin group and as 28,6±5,4 for the metformin+gliclazide group.

While there was not a statistically significant difference, the higher BMI values seen in patients using metformin+gliclazide can partially explain the higher glycemic values determined in this group. Another issue that is worth stressing here is that both groups had high BMI values, highlighting the importance of selecting antidiabetic agents that facilitate weight loss while evaluating treatment options and making lifestyle changes, particularly concerning dietary habits.

Glycemic targets must certainly be individualized. More flexible glycemic targets must be set in cases of low life expectancy, long diabetes duration, recurrent episodes of severe hypoglycemia, concomitant micro and macrovascular complications or other comorbid conditions.

In the case that glycemic control is not maintained in patients undergoing one of the current non-insulin antihyperglycemic therapies, individualized treatment alternatives and therapies in combination with insulin would be more appropriate. It is recommended that patients with type 2 diabetes mellitus are introduced to insulin therapy in situations such as type 1 diabetes mellitus, LADA and failed metabolic control with non-insulin antihyperglycemic medications, excess weight loss, severe hyperglycemic symptoms, hyperglycemic emergencies, acute myocardial infarction, inflammatory and systemic diseases, major surgery, pregnancy and lactation, severe liver and kidney failure, allergy to or strong side effects by non-insulin antihyperglycemic medications, clinically severe insulin resistance, and long-term high-dose corticosteroid use (23).

(Type 2 diabetes, commonly resulting from lifestyle and diet, thus has very close relation with lifestyle, this relation should be emphasized strongly) It is known that having adequate information as to type 2 DM and managing diabetes with a correct approach increase compliance with treatment and play an important role in the management of diabetes (24). Knowledge on type 2 DM and knowledge and awareness about correct nutrition help individuals with type 2 diabetes establish metabolic self-control and make dietary choices that would optimize quality of life (25). In overweight or obese adults with type 2 DM, weight loss attempts that depend on lifestyle interventions result in less than 5% weight loss and weight loss targets are often not achieved (26).

In cases where more than 5% weight loss is achieved through lifestyle changes, studies report favorable effects on glycemic levels, blood pressure, and the lipid profile (26). In order to be able to achieve such levels of weight loss, intensive interventions including energy restriction, regular physical activity, and regular follow-up visits to health professionals were reported to be necessary. A study

done by Franz et al. (26) expressed that weight loss was not a realistic primary treatment strategy for most individuals with a diagnosis of type 2 DM and a high body mass index. However, they propose that nutritional therapy promotes a healthy diet and that reduced energy intake, regular physical exercise, and education reinforce the primary treatment strategies.

On the other hand, due to low dietary compliance by individuals diagnosed with Type 2 DM, a well-balanced, simple, and easily understandable dietary approach is recommended (27).

In conclusion; the findings of our study that indicate suboptimal glycemic control may be explained by the incorrect dietary habits of the patients, lack of sufficient exercise and walking, poor compliance with the medical treatment proposed by the physician, and mentally, lack of awareness regarding the severity of the disease.

Our opinion is that the desired states in diabetes management can be reached by maximum patient compliance with the lifestyle changes proposed for their disease, providing environments that facilitate patient education, and multidisciplinary teamwork by physicians, nurses, dieticians, psychologists, and other allied health professionals.

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Author's Contributions: **EO, NG, BY:** Analyzes and interpretation of the data, preparation of the manuscript, application of the statistical analyses

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Effects of vitamin B12 and folic acid deficiency on hemogram parameters in children

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Abstract

Objective: According to numerous studies, bicytopenia, pancytopenia, or isolated thrombocytopenia and anemia patients have folic acid (folate) and vitamin B12 (B12) deficiency. The purpose of this study is to analyze the effects of folate and B12 deficiency in childhood on several haemogram parameters such as platelet (PLT), mean platelet volume (MPV), hematocrit (HCT), mean corpuscular volume (MCV), and white blood cell (WBC) count.

Materials and Methods: The retrospective study included children who had applied to the pediatric outpatient clinic between 2015 and 2017. Patients were divided into 3 groups according to serum B12 and folate status. The results were evaluated by statistical methods.

Results: PLT and WBC levels of the folate and B12 deficiency group were found to be lower than the control group ($p=0.015$, $p<0.001$ respectively), and their MPV and HCT levels were higher (p: 0.015, $p<0.001$ respectively). MCV levels, however, were not different ($p>0.05$). No effect of PLT and MCV on folate levels was seen. Similarly, any effects of MPV, PLT and MCV independent variables on B12 levels were not observed. Although platelet and leukocyte count was decreased in folate and B12 deficiency, thrombocytopenia and leukopenia were observed only three patients.

Conclusion: Although peripheral blood cell lines are not always seen low during folate and B12 deficiency, and there is not obvious anemia and MCV highness at a patient with neurological and psychological symptoms, folate and B12 deficiency should be thought if there are leukocyte and thrombocyte levels lower than mean reference values.

Keywords: Children, Deficiency, Folate, Haemogram, Vitamin B12

Introduction

Vitamin B12 (B12) is necessary for the development of the fetus and the child. The exogenous intake of this vitamin is essential for human species who could not synthesize B12 (1). The vitamin is rarely found in food derived from plants; therefore, those following strict vegetarian diets are likely to have inadequate intakes of B12. Low B12 status may cause ineffective erythropoiesis because of the limited DNA synthesis due to inhibition of purine and thymidylate synthesis and this contributes to homocysteinemia and impairs the metabolic utilization of folate. As a result, B12 deficiency may produce various hematological, mucocutaneous, gastrointestinal or neurological signs and symptoms. The hematological sign is megaloblastic anemia. However, hematologic and neurologic signs may not emerge together all the time in B12-deficient subjects. In most deficient subjects, either anemia or neurologic signs predominate (2, 3). Folic acid (folate) is essential for normal embryonic growth and development. It is necessary to promote closure of the neural tube, defects of which result in malformations of the embryonic brain and/or spinal cord referred to as neural tube defects (NTDs).

Folate is widely distributed among food, particularly the ones with plant foliar origin, has an important role in the production of new cells through its functions in the synthesis of purine and thymidylate that are required for the de novo synthesis of DNA, and DNA replication and cell division. Disruption of these functions impairs cell division and results in macrocytic anemia of folate deficiency (3,4). Ineffective DNA synthesis depending on the deficiency of B12 and/or folic acid might also affect the development of megakaryocytes, as reflected by abnormal large polylobate megaloblastic megakaryocytes with a lack of cytoplasmic granules.

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Corresponding changes in the blood smear include anemia with oval macrocytes, anisocytosis, and poikilocytosis; leukopenia with hyper segmented polymorphonuclear cells; and thrombocytopenia (5). On the other hand, different results were reported on this study. In a retrospective design study involving 120 subjects suffering from anemia resulting from B12 deficiency, it was reported that only 28% of the subjects had thrombocytopenia, 29% had leukopenia and 17.3% had pancytopenia (6). In another study, it was declared that deficiency of B12 and/or folate might be a possible cause of isolated thrombocytopenia, an, increased platelet volume may also accompany with thrombocytopenia (7). The mean platelet volume (MPV), index of platelet reactivity, is frequently used in outpatient and inpatient healthcare facilities because of being a relatively low cost hemogram parameter and a useful one to determine platelet volume (8).

In this study, how the platelet count and the mean platelet volume (MPV) were affected from different levels of B12 and folate in children were analyzed. The relationship between B12 and folate with other hematological parameters such as red blood cell (RBC), mean corpuscular volume (MCV), and white blood cell (WBC) were also examined. In addition, the effects of independent variables such as age and gender on these parameters have been also evaluated.

Materials and Methods

This retrospective study was performed with an approval from local ethical committee (University of Health Sciences, Haseki Training and Research Hospital Ethical commission). The study included 371 children (204 females and 167 males) between the ages of 9 months and 18 years who had applied to the pediatric outpatient clinic with simple complaints between the dates 1.01.2015 and 31.12.2017. During this period, 806 patients received simultaneous vitamin B12, folate and hemogram. The study excluded 388 patients with acute and chronic infection and hematologic disease, and 47 patients whose results could not be reached due to a technical reason. The study groups were divided into 3 groups according to their B12 and folate levels. Cut-off points established by the kit manufacturer which used in this study. 180 pg/ml was accepted as cut-off point for B12 while 5.9 ng/ml was considered as cut-off point for folate. Accordingly, while Group 1 included 79 people (43 females, 36 males) whose folate (>5.9 ng/ml) and B12 levels (>180 pg/ml) were normal, Group 2 comprised of 191 people (103 females, 88 males) who suffered from B12 deficiency (<180 pg/ml). On the other hand, Group 3 consisted of 101 people (43 females, 58 males) who had folate deficiency. The hematological condition in both vitamin B12 and folate deficiency is as in vitamin B12 deficiency, even if folate is normal. Therefore, both low patients were not enrolled in the study. The necessary information and relevant test results of the patients were obtained from the hospital information management system. Those recorded in the system as having chronic disease, acute-chronic infection and hematologic problems were excluded from the study.

Serum folate and B12 levels were analyzed by using UniCel DxI 600 autoanalyser (Beckman Coulter, Inc. USA) with chemiluminescence method. The platelet count was determined by hydrodynamic centering factor whereas MPV values were obtained by calculating through the formula: MPV (fl)= [(PCT (%)/platelet count (×109/L)]. Platelet related tests were studied with Sysmex XE-2100 hematology analyzer (TOA Medical Electronics, Kobe, Japan).

It was confirmed that the internal quality control results of the tests were at ±2 standard deviation on the day of the analysis. The lowest detectable level of folate distinguishable from zero with 95% confidence was 0.5 ng/mL (1.1 nmol/L). Total imprecision of folate (CV%) was 4.34. The lowest detectable level of B12 distinguishable from zero with 95% confidence was 50 pg/mL (37 pmol/L). Total imprecision of B12 (CV%) was 8.4.

Statistical Analysis

Statistical evaluations were performed using the Statistical Package for Social Sciences (SPSS) 21 software (IBM, New York, USA). The Kolmogorov-Smirnov test was used to determine whether the numerical data showed normal distribution and Levene test was used to evaluate the homogeneity of the variances of each group. Categorical variables were assessed by Chi-square test whereas comparisons among study groups in terms of the numerical variables were performed with One-way ANOVA test.

Two-way ANOVA test and covariance analysis (ANCOVA) were used to evaluate the common effect of multiple independent variables on a given dependent variable. The gender distributions of the groups were evaluated by Student's T test. The correlation between hemogram parameters and folate/ B12 levels was evaluated by Pearson correlation test. The significance level of P value was accepted as <0.05. The results were expressed as mean ± standard deviation.

Results

There was no statistically significant difference between the groups in terms of mean age and gender. While MPV, PLT, WBC, and HCT showed significant differences among groups, there was not any significant difference between the groups in terms of MCV. Although platelet and leukocyte count was decreased in folate and B12 deficiency, thrombocytopenia and leukopenia were observed only three patients. (Table 1).

There was difference between normal group and groups with folate and B12 deficiency in terms of MPV level while there were not any differences between these two groups with deficiency. In terms of PLT levels, on the other hand, statistically significant difference was found only between the normal group and group with B12 deficiency. Although the mean PLT level in normal group was higher than the mean PLT level in the group with folate deficiency, this difference was not statistically significant. (Figure 1,2).

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Although the MCV level of normal group showed a remarkable highness than the deficiency groups, no significant difference was determined between the MCV averages of the three groups. When the HCT averages were compared, the difference between the Hct level averages of the normal group and the averages of both deficiency groups was significant. Similarly, there is a significant difference between the normal group and deficiency groups in terms of mean WBC.

While the HCT levels of deficiency groups were higher than the normal group, their WBC levels were lower. There were not any differences between deficiency groups in terms of the averages of these two parameters (Figure 3,4).

As a result of the multiple linear regression analysis where the effect of haemogram parameters as independent variables on the folate and B12concentrations were evaluated, it was determined that MPV, HCT and WBC had effects on folate concentrations.

A correlation between the increase in MPV and HCT levels and the decrease in folate levels was found whereas it was determined that there was a correlation between the decrease of WBC and folate below the normal range. However, PLT and MCV had no effect on folate concentration (R=0.401, p<0.001). Likewise, no effect of MPV, PLT and MCV independent variables on B12 level was monitored. Similar to the relations with folate, the increase of MPV and Hct levels were found to be related to the decrease in B12 level while a correlation between the decrease in WBC and lowness of B12 was determined (R=0.354, p<0.001). Furthermore, there was a highly strong negative correlation between age and especially folate level (r=-0.677, p<0.001), and a reasonable negative correlation was determined between B12 and age (r=-0.426, p<0.001).

No difference was found between folate and B12 levels in terms of gender. However, when the haemogram parameters were analyzed, differences between females and males were found in terms of HCT and MPV levels (Table2).

Table 1. Demographic characteristics and laboratory test results of study groups

Variables	Group 1	Group 2	Group 3	P value
Gender (F/M)	43/36	103/88	58/43	0.84
Age (years)	11.3±0.5	12.6±0.3	12.7±0.4	0,78
Folate (ng/ml)	16.2±4.4	8.4±5.0	4.7±0.8	<0.001
Vitamin B_{12} (pg/ml)	480±110	144±20	288±76	<0.001
MPV (fL)	9.6±0.9	10.0±0.9	10.0±1.0	0.002
PLT (10^3/µL)	318±87	276±79	290±80	0.015
MCV (fL)	93.1±9.6	80.1±5.6	79.4±5.2	0.07
HCT (%)	36.5±4.8	38.8±4.0	38.7±3.6	<0.001
WBC/ mm^3	8.1±2.6	6.9±2.3	6.7±2.0	<0.001

F: female, M: male, MPV: mean platelet volume, PLT: platelet, MCV: mid corpuscular volume, HCT: hematocrit, WBC: white blood cell.

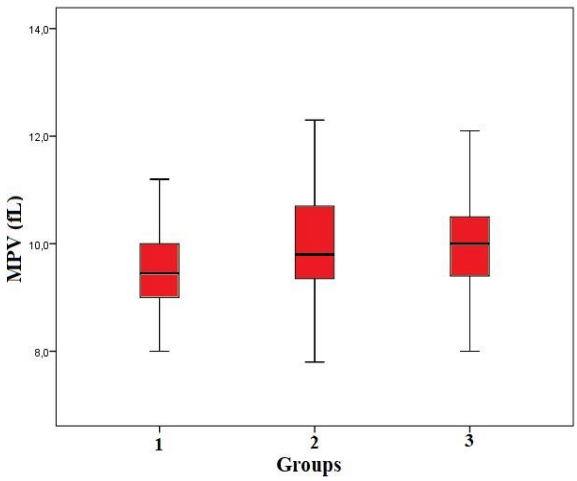

Figure 1. Box-plot graph showing mean platelet volume (MPV) of the groups

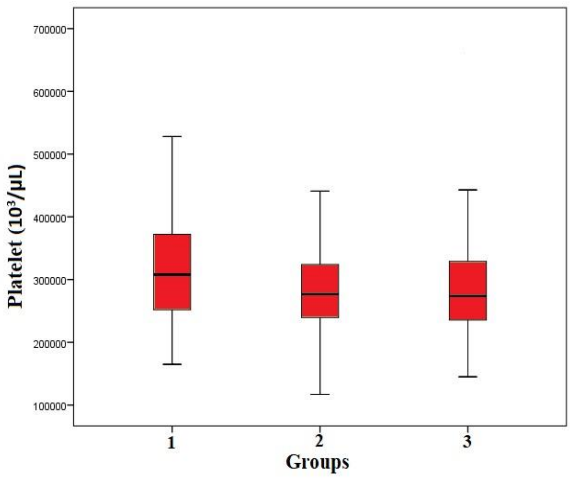

Figure 2. Box-plot graph showing the platelet count of the groups

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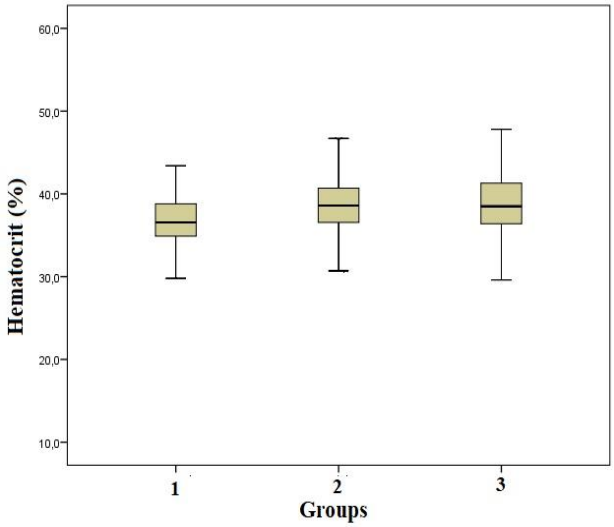

Figure 3. Box-plot graph showing hematocrit of the groups

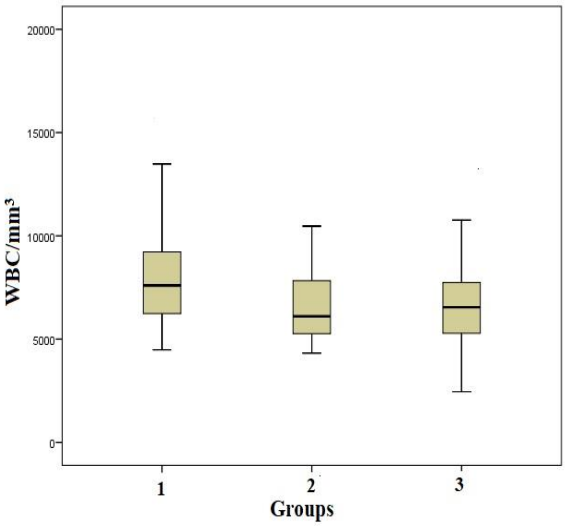

Figure 4. Box-plot graph showing white blood cell (WBC) of the groups

Table 2. Comparison of the values of the laboratory tests used in the study in females and males (Men±SD)

Variables	Female	Male	P value
Folate (ng/ml)	8.8±5.4	9.3±6.2	0.326
VitamineB_{12} (pg/ml)	280±196	261±166	0.307
MPV (fL)	10.0±1.0	9.7±0.9	0.005
Platelet (10^3/µL)	298±88	293±83	0.565
MCV (fL)	83.4±6.6	81.7±6.1	0.727
HCT (%)	37.6±2.9	39.0±5.0	<0.001
WBC/ mm^3	7.4±2.5	7.2±2.3	0.680

Discussion

Body needs B12 for tetrafolate production and methylation reactions that are required for DNA synthesis. In addition, folic acid is required together with B12 for the formation from homocysteine to methionine and the formation of shaped elements in the bone marrow. Therefore, it was reported that thrombocytopenia might occur as well as anemia and leukopenia as a result of the damage of DNA synthesis due to B12 and/or folate deficiency (9). In this study, it was observed that folic acid and B12 deficiency affected the platelets numerically and dimensionally. It was observed that platelet volume increased whereas platelet count decreased in the groups with deficiencies.

However, only a correlation between platelet volume increase and folate level decrease was determined. It was thought that the reason why there was no correlation between platelet count and the deficiency of these two vitamins could be the fact that the platelet decrease in the groups with folate and B12 deficiency was not at thrombocytopenia level, and that platelet levels of all the three groups showed changes within the reference ranges. Nevertheless, in a retrospective study by Jaggia A and Northern A, they argued that deficiency of B12 and/or folate might be a possible cause of isolated thrombocytopenia and platelet formation which is greater than normal levels (7).

The inverse correlation between platelet count and volume, which is a finding of this study, is similar to this study. In another study on a pediatric group, it was suggested that B12 deficiency may cause isolated thrombocytopenia without megaloblastic anemia and/or leukopenia (10).

In another retrospective designed study by Nafil H et.al., involving 120 subjects suffering from anemia resulting from B12 deficiency, it was reported that only 28% of the subjects had thrombocytopenia, 29% had leukopenia and 17.3% had pancytopenia (6). In this sense, different results have been obtained so far in the studies on this subject.

No correlation between corpuscular volume and folate and B12 levels was observed in the study. However, it was determined that HCT levels were higher in the groups where these vitamins were deficient, and that there was an inverse correlation between the deficiency of these two vitamins and HTC. Moreover, it was found out that leukocyte level was significantly lower in the groups with folate and B12 deficiency, and that there was a correlation. In some of the previous studies, only anemia due to B12 deficiency was observed, whereas other studies reported bicytopenia or pancytopenia (11,12).

In another study conducted by Refsum et. al., it was observed that the study group with B12 deficiency unexpectedly rarely had anemia and the reason for this was interpreted as that the adequate intake of folate suppressed

anemia (13). It is suggested to exclude B12 and folate deficiency primarily at the etiology of the patients applied with pancytopenia and bicytopenia in the literature (14).

However, it is needed to mention that 10% of the children in the study groups were suffering from anemia (HCT< 35) and there was no child with leukopenia in the study groups.

The differences among the haemogram parameters in the study represented the changes within reference ranges. Furthermore, it is necessary to consider that the folate and B12 deficiencies in the study groups were not at advanced levels. Nonetheless, in the literature, the lower limit level for serum B12 vitamin is stated to be as the 257 pg/mL in terms of homocysteine, and the 219 pg/mL in terms of urinary methyl malonic acids pillage. It is suggested to accept serum B12 lower limit level as the 250 pg/mL at routine practices for safety (15).

As the B12 averages of the deficiency group of the study were lower than these values, neurologic and psychiatric symptoms were started, but lower levels and low levels for longer period of time might be needed for the appearance of hematologic anomalies.

On the other hand, the retrospective design of the study prevented the clinical assessment of some tests and subjects. Measurement of plasma total homocysteine and serum methyl malonic acid levels as well as B12 levels for the diagnosis of B12 deficiency is also recommended by various guidelines (7).

In addition, the reliability of the study shall be enhanced by conducting a prospective design study of bone marrow examinations of patients. Another important factor is the duration of the symptoms of patients suffering from folate and/or B12deficiency. In our study, similar to some previous studies, there are limitations such as not knowing the duration of deficiency, not having bone marrow and methyl malonic acid and homocysteine levels done. The age average of the group without folate and B12 deficiency in the study was lower than that of the deficiency groups. However, no age based deficiency classification could be seen in the previous studies. We could only find a classification in accordance with suggested age for measurements of folate and B12 to be realized in plasma (16).

But we could not get use of this classification since we conducted the folate and B12 measurements in the study on serum samples. For this reason, cut-off values obtained with reference range studies by kit manufacturer were taken into consideration.

Conclusion

As a result, it was observed that folate and/or B12 deficiency decreased platelet count and WBC while increasing platelet volume and HCT. On the other hand, it was observed that corpuscular volume was not affected by the deficiency of these two vitamins.

Moreover, it was determined that these changes were not at a level to cause thrombocytopenia, leukopenia, or anemia.

Although one or several of the peripheral blood cell lines are not always low during folate and B12 deficiency, and there is not obvious anemia and MCV highness at a patient with neurological and psychological symptoms, the probability of folate and B12 deficiency should be considered if there are leukocyte and thrombocyte levels lower than mean reference values. In parallel with the decrease in platelet count, MPV values are also higher. However, further studies are also suggested considering the limitations mentioned above.

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Author's Contributions: **KŞ, ME,:** Patient examinations, **CC, MK:** Biochemical Analysis**, KŞ:** interpretation of the data, preparation of the manuscript, application of the statistical analyses

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The bacterial profile and antibiotic sensitivity of the isolated pathogens from medical equipment and surfaces in the children's emergency room of a Nigerian hospital

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Abstract

Objective: Nosocomial infections are those acquired in hospitals or healthcare service units that first appear 48 hours or more after admission or within 30 days after discharge following in-patient care. Knowledge of the bacterial profile and sensitivity patterns of any hospital environment is a key factor in infection control and good antibiotic stewardship.

Material and Methods: This hospital-based cross-sectional study was conducted in the Children's Emergency Room (CHER) of Enugu State University Teaching Hospital, Enugu, Nigeria. Samples for culture were collected from equipment and hospital surfaces. Antimicrobial susceptibility testing was determined for each isolate by the Agar diffusion method using Standard Nutrient Agar 1 discs.

Results: Bacterial growth was observed in 83 (70.3%) specimens. *Staphylococcus aureus* (53.4%) was the most common isolate cultured followed by Coagulase-negative *Staphylococcus* (18.8%), then *Escherichia coli* (13.9%). Among *Staphylococcus aureus* isolates, 25.9% were MRSA. Ampicillin resistance of the gram negatives was high. All the Gram-negative isolates were susceptible to Ciprofloxacin and Ceftriaxone.

Conclusion: *Staphylococcus aureus*, Coagulase-negative *Staphylococcus*, and *Escherichia coli* were the commonest isolates. More efforts are needed to ensure improved hygiene standards in order to reduce the burden of nosocomial infections.

Keywords: Bacterial profile, sensitivity patterns, surfaces, Nigeria

Introduction

The hospital environment is a significant reservoir of pathogens for transmission to patients in different ways including contaminated surfaces. This is more so in emergency rooms because of the high influx of patients, patients' relations and health care providers. They may harbor potential pathogens and contaminate surfaces, or equipment they come in contact with. Contaminated surfaces have been reported to increase the prevalence of nosocomial infections especially at both extremes of life (1). Nosocomial infections, otherwise known as hospital-acquired infections (HAI) are those infections acquired in hospitals or healthcare service units, that first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care (2). Many surfaces and equipment in the hospital environment may not be adequately decontaminated and can become reservoirs of pathogens.

It has been reported that commonly used disinfection techniques are sometimes incapable of eradicating fomites reservoirs of nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) (3). This is a major public health concern because antimicrobial resistant organisms are increasingly responsible for higher morbidity and mortality rates from nosocomial infections (2-4). There is increasing evidence that contaminated inanimate surfaces especially those frequently touched by hand, can contribute to the spread of healthcare associated pathogens (5-7). Every year approximately two million patients in the United States acquire nosocomial infections, and at least 90,000 of them die (8-10). There is poor documentation about the prevalence of hospital acquired infections in developing countries; however, published data show that the burden of HAI is greater in Africa than in developed countries (6,11). There is also paucity of data on the

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bacterial profile of frequently used medical equipment and surfaces in hospitals especially in the context of developing countries; only nine studies have been carried out at in over 50 teaching hospitals in Nigeria (1,5,12-18).

Knowledge of the bacterial profile and sensitivity patterns of any hospital environment is a key factor in infection control and good antibiotic stewardship. De-escalation to pathogen-directed therapy is one of the "4 D's of optimal antimicrobial therapy." It is based on the clinical response, culture and susceptibility results as well as local resistance patterns in order to avoid the emergence of bacterial resistance (19). In addition, the spread of pathogenic strains can also be controlled by appropriate hospital hygiene measures (20). Thus this study aimed to determine the profile of nosocomial bacteria isolated from medical equipment and surfaces in the children's emergency room of Enugu State University Teaching Hospital and their sensitivity patterns in order to fight against healthcare-associated infections.

Materials and Methods

This hospital-based cross sectional study was conducted in the Children's Emergency Room (CHER) of Enugu State University Teaching Hospital, Enugu, Nigeria (ESUTH) between April and May 2019. It is the Enugu State owned Teaching Hospital, located at the center of Enugu, the capital city of Enugu State. It is a major referral center for the state and other border states. The Children's Emergency Room is a 14-bedded ward, and it attends to an average of 70 patients every week. Ethical approval for the study was obtained from Research and Ethics Committee of the ESUTH, Enugu before the commencement of the study (ESUTHP/C-MAC/RA/034/104; 11th April 2019). Information about cleaning of hospital surfaces was obtained from the cleaning staff of the emergency room.

Data collection and laboratory methods: The sample size was 118 taking into account the number of Bed surfaces (7 side bed-rails and 4 foot bed-rails), Electrical appliances (11 fan switches, 8 light switches, 1 X-Ray viewing box switch, 3 suction machine plug-ins, 2 oxygen concentrator plug-ins, 2 nebulizer plug-ins, 1 electric kettle plug-in, 1 X-Ray viewing box plug-in, 1 drugs refrigerator handle), Furniture surfaces(6 desks surfaces, 10 chairs arm-rests, 3 table drawer handles, 7 bedside tables), Door knobs(14),Wall/floor surfaces (n=25) (8 wall surfaces, 5 floor surfaces, 4 curtain poles, 8 pillars), and Portable medical apps n=12 (4 drip stands, 3 "veronica" bucket taps, 1 bassinet scale, 1 food trolley, 2 ward stethoscope, 1 ward mercury in-glass thermometer) in the ward. See table 1.

Table 1: Swabbed surfaces

Surface	Number
Bed surfaces	11
Electrical appliances	30
Furniture surfaces	28
Door knobs	14
Portable medical appliances	12
Wall/ floor surface	25

Samples were collected from the surfaces using sterile cotton swabs moistened with normal saline (0.9% w/v). Collection commenced in the morning, two hours after routine cleaning. The samples were sent to Spectrum Diagnostic and Research Laboratories (Proficiency testing by European Society for External Quality Assessment (ESfEQA) and inoculated into Cystine–lactose–electrolyte-deficient agar (CLED), Salmonella Shigella Agar and blood agar plates. The inoculated agar plates were incubated at 37^{o}C for 24 – 48hrs for primary bacterial isolation. Distinct bacteria strains were selected on the basis of colony, morphology, gram staining, lactose fermentation, coagulase and or catalase test. Full identification of the bacteria was done by conventional biochemical tests depending on the organism isolated. Antimicrobial susceptibility testing was determined for each isolate by the Agar diffusion method using Standard Nutrient Agar 1 discs containing the following antibiotics: Cefoxitin (30µg), Ciprofloxacin (5µg), Erythromycin (5µg), Gentamicin (10µg), Amoxicillin-Clavulanate (30µg), Ampicillin (10µg), Nitrofurantoin (300µg), Levofloxacin (5µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefuroxime(30µg), Ofloxacin (5µg), Ofloxacin+Ornidazole (10µg), Cefixime+Clavulanate (30µg), Cloxacillin (5µg). Methicillin resistant *Staphylococcus aureus* (MRSA) was detected by Cefoxitin disc using diffusion method; the Cefoxitin disc was produced by Oxoid Ltd. Company, UK.

Data Analysis and Interpretation: The data obtained were analysed using SPSS version 20.0 (Chicago IL). Frequency distribution statistical analysis was used to compute the results. Results were presented in prose, tables and charts.

Results

Out of 118 samples collected from various sites, bacterial growth was observed in 83 (70.3%) specimens while the remaining 35(29.7%) did not show bacterial growth. A total of 101 bacterial isolates were cultured from the 83 sites. Mixed bacterial flora were isolated from various sites. There were 72.2% gram positive bacteria and 27.8% gram negative bacteria. *Staphylococcus aureus* was the most common isolate cultured from 54 different sites. Details of specimen and bacterial isolates are depicted in Table 2. Majority of Staphylococcus aureus isolates were from furniture surfaces 27.8% (15/54), followed by walls/floor surfaces 24.1% (13/54), bed surfaces 14.8% (8/54), electrical appliances 14.8% (8/54), door knobs 9.3% (5/54) and portable medical appliances 9.3% (5/54). Among *Staphylococcus aureus* isolates, 25.9% (14/54) were MRSA and remaining were methicillin-susceptible *Staphylococcus aureus* (MSSA). The antimicrobial resistance pattern of Staphylococcus aureus isolates is shown in Table 3. All the isolates of *Staphylococcus aureus* were susceptible to Levofloxacin and Cefixime+Clavulanate. Among Gram negative bacteria, Escherichia coli, *Klebsiella* species and *Proteus* species were isolated. All the Gram negative isolates were susceptible Ciprofloxacin and Ceftriaxone. The antimicrobial resistance pattern of Gram negative isolates is depicted in Table 4. Figure 1 shows the overall distribution of the bacterial isolates.

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Table 2: Swabbed Surfaces and their bacterial isolates

Swabbed Surfaces		*S. Aureus* (n=54)	*E. Coli* (n=14)	*Klebsiella* S. (n=13)	*CoNS** (n=19)	*Proteus* S. (n=1)	Total
Bed surfaces	11	8 (14.8%)	1 (7.1%)	1 (7.7%)	1 (5.3%)	-	11(100%)
Electrical apps	30	8 (14.8%)	2 (14.3%)	7 (53.8%)	7 (36.8%)	1 (100%)	25 (83.3%)
Furniture	26	15 (27.8%)	5 (35.7%)	3 (23.1%)	5 (26.3%)	-	28 (107.7)
Door knobs	14	5(9.3%)	2 (14.3%)	1 (7.7%)	-	-	8 (57.1)
Wall/floor surfaces	25	13 (24.1%)	3 (21.4%)	-	4 (21.1%)	-	20 (80%)
Portable medical appliances	12	5 (9.3%)	1 (7.1%)	1 (7.7%)	2 (10.5%)	-	9 (75%)

*Coagulase-negative staphylococci (CoNS)

Table 3: Antibiotic resistance pattern of *Staphylococcus aureus* (MRSA and MSSA) isolates

Antibiotics	*S. aureus* isolates (n=54) Frequency (%)	MRSA isolates (n=14) Frequency (%)	MSSA isolates (n=40) Frequency (%)
Augmentin	1	0	1 (100)
Ofloxacin	8	2 (25)	6 (75)
Cloxacillin	9	6 (66.7)	3 (33.3)
Erythromycin	29	7 (24.1)	22 (75.9)
Ceftriaxone	3	3 (100)	0
Gentamycin	2	2 (100)	0
Cefuroxime	1	1 (100)	0
Ceftazidime	45	12 (26.7)	33 (73.3)
Oflox + Ornidazole	0	0	0
Levofloxacin	0	0	0
Cefixime + Clavulanate	0	0	0

Table 4: Antibiotic resistance pattern of Gram negative isolates

Antibiotics	*E. coli* n=14 (%)	*Klebsiella* n=13 (%)	*Proteus* n=1
Ampicillin	8 (57.1)	8 (61.5%)	0
Nitrofurantoin	1 (7.1%)	0	0
Gentamycin	1 (7.1%)	0	0
Cefuroxime	1 (7.1%)	3 (23.1%)	0
Ceftazidime	2 (14.3%)	1 (7.7%)	0
Ciprofloxacin	0	0	0
Ceftriaxone	0	0	0

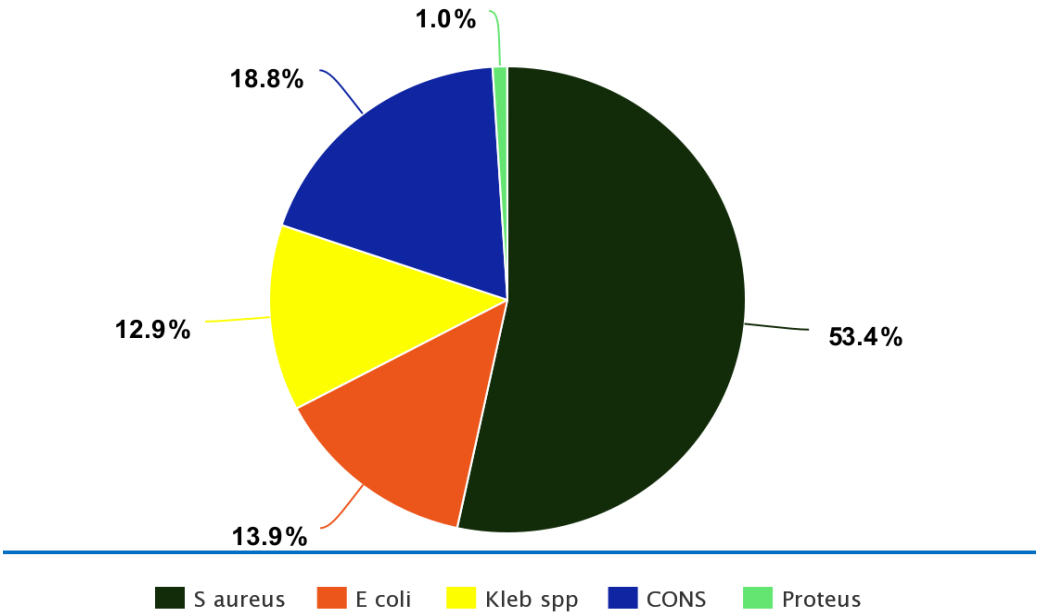

Figure 1: Bacterial isolates

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Information obtained from the cleaning staff of the emergency room, revealed that the floors are mopped twice or thrice a day with detergent solution and occasionally with bleach. There are no standardized practices of cleaning/disinfecting table tops and benches, surface of electrical appliances, wall surfaces, ceilings, door knobs and portable medical appliances. The cleaning staff received no periodic training or assessment for competence.

Discussion

The bacteriological profile of surfaces and commonly used equipment in this study, yielded a wide range of organism including *Staphylococcus aureus*, Coagulase negative staphylococcus (CoNS), *Escherichia coli (E. coli), Klebsiella species, and Proteus species.* The overall prevalence of bacterial contamination of hospital surfaces and equipment was 70.3%. This is similar to results obtained by Saka et al. (69.7%), Okon et al. (70%) and Bhatta et al. (78%) at Ilorin, Maiduguri and Nepal respectively (5,8,12). However, it was significantly higher than the result obtained from Ethiopia (43.8%) by Worku et al. (3). The difference may be explained by the presence of sanitary team leaders who supervise sanitary teams employed by the government of Ethiopia, to ensure disinfection of hospital surfaces.

The predominant bacterial contaminant in this study was *Staphylococcus aureus* accounting for 53.4% of the organism isolated followed by CoNS. It was the most frequent isolate on all the surfaces and equipment cultured. Similarly, Saka et al. (5), Bhatta et al. (8), and Muhammed et al. (13) reported predominance of *Staphylococcus aureus* in their various studies at Ilorin, Sokoto and Nepal respectively. The higher prevalence of *Staphylococcus aureus* may be due to ubiquitous distribution in human body as part of the normal flora of the anterior nares, nasopharynx, and the skin (21). Also *Staphylococcus aureus* has predilection for inanimate surfaces and is relatively resistant to drying, heat and sodium chloride (5). On the other hand, Okon et al. (12) reported a predominance CoNS at Maiduguri, and Gracia- cruz et al. (22) in Mexico reported predominance of *Klebsiella spp*. Coagulase negative staphylococcus was the second most common isolate in this study, accounting for 18.8% of isolates. Because CoNS may contaminate clinical specimens, care has to be exercised in assessing its significance especially from superficial sites (23). However, it has been reported as the most common cause of colonization of central lines and hence central line associated blood stream infections (24). Furthermore, it has been found to cause pronounced systemic infections in immunocompromised hosts (23). Of the gram negative organisms identified in this study, *Escherichia coli* was the most common isolate accounting for 13.9%. Others were *Klebsiella spp* (12.9%) and *Proteus spp* (1%). Similar studies have shown the presence of *Escherichia coli, Klebsiella spp, and Proteus spp* on inanimate objects in hospitals (5,3,13). These organisms especially *Escherichia coli* are well implicated in nosocomial infections (25).

Furniture surfaces yielded the most isolates. These surfaces are frequented by patients, visitors and health care workers making for easy spread of the potential pathogens among unsuspecting users. 53.5% (15/28) specimens collected from furniture surfaces showed growth of *Staphylococcus aureus* and 17.8% (5/28) yielded *Escherichia coli*. Bed rails contamination rate was 100% (11/11) of which *Staphylococcus aureus* was the most predominant isolate. Similarly, high bed rails contamination rate was reported by Saka et al. (5).

Door handle contamination in this study was recorded to be 57.8% (8/14). The implicated organisms were *Staphylococcus aureus. Escherichia coli*, and *Klebsiella spp*. Similarly, Odigie et al. (18) reported *Staphylococcus aureus*, *Pseudomonas spp*, and *Escherichia coli* as common isolates from door handles. The risk of transmission of pathogens through door handles is increased as nursing staff, clinicians, and visitors frequently touch them (8). Majority of the medical devices in the present study yielded bacterial growth with *Staphylococcus aureus* being the most predominant isolate. Similarly, Uneke et al. (16) and Shiferaw et al. (26) reported a prevalence of 79% and 87% respectively. This is not surprising as it has been reported that health care workers and medical students have been found not to follow standard cleaning protocols for medical equipment such stethoscopes (3).

There was a high percentage of ceftazidime resistant *Staphylococcus aureus* (83.3%). Although there is paucity of data in clinical settings about *Staphylococcus aureus* resistance to ceftazidime, in vitro studies have shown that ceftazidime is less active against *Staphylococcus aureus* (27). Among *Staphylococcus aureus* isolates, 25.9% were MRSA. The presence of MRSA is a source of concern because of the risk of MRSA associated nosocomial and community infections. The organism can survive for days in hospital settings due to the ability to form biofilm on inanimate objects which prolongs their survival and spread (8,20). All the cultured gram negative organisms were sensitive to Ciprofloxacin and Ceftriaxone. There was no documented resistance of *Staphylococcus aureus* to Levofloxacin and Ofloxacin+Ornidazole. A high rate of erythromycin resistant *Staphylococcus aureus* was also demonstrated in this study (53.7%). Similarly, Bhatta et al (8) reported 56.8% resistance to erythromycin by *Staphylococcus aureus*. This resistance to erythromycin may be explained by the use of other macrolides to treat infections caused by bacteria other than *Staphylococcus aureus*. Thus commensal staphylococci in humans will be exposed to macrolides and this may contribute to erythromycin resistance being commonly encountered in clinical isolates (28).

Ampicillin resistance of the gram negatives was significantly high with 57.1% resistance documented by *Escherichia coli*. A similar result was obtained by Hammuel et al. (14). This resistance to ampicillin is due to production of β-lactamase enzyme by *Escherichia coli* and it is a major therapeutic challenge today in the treatment of hospitalized and community-based patients (29).

Standard cleaning protocols for hospitals emphasize consistency and thorough scrubbing of surfaces. For instance, the South Australian health cleaning policy

requires daily cleaning with detergent and disinfectant of all elements in a patient's room, spot cleaning of walls and ceiling as spills occur (30). However, there are no standard cleaning protocols for most hospitals in Nigeria. Based on information obtained from the cleaning staff in the present study, there was no awareness of established cleaning protocols and the need for periodic training or assessment programs. They were also unaware of the need for consistency and thoroughness while cleaning surfaces. Worku et al (3). in Ethiopia documented similar findings by members of the sanitary team who had no idea of regular cleaning and disinfection of walls and ceilings except in situations where there was visible contamination. It has been recommended that cleaning staff be properly trained and knowledgeable in hygiene matters appropriate to their work activities with yearly assessments for competency (30).

Conclusion

Staphylococcus aureus, Coagulase negative staphylococcus and Escherichia coli were the commonest isolates. TheSre was high ampicillin resistance by gram negatives but all the Gram negative isolates were susceptible to Ciprofloxacin and Ceftriaxone. There were no established cleaning protocols or periodic assessment of cleaning staff to identify competency issues. More efforts are needed to ensure improved hygiene standards in order to control the contamination of surfaces and reduce the burden of nosocomial infections.

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Dynamic thiol/disulfide homeostasis in gestational diabetes mellitus: Is it related with adverse perinatal outcomes?

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Abstract

Objective: To specify the significance of thiol/disulfide homeostasis in the aspect of gestational diabetes mellitus (GDM) and GDM-related complications.

Material and Methods: This study is a prospective review of the data of 61 healthy and non-pregnant women, 58 healthy pregnant women, and 62 pregnant women with GDM.

Results: The patients with gestational diabetes mellitus had significantly higher disulfide/native thiol and disulfide/total thiol concentrations than non-pregnant patients ($p<0.001$ for both) and healthy pregnant patients (p: 0.015 and p: 0.018, respectively). Besides, in GDM group had significantly lower native thiol/total thiol concentrations than non-pregnant patients and healthy pregnant patients ($p<0.001$ and p: 0.016, respectively). There were positive and significant correlations between disulfide levels and HbA1c concentrations (r=0.26, p: 0.042), and between disulfide and oral glucose tolerance test first hour concentrations (r=0.26, p: 0.039). The receiver operating characteristic curve analyses for native thiol, total thiol, and disulfide were unable to predict adverse perinatal outcomes in this cohort.

Conclusion: The significantly higher concentrations of disulfide/native thiol and disulfide/total thiol in women with GDM could be considered as the presence of increased oxidative stress. However, these markers failed to predict adverse perinatal outcomes.

Keywords: gestational diabetes mellitus; oxidative stress; perinatal outcome; pregnancy; thiol/disulfide homeostasis

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first diagnosis in the second or third trimester of pregnancy that is clearly not preexisting diabetes (1). Hyperglycemia caused by GDM is responsible for both maternal, fetal and neonatal complications. In the short term, GDM increases the risk of maternal hypertensive disorders, fetal macrosomia, shoulder dystocia, amniotic fluid anomalies, fetal distress, and cesarean delivery (2). Long term, the risk of developing type 2 diabetes mellitus is increased by 50% in women with GDM (3). Moreover, the offspring of women with GDM are at risk for obesity, glucose intolerance, type 2 diabetes mellitus, and hypertension (4).

Between 24-28th gestational weeks, GDM screening is recommended to all pregnant women who have not been diagnosed with pregestational diabetes after the first antenatal visit.

While only two-step screening tests were used until 2010, after 2010, GDM screening is performed in many centers using single-step 75 g oral glucose tolerance test (OGTT) with the recommendations of 'The International Association of Diabetes and Pregnancy Study Groups (IADPSG)' (5-7). The prevalence of GDM in Turkey varies between 1.2% and 27.9%, depending on the geographic location of the study and the diagnostic tests used (8). Compared with previous years, the prevalence of GDM is increasing due to increased obesity and advanced maternal age all around the world (9).

Previous studies have shown a relationship between oxidative stress and gestational diabetes (3,4). Failure to maintain the balance between the oxidant and antioxidant production of biologic systems results in oxidative stress.

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Pregnancy is one of the situations in which this balance is impaired in favor of oxidants because oxygen consumption in pregnancy increases significantly and excessive amounts of free oxygen radicals are produced in the mitochondrial rich placenta (10). Pregnant women with GDM are exposed to more oxidative stress than healthy pregnant women due to the excess production of reactive oxygen species (ROS) or inadequate protective mechanisms (3,4).

Thiol-disulfide balance is an antioxidant system that protects the cells and minimizes the effects of oxidative damage. In recent years, many studies have reported how thiol/disulfide homeostasis is altered in prediabetes (11), diabetes mellitus (12), and GDM (13). Besides, in the literature, it was reported that decreased native thiol levels predicted adverse pregnancy outcomes in GDM cases diagnosed by two-step diagnostic test (13). In the presence of oxidative stress, thiols react with oxidizing agents and mediate the formation of reversible disulfide bonds between proteins (14). This process mediates the formation of reversible disulfide bonds between proteins. When the oxidative stress is eliminated, the disulfide bonds return to thiol groups again. This cycle preserves dynamic thiol/disulfide homeostasis, which plays an important role in the stabilization of antioxidant defense, apoptosis, and protein structures (15,16).

To the best of our knowledge, there is no study investigating dynamic thiol/disulfide homeostasis in GDM patients diagnosed using a single-step screening test. The aim of this study was to show the changes in dynamic thiol/disulfide homeostasis in GDM cases diagnosed using single-step screening test and to determine whether it was associated with increased adverse pregnancy complications.

Materials and Methods

This multicenter study was performed from July 2018 to November 2018 at the Department of Obstetrics and Gynecology, Izmir Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology, Rize Recep Tayyip Erdogan University Hospital and Department of Clinical Biochemistry, Ankara Yildirim Beyazit University Hospital. The study protocol was undertaken in accordance with the principles of the Declaration of Helsinki and ethical approval was granted (No: 2018/6-13). All participants were asked to sign written informed consent forms.

Women who had diabetes mellitus, multiple pregnancies, fetal anomalies, hypertension, cerebrovascular disease, deep vein thrombosis, pulmonary embolism, hematologic diseases, thyroid or heart disease, chronic liver or renal disease, cancer, autoimmune disorders, inflammatory diseases, and women who smoked and/or consumed alcohol were excluded.

Study design: This is a prospective review of 61 healthy and non-pregnant women, 58 healthy pregnant women, and 62 pregnant women with GDM. All pregnant women admitted to the study centers routinely undergo GDM screening at 24 and 28 weeks using the 75 g OGTT. According to the IADPSG criteria, GDM is diagnosed when at least one of the following conditions are present: fasting glucose concentration higher than 92 mg/dl, 1-hour glucose concentration higher than 180 mg/dl, and/or 2-hour glucose concentration higher than 153 mg/dl (5). The patients in group 1 were consecutively recruited from healthy and non-pregnant women who were admitted to the antenatal polyclinic during the study period. The healthy pregnant women (group 2) and pregnant women with GDM (group 3) were consecutively recruited from patients who underwent the 75 g OGTT for screening gestational diabetes.

Anthropometric measurements were performed with light clothing and no shoes. In each center, all subjects' height and weight measurements were performed by the same qualified researcher using a weekly calibrated weighing scale. Body mass index (BMI) (kg/m2) was calculated by dividing weight (in kilograms) by the square of height (in meters). A calibrated mercury sphygmomanometer was used to measure systolic and diastolic blood pressures. The gestational age of the participants was verified with first trimester ultrasonography. Any adverse pregnancy outcomes, including polyhydramnios, preterm delivery, small for gestational age (SGA), macrosomia, intrauterine growth restriction (IUGR), preeclampsia, need for neonatal intensive care unit, and postpartum hemorrhage were recorded.

Measurement of serum thiol/disulfide homeostasis levels: All peripheral blood samples were collected between 08:00 AM and 10:00 AM after 10-12 hours of fasting, from the antecubital vein using a 20-gauge needle. Blood samples were quickly centrifuged at 1500 rpm for 10 minutes to determine thiol/disulfide hemostasis parameters. Plasma and serum samples were then separated. Serum samples were collected at -80°C until the thiol/disulfide hemostasis measurements were analyzed.

A simple new fully automated colorimetric method was applied to evaluate the serum concentrations of native and total thiol and the ratio of disulfide to native and total thiol. This method is similar to the method developed by Erel and Neselioglu, where dynamic disulfide bonds are reduced by sodium borohydrate to functional thiol groups (17). Serum samples were automatically performed by a clinical chemistry analyzer (Roche, Cobas 501, Mannheim, Germany). The results are given as µmol/L. Using this method, the concentrations of natural thiol, total thiol, and disulfide were determined. Then, the disulfide-natural thiol, disulfide-total thiol, and natural thiol percentages were calculated in all groups.

Statistical analysis: Collected data were analyzed using the Statistical Package for the Social Sciences version 25.0 (SPSS IBM, Armonk, NY, USA). Descriptive statistics are presented as mean ± standard deviations, frequency distributions, and percentages. The Chi-square test was used in the analysis of categorical variables. The normality of distribution of the variables was tested using the Kolmogorov–Smirnov or Shapiro–Wilk test. Equality of variances was checked using the Levene test. One-way analysis of variance, Welch analysis of variance, and the Kruskal–Wallis test were used to determine the significant differences between the three groups. Post hoc tests for

pairwise comparisons were also performed. The Pearson test was used to investigate the correlations among variables. The optimal cut-off points for thiol/disulfide homeostasis parameters in distinguishing the adverse pregnancy outcomes of patients with GDM were further evaluated using receiver operating characteristic curve (ROC) analyses. A probability level of p<.005 was considered to be statistically significant.

Results

The baseline demographic, anthropometric, and biochemical characteristics of the groups are summarized in Table 1. The patients in group 1 were significantly older than those in group 2 and group 3 (p: 0.001 and p: 0.016, respectively). The patients in group 1 had significantly lower BMI than patients in group 3 (p: 0.006). The patients in group 1 had significantly lower waist circumferences than those in group 2 and group 3 (p: 0.001 and p<0.001, respectively).

The patients in group 3 had significantly higher fasting plasma glucose, OGTT first hour, OGTT second hour, HbA1c and CRP concentrations than those of patients in group 2 (p<0.001, p<0.001, p<0.001, p: 0.001, and p: 0.001, respectively). The outcomes related with thiol/disulfide homeostasis are listed in figure 1. Group 1 had significantly higher native thiol and total thiol concentrations than group 2 and group 3 (p<0.001 for all). Group 3 had significantly higher disulfide concentrations than group 1 (p: 0.04). Group 1 had significantly lower disulfide/native thiol and disulfide/total thiol concentrations than group 2 (p: 0.016 and p: 0.009, respectively). Group 1 and group 2 had significantly lower disulfide/native thiol and disulfide/total thiol concentrations than group 3 (p<0.001, p<0.001, p: 0.015, and p: 0.018, respectively). Group 1 had significantly higher native thiol/total thiol concentrations than group 2 (p: 0.013). Group 3 had significantly lower native thiol/total thiol concentrations than group 1 and group 2 (p<0.001 and p: 0.016, respectively).

Table 1. Demographic, anthropometric and biochemical characteristics of groups (mean ± SD)

	Healthy women (n=61)	Healthy pregnant (n=58)	GDM (n=62)	p
Age,years	35.4±10.0	28.7±6.1	31.7±5.0	<0.001* <0.001$^{\alpha}$, 0.016$^{\beta}$, 0.068$^{\Upsilon}$
Body mass index (kg/m^2)	25.20±4.47	25.78±3.73	27.49±3.93	0.006* 0.712$^{\alpha}$, 0.006$^{\beta}$, 0.058$^{\Upsilon}$
Systolic blood pressure (mmHg)	109.3±9.6	109.4±12.6	108.3±10.4	0.834*
Diastolic blood pressure (mm Hg)	66.9±9.1	67.8±9.6	66.3±8.7	0.677*
Waist circumference (cm)	74.7±7.2	79.2±7.4	81.1±5.9	<0.001* 0.001$^{\alpha}$, <0.001$^{\beta}$, 0.299$^{\Upsilon}$
Fasting plasma glucose (mmol/L)	89.4±10.3	78.0±8.95	91.76±16.2	<0.001* <0.001$^{\alpha}$, 0.541$^{\beta}$, <0.001$^{\Upsilon}$
Oral glucose tolerance test 1st hour (mmol/L)	123.2±33.2	119.9±25.1	206.2±29.1	<0.001* 0.807$^{\alpha}$, <0.001$^{\beta}$, <0.001$^{\Upsilon}$
Oral glucose tolerance test 2nd hour (mmol/L)	98.5±23.9	95.7±21.8	167.1±29.2	<0.001* 0.813$^{\alpha}$, <0.001$^{\beta}$, <0.001$^{\Upsilon}$
HbA1c (%)	5.26±0.42	5.10±0.38	5.38±0.42	0.001* 0.074$^{\alpha}$, 0.278$^{\beta}$, <0.001$^{\Upsilon}$
C-reactive protein (mg)	0.37±0.33	0.61±0.60	0.99±0.67	<0.001* 0.051$^{\alpha}$, <0.001$^{\beta}$, 0.001$^{\Upsilon}$
Creatinine (µmol/L)	0.59±0.10	0.54±0.11	0.56±0.12	0.041* 0.034$^{\alpha}$, 0.248$^{\beta}$, 0.614$^{\Upsilon}$
Blood urea nitrogen (mg/dl)	21.02±9.36	15.39±4.35	13.88±4.49	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.416$^{\Upsilon}$
Albumin (g/L)	6.06±1.05	3.75±1.22	3.52±0.99	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.480$^{\Upsilon}$
Total cholesterol (mmol/L)	184.0±33.6	232.8±47.1	234.3±55.4	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.980$^{\Upsilon}$
Triglyceride (mmol/L)	97.6±46.1	184.6±69.3	234.6±84.5	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, < 0.001$^{\Upsilon}$
High density lipoprotein (mmol/L)	17.2±2.2	13.5±1.8	12.1±1.5	<0.001* <0.001$^{\alpha}$, 0.014$^{\beta}$, 0.036$^{\Upsilon}$
Low density lipoprotein (mmol/L)	107.7±27.6	120.8±44.3	128.2±54.3	0.032* 0.231$^{\alpha}$, 0.026$^{\beta}$, 0.615$^{\Upsilon}$

Group 1; healthy women, group 2; healthy pregnant, group 3; pregnant with GDM. α Statistical significance between group 1 and group 2, β Statistical significance between group 1 and group 3, γ Statistical significance between group 2 and group 3, *p<0.05 was accepted to be statistically significant

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The perinatal outcomes of group 2 and group 3 are displayed in Table 2. The weight gain during pregnancy, birthweight, and macrosomia were significantly higher in group 3 than in group 2 (p: 0.006, p<0.001, and p<0.001, respectively). There were no cases of preeclampsia, fetal anomaly or neonatal death in groups.

There were positive and significant correlations between disulfide and HbA1c concentrations (r=0.26, p: 0.042), and between disulfide levels and OGTT first hour concentrations (r=0.26, p: 0.039) (Table 3).

The area under the ROC curve for native thiol was 0.46 (95% CI: (0.304-0.615), p: 0.622). The area under the curve for total thiol was 0.478 (95% CI: (0.322-0.635), p: 0.789). The area under the curve for disulfide was 0.532 (95% CI: (0.362-0.701), p: 0.694). These values were unable to predict adverse perinatal outcomes in this cohort.

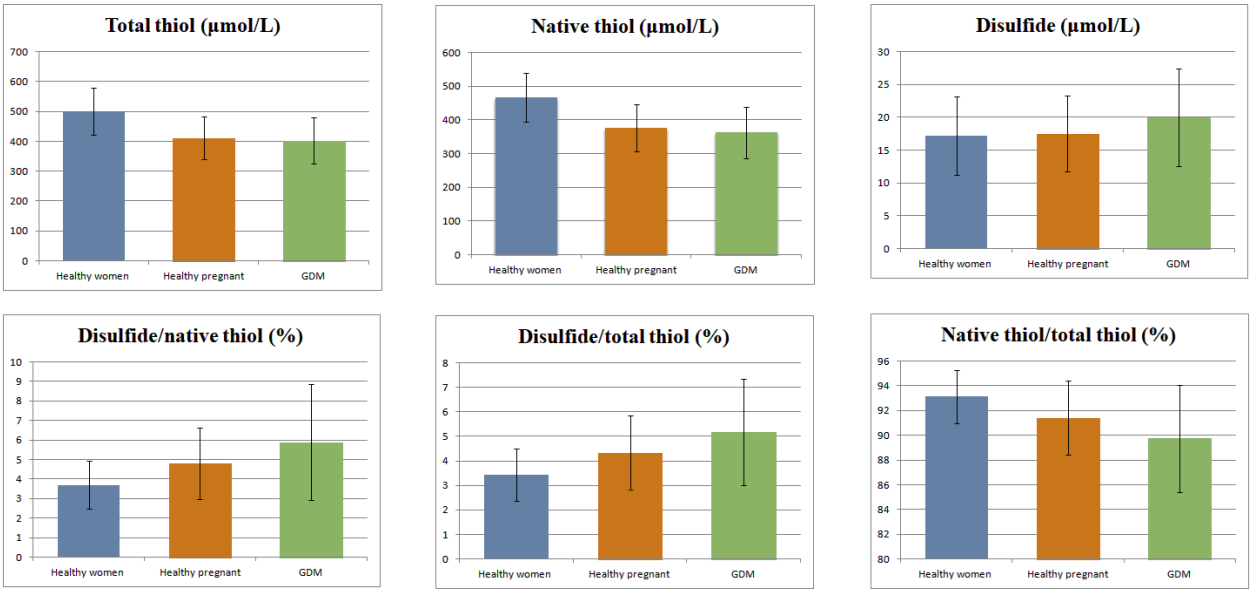

Figure 1. Thiol/disulfide homeostasis of groups (mean ± SD)

Table 2. Perinatal outcomes of GDM and healthy groups (mean ± SD)

	Healthy pregnant (n=58)	GDM (n=62)	p
Gravidity (n)	1.6±0.7	1.7±0.5	0.277
Weight gain (kg)	6.79±4.53	9.24±5.09	0.006
Route of delivery (n, %)			
Vaginal delivery	42 (72.4)	41 (66.1)	0.456
Caesarian section	16 (27.6)	21 (33.9)	
Gestational age at birth (weeks)	38.4±1.3	38.6±1.3	0.528
Birthweight (g)	3184.5±300	3693.6±373.7	<0.001*
Female newborns (n, %)	30 (51.7)	31 (50.0)	0.850
Apgar (1st minute)			
<7	10 (17.2)	9 (14.5)	0.683
≥7	48 (82.8)	53 (85.5)	
Apgar (5th minute)			
<7	6 (10.3)	3 (4.8)	0.252
≥7	52 (89.7)	59 (95.2)	
Polyhydramnios (n, %)	0 (0.0)	5 (8.1)	0.058
Macrosomia (≥4000 g) (n, %)	0 (0.0)	15 (24.2)	<0.001*
Small for gestational age (n, %)	4 (6.9)	3 (4.8)	0.631
Intrauterine growth restriction (n, %)	5 (8.6)	3 (4.8)	0.481
Postpartum hemorrhage (n, %)	7 (12.1)	4 (6.5)	0.352
Postpartum hemorrhage (n, %)	1 (1.7)	3 (4.8)	0.619
Need for neonatal intensive care unit (n, %)	3 (5.2)	2 (3.2)	0.672

*p<0.05 was accepted to be statistically significant

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Table 3. Correlations among variables

	HbA1c	FPG	OGTT 1st hour	Insulin
Native Thiol				
Group 1	r=0.21, p: 0.103	r=0.10, p: 0.437	r= -0.05, p: 0.703	r=0.02, p: 0.855
Group 2	r= -0.04, p: 0.766	r=0.01, p: 0.922	r=0.11, p: 0.406	r=0.01, p: 0.969
Group 3	r= -0.07, p: 0.573	r=0.02, p: 0.910	r=0.05, p: 0.683	r=0.21, p: 0.106
Total Thiol				
Group 1	r=0.19, p: 0.139	r=0.13, p: 0.338	r=0.07, p: 0.159	r=0.04, p: 0.735
Group 2	r= -0.06, p: 0.655	r=0.01, p: 0.969	r=0.08, p: 0.539	r=0.01, p: 0.959
Group 3	r= -0.02, p: 0.860	r=0.03, p: 0.821	r=0.10, p: 0.425	r=0.19, p: 0.903
Disulfide				
Group 1	r= -0.04, p: 0.785	r=0.20, p: 0.129	r=0.16, p: 0.221	r=0.14, p: 0.279
Group 2	r=0.13, p: 0.334	r= -0.04, p: 0.767	r= -0.16, p: 0.228	r=0.01, p: 0.964
Group 3	r=0.26, p: 0.042*	r=0.07, p: 0.565	r=0.26, p: 0.039*	r= -0.11, p: 0.406

FPG; fasting plasma glucose, **HbA1c;** glycated hemoglobin, **OGTT 1.** hour; oral glucose tolerance test first hour. **Group 1;** healthy women, **Group 2;** healthy pregnant**, Group 3;** pregnant with GDM, ***p<0.05** was accepted to be statistically significant

Discussion

The placenta is a source of physiologic oxidative stress in normal pregnancy, but also a rich source of antioxidants (18). Therefore, the placenta plays a major role in maintaining the balance between oxidant and antioxidant systems with enzymatic and non-enzymatic scavengers during pregnancy. These moderate changes in oxidative stress are essential for the maintenance of pregnancy (4, 19). In our study, the women with uncomplicated pregnancies had significantly higher disulfide/native thiol and disulfide/total thiol ratios and significantly lower native thiol/total thiol ratios than healthy women. This finding indicates that oxidative stress is relatively increased in healthy pregnancies.

It has been reported that GDM is associated with excessive oxidative stress, which can be attributed to the overproduction of free radicals and interruption of anti-oxidant defense mechanisms within the placenta. Karacay et al. assessed maternal oxidative damage and anti-oxidant status by measuring lipid peroxidation products, protein oxidation markers, myeloperoxidase and lipid hydroperoxidase between 24-36 weeks of gestation (19). It was found that oxidative markers were significantly increased and anti-oxidant status was significantly reduced in GDM (19). Another study investigated the oxidative stress level during the second and third trimester of pregnancy in patients with GDM (20). It was specified that lipid peroxidation and protein oxidative damage was significantly increased in patients with GDM compared with healthy pregnant women (20). Yildirim et al. observed significantly higher disulfide concentrations and reduced thiol concentrations in patients who were diagnosed as having GDM according to two-step antenatal diabetes screening (21). In our study, disulfide/native thiol and disulfide/total thiol ratios were significantly higher and native thiol/total thiol ratios were significantly lower in patients with GDM.

Moreover, there was a significant and positive correlation between disulfide and HbA1c concentrations and between disulfide and OGTT first hour concentrations. This finding implies that increased oxidative stress might participate in the pathogenesis of GDM.

It is a known fact that increased oxidative stress triggers chronic inflammation through a very complex mechanism consisting of inflammatory mediators such as adhesion molecules and interleukins (22). The pathophysiology of GDM-associated complications is not clearly understood, but the positive feedback cycle involving oxidative stress and chronic systemic inflammation probably plays an important role (23-25). Ozler et al. investigated the predictive power of thiol/disulfide homeostasis parameters for perinatal complications in patients with GDM who were diagnosed with the two-step protocol (13). It was revealed that disulfide concentrations, and disulfide/native thiol and disulfide/total thiol ratios were significantly increased and native thiol/total thiol ratios were significantly decreased in the cord blood of babies born to women with GDM (13). In addition, patients with GDM who had high BMIs before pregnancy were found to have decreased native thiol concentrations at 24-28 weeks of pregnancy and an increased risk for adverse perinatal outcomes (13). Rueangdetnarong et al. assessed an oxidative stress marker (isoprostane) and an inflammatory marker (tumor necrosis factor-α) in GDM (26). Although these markers were increased in patients with GDM during the 24th to 28th weeks of pregnancy, the concentrations of the aforementioned markers in fetal cord blood were statistically similar to those of healthy controls (26). In addition, all perinatal and neonatal outcomes were statistically comparable despite the increase in oxidative stress (26). In our study, thiol/disulfide homeostasis parameters determined during the 24th-28th weeks of gestation failed to predict adverse pregnancy outcomes in patients with GDM, except fetal macrosomia.

This study was designed to evaluate the possible role of thiol/disulfide homeostasis in the occurrence of GDM-related complications. To the best of our knowledge, this is the first study to specify the status of oxidative stress by evaluating thiol/disulfide homeostasis in patients with GDM whose diabetes was diagnosed according to IADPSG criteria. The inclusion of healthy non-pregnant women and healthy pregnant women as control groups might provide an advantage for this study. However, this study has several limitations. First, the study cohort is relatively small, thus serious adverse perinatal outcomes such as preeclampsia, neonatal death or fetal anomalies were not observed. This makes the study inadequate to comment in terms of pregnancy complications. The second limitation is the use of a single-step test for the diagnosis of GDM in this study. In contrast, similar studies in literature used the two-step test for the diagnosis of GDM. The adoption of different diagnostic tests may lead to variations in the description of perinatal outcomes and their probable relationship with oxidative stress markers. The third limitation is the lack of data related with good and poor glycemic control. The effects of diabetic diet and/or insulin treatment on oxidative biomarkers have not been evaluated. The fourth limitation is the absence of histopathologic data indicating the severity of oxidative stress and inflammation in placental tissues. The fifth limitation is the lack of longitudinal data related with oxidative stress markers in the first trimester, last trimester, puerperium or cord blood of newborn.

Conclusion

Pregnancies complicated with GDM had significantly higher concentrations of disulfide/native thiol and disulfide/total thiol and lower concentration of native thiol/total thiol than healthy pregnancies. This finding could be considered as the presence of increased oxidative stress in patients with GDM. However, these markers failed to predict adverse perinatal outcomes. Further research is required to understand the role of oxidative stress in the emergence of GDM-related complications.

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Evaluation of benign acute childhood myositis by ultrasound elastography

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Abstract

Objective: Herein, we aimed to determine the diagnostic contribution of ultrasound elastography (UE) technique to the assessment of muscle stiffness in pediatric patients with myositis.

Material and Methods: This study enrolled 16 patients who presented to our hospital's Pediatric Neurology Outpatient Clinic with the complaint of inability to walk and who had a clinical presentation of benign acute childhood myositis (BACM). The patients were referred to the Radiology Department to undergo muscle ultrasonography (USG), where they underwent UE of the gastrocnemius muscle (GCM).

Results: Children with myositis and healthy children are similar age (7.06 ± 1.52 year (5–11) vs. 7.00 ± 1.59 year (5–11) year) (P: 0.908) and body mass index (BMI) (20.04 ± 1.58 (18.6–24.2) vs. 22.08 ± 1.43 (19.9–24.4) (P: 0.946). The mean serum creatine kinase (CK) was measured as 1520.3 ± 1163.6 U/L (min: 456, max:4100) in children with myositis. In the children with myositis, the thickness of the medial and lateral GCM increased compared with that in control group (medial; 18.15 ± 3.02 mm vs 13.10 ± 2.26 mm, p<0.001, lateral; 13.51 ± 3.07 mm vs 9.34 ± 1.86 mm, p<0.001). The medial and lateral GCM ratio in group 1 was slight bigger than that in group 2 (medial; 1.10 ± 0.37 vs 1.00 ± 0.34, p: 0.274, lateral; 1.22 ± 0.44 vs 1.10 ± 0.29, p: 0.243). GCM strain values were mildly elevated in patients with myositis compared to controls.

Conclusion: In the children with myositis, the thickness of the medial and lateral GCM increased compared with that in control group. GCM strain ratio values were slightly higher in myositis patients compared to the control group. We think that the increase in muscle thickness values is mainly secondary to the edema seen in myositis. In addition, UE is a clinically applicable quantitative analysis for changes in myositis.

Keywords: child, elastography, myositis, ultrasonography

Introduction

Benign acute childhood myositis (BACM) is a benign self-limiting condition affecting school-age children that heals with supportive care without complications, which usually develops suddenly after an episode of upper respiratory tract infection (URTI) and manifests itself with bilateral calf pain and difficulty in walking (1, 2). Although many causes of URTI (respiratory syncytial virus, adenoviruses, herpes simplex virus, Epstein Barr virus, cytomegalovirus, mycoplasma, and rotavirus) may lead to myositis, the disorder most commonly occurs due to influenza viruses (2, 3). It was first described in 1957 by Lundberg among 74 pediatric cases (4). It is more common among boys. Its typical laboratory finding is elevated serum CK level (5, 6). It's important to make its differential diagnosis from Guillain-Barré syndrome and more severe disorders that cause myoglobinuria (6). In acute benign myositis muscle biopsy frequently yields nonspecific findings and thus it is unnecessary (7). Moreover, as myositis may be unevenly distributed in affected muscles among cases requiring a muscle biopsy, muscle imaging is especially useful for determining single or multiple muscle involvement and selecting the biopsy site (8).

The aim of the present study was to measure muscle stiffness with UE technique among children with BCAM and compares it with that of healthy children.

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Material and Methods

This study enrolled a total of 32 children, 16 of whom had been diagnosed with BCAM after being referred by various pediatric clinics to Pediatric Neurology Clinic for inability to walk, and 16 healthy volunteers. Because all participants in this study were younger than 18 years, informed written consent and verbal assent were obtained, respectively, from, parents and children before participation in the study.

Clinical Assessment: Acute benign myositis was diagnosed on the basis of the criteria including sudden-onset bilateral calf pain, inability to walk or walking disturbance, history of URTI prior to and/or during the attack, moderately elevated serum CK level, and the absence of a marked abnormality of the nervous system or in muscle examination. Patients whose clinical condition did not start to improve within 48 hours, those with a family or past history of muscular or rheumatic disease or any systemic disorder, and those with a history of long-term medication use or trauma were excluded.

The patients' demographic information, haemogram, full urine analysis, C-reactive protein (CRP), kidney function tests and serum muscle enzymes around the USG examination, including CK, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were recorded. Both patients and volunteers were instructed to sit still for 30 min before the USG examination.

Imaging Assessments: Axial B-mode USG and real-time elastography (RTE) images were obtained using a digital sonography scanner (Aplio 400, Toshiba Medical Systems Corporation, Otawara, Japan) supplied with SE software with a 12 MHz linear array transducer of the medial and lateral GCM. All measurements were performed by the same radiologist (10 years of experience for ultrasonography and 7 years of experience for strain elastography). Ultrasound gain, depth, focal points and transducer frequency settings were kept constant in all image scans. The thickness of GCM was measured by using an electronic caliper at real-time B-mode USG (Fig.1).

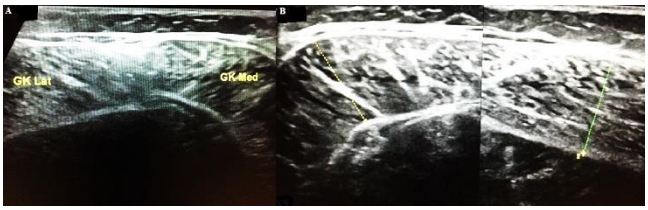

Figure 1: B-mode ultrasound image of lateral (A) and medial (B) gastrocnemius muscle shows the thickness by an electronic caliper.

Manually, RTE image was obtained by applying light repetitive compression in the form of rhythmic compression and relaxation cycles with the transducer. The RTE image appeared as a translucent, colour-coded, real-time image superimposed on the B-mode image. On the elastogram, while areas of low strain were displayed in red, areas of high strain were displayed in blue. More than 3 RTE images were obtained with repetitive compression.

A 4-mm- diameter circular regions of interest (ROI) were used for measuring SR. A 4-mm- diameter circular regions of interest (ROI) were used for measuring SR. For each patient, the ROI of the medial or lateral GCM (A) and the ROI of adjacent subcutaneous fatty tissue (B) were compared and the SR value (B / A) was automatically calculated by the sonography scanner. For each of the three images the mean values were obtained.

Statistical Analysis: Differences between bilateral medial and lateral GCM SR between children with myositis and healthy children was of primary interest. Data were analyzed using SPSS v. 22.0 software (SPSS Inc., Chicago, IL). Variables are expressed as mean ± standard deviation (SD). When parametric test assumptions are provided, Independent Samples T Test was used to compare independent group differences. If the parametric test assumptions were not met, Mann-Whitney U test was used to compare independent group differences. The P value less than 0.05 was considered statistically significant.

Results

A total of 32 children participated in this study: 16 children with myositis and 16 healthy children with typical development. Among 16 patients enrolled in the study, 12 (80%) were male and 4 (20%) were female. The age range was 5-11 years, with a mean age of 7.06 ± 1.52 years. Children with myositis and healthy children are similar age (7.06 ± 1.52 year (5–11) vs. 7.00 ± 1.59 year (5–11) year) (P=0.908) and body mass index (BMI) (20.04 ± 1.58 (18.6–24.2) vs. 22.08 ± 1.43 (19.9–24.4) (P: 0.946). All patients had pain and stiffness in the calf muscles but no muscle weakness. None of the patients had abnormal signs in neurological examination. In all cases signs and symptoms began to improve within 24-48 hours and completed by three day at the latest. The mean serum CK was measured as 1520.3 ± 1163.6 U/L (min: 456, max:4100) in children with myositis.

USG and elastographic images of medial and lateral GCM were taken in both groups and muscle stiffness was assessed with the RTE technique. In the children with myositis, the thickness of the medial and lateral GCM increased compared with that in control group (medial; 18.15 ± 3.02 mm vs 13.10 ± 2.26 mm, p<0.001, lateral; 13.51 ± 3.07 mm vs 9.34 ± 1.86 mm, p<0.001). There was no significant difference between children with myositis and healthy children with typical development for SR. The medial and lateral GCM ratio in group 1 was slight bigger than that in group 2 (medial; 1.10 ± 0.37 vs 1.00 ± 0.34, p:0.274, lateral; 1.22 ± 0.44 vs 1.10 ± 0.29, p:0.243).

While subcutaneous fat predominantly appears as a green/red mosaic, abnormal muscle is stiffer (blue) tissue in an elastogram in the muscles of patients with myositis (Figure 2). Also, there was no significant difference between right and left GCM for SR. Demographic characteristics, thickness of muscle, and SR of children with myositis compared to healthy children with typical development are in Table I.

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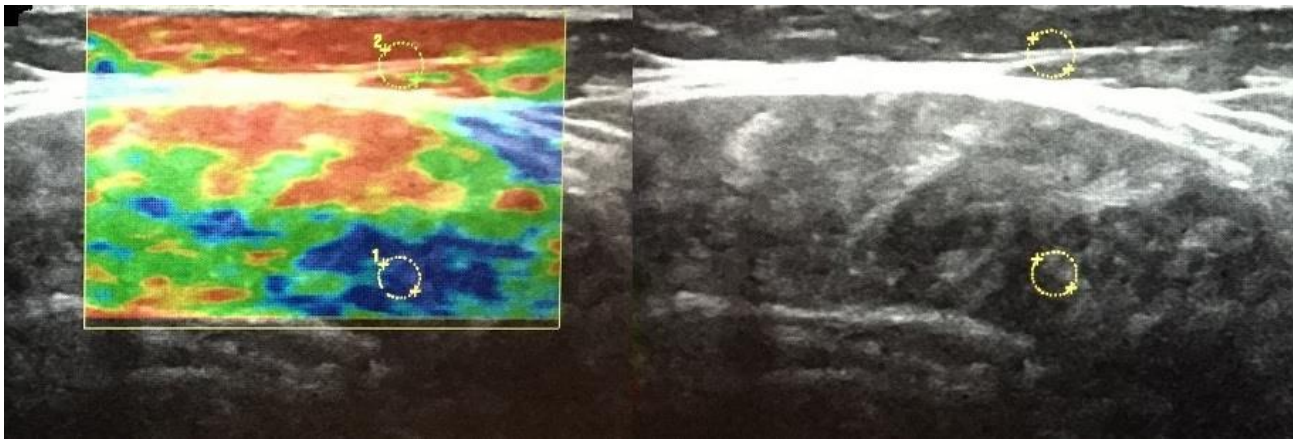

Figure 2: Ultrasound elastography image of gastrocnemius muscle from 9 year-old boy with myositis. Elastogram shows that subcutaneous fat appears green/red. Affected areas of the muscle are stiffer (blue) than normal muscle (green/red).

Table I: Demographic characteristics, thickness of muscle, and strain ratio of children with myositis compared to healthy children with typical development.

	BACM Patients (n=16) mean ± std. dev	Control (n=16) mean ± std. dev	p
Year	7.06 ± 1.52	7.00 ± 1.59	p>0.05
BMI	20.04 ± 1.58	22.08 ± 1.43	p>0.05
Lateral GKM thickness (mm)	13.51 ± 3.07	9.34 ± 1.86	**p<0.05**
Medial GKM thickness (mm)	18.15 ± 3.02	13.10 ± 2.26	**p<0.05**
Lateral GKM strain ratio	1.22 ± 0.44	1.10 ± 0.29	p>0.05
Medial GKM strain ratio	1.10 ± 0.37	1.00 ± 0.34	p>0.05

Discussion

Evaluation of gastrocnemius muscles by UE may be potentially useful for managing myositis by providing quantitative information on disease. In this study, the clinical applicability of elastography method in GC muscles was examined in 16 patients with myositis and 16 healthy controls. In the children with myositis, the thickness of the medial and lateral GCM increased compared with that in control group. We think that the increase in muscle thickness values is mainly secondary to the edema seen in myositis. There was no significant difference between children with myositis and healthy children with typical development for SR. However, GCM strain ratio values were mildly higher among patients with myositis than the controls. Based on these results, we believe that our statistical power was low due to a small sample size. Significant results in strain ratio may be obtained in future studies with larger sample size. UE is a clinically applicable quantitative analysis for changes in myositis.

In acute benign myositis, muscle biopsy commonly yields a nonspecific result and is unnecessary. Bove et al. took biopsy samples from 12 cases of acute benign myositis and showed signs of nonspecific minimal inflammatory infiltration and necrosis in 11 cases and a normal microscopic appearance in one case (7). As myositis may have a heterogeneous distribution, muscle imaging is especially helpful in determining single or multiple muscular involvement and selecting the biopsy site (8).

Thanks to its high spatial resolution, perfusion CT study with intravenous radiopaque contrast agent can assess both muscular and skeletal tissue. However, repeat screening protocols results in the exposure of patients to a substantial amount of radiation. Furthermore, the risk of adverse events with contrast material is higher in CT compared to contrast materials used for MRI and ultrasonography (9).

Advanced magnetic resonance imaging (MRI) techniques can assess a variety of muscular pathologies including acute or chronic muscle injuries, intramuscular collections, and soft tissue masses (10). MRI may also be useful to diagnose focal myositis (11, 12) and other inflammatory myopathies like BCAM that most commonly affects the gastrocnemius and soleus muscles (13, 14). In MRI, muscles with inflammatory edema appear hyper intense in T2 weighted images. Additionally, certain techniques such as fat suppression T2 weighted images or short tau inversion recovery (STIR) series can be used to eliminate fat signal.

Although T2 weighted signal hyper intensities commonly represent edema as a sign of early myositis, this sign may be misleading because it may also be observed in metabolic and traumatic myopathies, neuropathies, muscle dystrophies, myotonic dystrophy, rhabdomyolysis, diabetic muscle infarction, and even after physical exercise (15). However, MRI has some disadvantages such as being affected by motion artifacts, difficulties experienced by

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patients with claustrophobia or loud sound phobia, and a high cost (8).

Compared to computerized tomography (CT), ultrasonography has the advantages of being cheap and easy-to-perform. It has a high spatial resolution and offers an opportunity of real-time imaging without exposure to ionizing radiation (8). A study found no significant difference between muscle USG's sensitivity (83%) than that of electromyography (92%) or serum CK activity (69%) for myositis. That study found that USG had a positive predictive value of 95% and a negative predictive value of 89% (16). Different types of inflammatory myopathies present with typical, although not entirely specific, USG properties. In prolonged muscle disease, muscle mass is reduced due to muscle atrophy and echogenicity increases due to fat infiltration. Nevertheless, echogenicity may also decrease in less active myositis (8). As contrast-enhanced USG can diagnose edema and increased muscle perfusion in many patients with myositis, it can be used to increase diagnostic specificity when edema-like changes were detected in muscles by MRI (16).

UE is a quantitative USG study based on the principle that, depending on the elastic properties of a tissue, stress applied to that tissue causes a change in it. It is cheap, easy to apply, takes short time and has no adverse effect (17). SE is an operator-dependent technique where training and experience are crucial. In this technique, probe movements should be in the same direction and at a regular frequency (18, 19).

UE has been used frequently to evaluate tendons, plantar fascia pathologies and soft tissue masses (20-22). Measurement of passive muscle stiffness is perhaps the simplest measurement method but it should be performed in a standardize manner (23). In children, elastography study performed prior to and after standard exercise found a higher muscle elasticity (24). In contrast, another study in adults reported that muscle elasticity was found to decrease before and after muscle exercise (25). It is difficult to compare these contradictory results with each other due to the inadequate standardized application of strain elastography and the differentiated evaluation of elastograms (20, 26, 27).

This study has some limitations. First, a relatively low number of patients with myositis were studied, and the patients are relatively heterogeneous in regards to age and gender. Therefore the statistical significance is limited. Further studies should preferably be designed with a larger number of cases. Elastography is user-dependent, requires a learning curve to a certain extent, and is flawed by technical problems related to image reproduction due to pressure imbalance applied with free hand technique. To reduce the impact of this problem, SR measurements were performed by a single radiologist. UE is technically very challenging in terms of the proper application of the technique. Experience with technical problems and situations to solve these problems will be guided by the use of free hand compression elastography. Lastly, elastography measurements are not truly quantitative and are considered subjective in nature.

Conclusion

In this study we aimed to establish the diagnostic value of elastography technique in assessing muscle stiffness among patients with myositis. We believe that the increase of muscle strain ratio values is mainly caused by myositis. SE is a promising technique for assessing changes in muscle elasticity. Although studies or case reports have been recently published that assess the elastic properties of musculotendinous tissue, we did not come across any study that specifically examined the role of elastographic examinations in patients with BCAM (28, 29). Although there is a need for more comprehensive studies with larger sample size are needed in this field, we are of the opinion that elastography technique may be a helpful tool in addition to USG findings among patients with myositis. RTE is one of the available elastography techniques and may particularly evolve to become a useful ancillary technique for investigation of muscular disorders.

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Author's Contributions: GG, OG; Research concept and design, Patient examinations, Research the literature, preparation of the article **GG;** Revision of the article.

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Colistin-induced nephrotoxicity and risk factors in intensive care unit: estimating from the routine laboratory findings

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Abstract

Objective: In this study we aimed to evaluate the patients treated with colistin in an intensive care unit (ICU) and risk factors emergence of acute renal failure (ARF) after colistine treatment.

Materials and Methods: Patients treated with colistine in the ICU between June 2016 and September 2018 were reviewed in this retrospective study. The 37 patients who were received colistine more than 3 days due to detection of Acinetobacter baumannii in culture of tracheal aspirate specimen were included in this study. Sociodemographic and clinical data and also biochemical parameters, glomerular filtration rates (GFR), APACHE-II, RIFLE and AKIN scores were examined. Patients were divided into two groups as ARF-developing and non-ARF-developing. Follow - up parameters were compared between these two groups.

Results: The patient group consisted of 26 males and 11 females. The mean age of the patients was 61.0 ± 19.33 years and %45 of the patients developed ARF. Mean APACHE-II score was 20.7±5.6. Mean age was significantly older in ARF patients. Onset day of colistine was significantly lower in patients with ARF. Significant relationships were found with the creatinine, albumin, AST, ALT and BUN parameters between ARF.

Conclusion: Older age and early initiation of colistin treatment in the ICU should be considered to be risky for ARF development. Before colistin treatment BUN, creatinine, CRP, albumin and AST levels should be considered to be risky for ARF development. After colistin treatment ALT, BUN, creatinine, urine output, platelet, AST, arterial blood gas base excess levels, urine pH, and protein amount in urine should be followed carefully.

Keywords: Colistin, nephrotoxicity, acute renal failure, risk factors

Introduction

An acute increase in serum creatinine levels with acute decrease in glomerular filtration rate is defined as acute renal failure (ARF) (1). Determining the cause of kidney damage and early/ rapid intervention for failure is very important to prevent progression of kidney injury (2). ARF is a serious and widespread complication that is between 5 and 7 % of hospitalized patients with a mortality rate of % 50 to 70 (3). The most common causes of ARF in hospitalized patients are drugs, decreased renal perfusion, surgical and radiographic contrast agents. Among the drugs that cause ARF in hospitalized patients; aminoglycosides, nonsteroidal anti-inflammatory drugs, cyclosporine, piperacilin tazobactam, amphotericin B, angiotensin converting enzyme inhibitors, combinations of trimethoprim with sulphonamides are the most common (4). Colistin is a polypeptide antibiotic which has high activity against multiple drug-resistant gram-negative bacteria (5).

Because of the renal toxicity of colistin, clinical use in the past has been nearly abandoned and has been used only for topical applications (5, 6). However, increased multiple drug-resistant infections and carbapenem-resistant gram-negative bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae) over the last ten years have led to an increase in the clinical use of colistin (7, 8).

Nephrotoxicity is defined as; patients develop one of the following criteria's while patients' renal function is normal (serum creatinine of 1.3 mg/dL. in women and 1.5 mg/dL in men):1. Increase in serum creatinine by ≥0.3 mg/dL within 48 h, 2. Increase in serum creatinine to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, 3. Urine volume <0.5 mL/kg/h for 6 h (9).

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Acute renal failure (ARF) is defined as severe acute reduction in kidney function, with severe azotemia in biochemical findings and oliguria or anuria as clinical symptoms frequently (10). In this study we aimed to evaluate the patients treated with colistin in an intensive care unit (ICU) and risk factors emergence of ARF after colistine treatment.

Material and Methods

Patients treated with colistine in the ICU were included in this retrospective study between June 2016 and September 2018 in Sanko University Medical Faculty Training Hospital. The approval for the study was obtained from the local Ethics Committee of Sanko University before collection of data. Written consent was not obtained because of retrospective file review design.

Study population: Patients who were received colistin more than 3 days (we administered colistin as 5mg/kg loading dose and after 2.5 mg/kg twice in a day) due to detection of Acinetobacter baumannii in culture of tracheal aspirate specimen, were included this study.

Parameters: Data of age, gender, rate of arrest, comorbid diseases, intubation situation and duration, glomerular filtration rates (GFR), APACHI-II, RIFLE and AKIN scores, onset day of colistine, positive inotrope usage, treatment response, sepsis situation, furosemide, N.-acetyl cysteine usage, hemodialysis/hemodiafiltration history (Because of the hemodynamic instability we used hemodiafiltration instead of hemodialysis for metabolic acidosis), biochemical parameters, arterial blood gas values were examined. In addition, cardiac arrest and CPR history before ICU charge, the onset day of renal dysfunction after colistine were analyzed. All these parameters and the relationship between renal dysfunction after colistine were evaluated. RIFLE criteria were used to evaluate the renal dysfunction.

Assessment tools: RIFLE (Risk, Injury, Failure, Loss, and End-stage kidney disease) criteria: This scale is used by the clinician and assesses the renal failure injury according to the decrease in GFR rates (11). It's also used to colistine associated nephrotoxicity (12).

APACHE (Acute Physiology, Age, Chronic Health Evaluation): This scale is used by the clinician and assesses the severely ill hospitalized patients. This scale assess the risk estimates for hospital mortality of patients in ICU (13)

Statistical analysis: Descriptive statistics were used to evaluate the demographic characteristics. The Mann-Whitney U Test was used to compare numerical variables that did not have a normal distribution in two groups. The Chi-square test was used in the comparison of categorical variables. Windows version of SPSS 21.0 package software was used in the analyses. $P<0.05$ was considered significant.

Results

One hundred-ten patients had been treated with colistin. Totally 3 patients had been survived. But 37 patients' hospital files were able to include the study. The patient group consisted of 26 males and 11 females. The mean age of the patients was 61.0 ± 19.33 years. Demographic data is shown in the table 1. Two of 37 patients had been diagnosed with acute pancreatitis, 16 of them had been diagnosed with cranial problems (intra-cranial hemorrhages, cerebral infarcts) 5 of them had been diagnosed with cardiopulmonary arrest and 14 of them had been diagnosed with pulmonary diseases (table 1). Pulmonary diseases were more related to ARF. Also ARF was found related to older ages. ARF was seen in male patients more, but it was not statistically significant (Table 2). Seventeen of 37 patients developed ARF. ARF had been developed on average 5th day (15-81 days). Mean APACHE-II score was 20.7±5.6. According to RIFFLE classification 1 patient had end stage renal disease (ESRD), 2 renal failures, 1 renal injury, 6 renal losses and 6 renal risks. All patients had been treated with mechanical ventilator (trans-tracheal intubated or tracheostomy). Mean mechanical ventilator duration under intubation/tracheostomy was 19.05± 20.99 days (Table 1).

There were no significant differences between patient with and without ARF according to gender (Table 2). Mean age was significantly older in patients with ARF then without ARF (Table 3). There were no significant differences between patient with and without ARF according to diagnosis, cardiopulmonary arrest situation, smoking, septic status, inotropic agents, furosemide, opaque substance usage, hemodialysis usage, nutrition type. Hemodiafiltration treatment ratio at first day of ICU was higher in patients with ARF than without ARF. Beginning of the day of colistine was significantly lower in patients with ARF then without ARF. (Table 2).

Relationship between continues variables and ARF had been evaluated at first day, the day before colistin onset, the day after colistin onset, 3rd and 5th days of colistin onset. The patients with ARF had significantly lower albumin and higher BUN, creatinine and CRP levels than patients without ARF at first day (Table 3). The patients with ARF had significantly lower albumin and higher AST levels at the day before colistin onset (table 4). The patients with ARF had significantly higher ALT, BUN, creatinine levels and urine output amount at the day after colistin onset, (Table 5). The patients with ARF had significantly lower albumin and higher AST, BUN, creatinine, platelets levels, and urine output amount at the 3rd day of colistin onset (table 6). The patients with ARF had significantly higher AST, ALT, and BUN, lower arterial blood gas base excess levels, creatinine, and urine pH, protein amount in urine and urine output amount at the 5th day of colistin onset (Table 7). There was no significant differences between patients with and without ARF according to levels of continue variables as total bilirubin, hemoglobin, daily fluid balance, lactate, Na, osmolality, pCO2, PO2, troponin, WBC, urine density, erythrocyte amount of urine in any days of the study. APACHE II scores were higher in ARF group but were not statistically significant. The onset day of colistin was significantly lower in ARF group (table 8, Graph1).

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Table 1. Demographic data and some clinical variables

Variables	Mean ± SD	Median (Min–Max)	Variables	Grup	n (%)
APACHE-II	21.41 ± 6.14	21 (10 - 39)	RIFLE	esrd	1 (6.3%)
Days under intubation	19.05 ± 20.99	14 (0 - 110)		failure	2 (12.5%)
GFR	4769.56 ± 13587.69	101.5 (101.5 - 96.7)		injury	1 (6.3%)
Onset day of colistin	25.08 ± 21.68	17 (6 - 110)		loss	6 (37.5%)
Onset day of acute renal failure	5.24 ± 3.38	5 (1 - 15)		risk	6 (37.5%)
			AKIN	1	6 (37.5%)
				2	2 (12.5%)
				3	8 (50%)
			Gender	M	26 (70.3%)
				F	11 (29.7%)

Table 2. Demographic Data And Some Clinical Variables according to AFR

Variables	Group	AFR-	AFR+	p*
Gender	M	12 (46.2%)	14 (53.8%)	0.262
	F	8 (72.7%)	3 (27.3%)	
Diagnosis in ICU	Gastrointestinal	0 (0.0%)	2 (100.0%)	0.058*
	Cranial	12 (75.0%)	4 (25.0%)	
	Post-CPR	3 (60.0%)	2 (40.0%)	
	Pulmonary	5 (35.7%)	9 (64.3%)	
Cardio-pulmonary resuscitated	No	15 (55.60%)	12 (44.40%)	0.99*
	Yes	5 (50.00%)	5 (50.00%)	
Smoking	No	11 (64.7%)	6 (35.3%)	0.386
	Yes	9 (45.0%)	11 (55.0%)	
Survival	No	1 (50.00%)	1 (50.00%)	0.99*
	Yes	19 (54.30%)	16 (45.70%)	
Inotropic agent usage	No	13 (65.0%)	7 (35.0%)	0.264
	Yes	7 (41.2%)	10 (58.8%)	
Hemodialysis	No	18 (62.1%)	11 (37.9%)	0.109*
	Yes	2 (25.0%)	6 (75.0%)	
Hemodiafiltration	No	19 (63.3%)	11 (36.7%)	**0.033***
	Yes	1 (14.3%)	6 (85.7%)	
Furosemide usage	No	14 (58.3%)	10 (41.7%)	0.716
	Yes	6 (46.2%)	7 (53.8%)	
Radiopaque usage	No	12 (44.4%)	15 (55.6%)	0.073*
	Yes	8 (80.0%)	2 (20.0%)	
p Pearson Chi-Squared Test, p* Fisher Exact Test				

Table 3. Demographic data and other variables

	Mean ± SD		
	Median (Min–Max)		
Acute renal failure	**No (20)**	**Yes (17)**	**p**
APACHE-II	20.7 ± 5.6	22.24 ± 6.81	0.46
	21.5 (13 - 31)	21 (10 - 39)	
Days under intubation	23.65 ± 26.51	13.65 ± 9.96	0.34
	16 (0 - 110)	13 (2 - 47)	
GFR	115.88 ± 51.16	102.44 ± 88.80	0.78
	107.35 (49.6 - 230)	94.1 (49.2 – 43.27)	
Onset day of colistin	29.6 ± 24.75	19.76 ± 16.55	**0.05**
	19 (9 - 110)	15 (6 - 67)	
Age	53.05 ± 20.36	70.35 ± 13.29	**0.01**
	49.5 (15 - 86)	76 (47 - 86)	

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Table 4. Laboratory Findings at The Arrival Of ICU

	Mean ± SD Median (Min–Max)		p
ARF	**NO (20)**	**YES (17)**	
Albumin	3.58 ± 0.76	2.93 ± 0.49	**0.004**
	3.45 (2.4 - 4.9)	3 (2.1 - 4)	
ALT	32.7 ± 35.95	25.71 ± 15.0	0.615
	18 (6 - 131)	25 (6 - 59)	
AST	32.8 ± 30.77	22.59 ± 8.59	0.749
	23 (11 - 139)	19 (9 - 37)	
Daily fluid balance	520.6 ± 581.75	912.69 ± 1307.28	0.604
	575 (-1276 - 1180)	800 (-800 - 4440)	
Base excess	-2.61 ± 4.53	-3.74 ± 4.81	0.467
	-3.2 (-8.6 - 7.1)	-4 (-14.1 - 6.7)	
BUN	19.22 ± 8.19	35.84 ± 21.72	**0.002**
	19.8 (7 - 39)	28.5 (13.6 - 100)	
Bilirubin	0.62 ± 0.33	0.8 ± 0.9	0.830
	0.6 (0.2 - 1.5)	0.5 (0.2 - 4.1)	
CRP	40.06 ± 47.03	106.8 ± 84.55	**0.008**
	19.65 (3 - 174)	91.5 (5.7 - 280)	
Hemoglobin	12.57 ± 3.42	11.97 ± 2.09	0.329
	12.1 (1.8 - 18.1)	11.8 (8.1 - 16.4)	
Potassium	4.07 ± 0.61	4.26 ± 0.58	0.342
	4.05 (2.8 - 5.3)	4.2 (3.3 - 5.5)	
Creatinine	0.81 ± 0.29	1.72 ± 1.49	**0.016**
	0.8 (0.39 - 1.5)	1.1 (0.67 - 5.3)	
Lactate	1.64 ± 0.87	2.09 ± 1.78	0.511
	1.55 (0.5 - 3.6)	1.5 (1 - 8.5)	
Sodium	140.15 ± 4.83	139.65 ± 3.0	0.712
	140 (130 - 152)	140 (135 - 147)	
Osmolality	299.25 ± 64.08	288.45 ± 16.36	0.963
	287.5 (267 - 567)	281 (268 - 333)	
PCO_2	43.28 ± 16.02	40.84 ± 15.12	0.670
	36.5 (26 - 81)	37.5 (20 - 76)	
Platelets	262036.5 ±104871.58	259711.76±131847.97	0.503
	279580 (77800 - 470000)	236000 (81600 - 498000)	
PO_2	131.94 ± 80.75	133.24 ± 84.72	0.951
	114 (57 - 384)	104 (36.3 - 364)	
Troponin	0.09 ± 0.13	0.19 ± 0.62	0.225
	0.02 (0 - 0.4)	0.04 (0.01 - 2.6)	
WBC	12796.38 ± 9237.0	13329.82 ± 8514.33	0.857
	13105 (8.1 - 34600)	13050 (6.9 - 36120)	
Urine output	1613.0 ± 772.91	1264.28 ± 546.97	0.127
	1400 (800 - 4250)	1100 (400 - 2590)	
Urine density	1017.9 ± 12.48	1014.18 ± 7.56	0.492
	1016.5 (1004 - 1055)	1014 (1003 - 1030)	
Urine erythrocyte	78.85 ± 119.74	127.41 ± 136.24	0.175
	0 (0 - 330)	100 (0 - 330)	
Urine pH	6.85 ± 1.03	6.47 ± 0.94	0.253
	7 (5 - 8.5)	6.5 (5 - 8)	
Urine protein	55.5 ± 125.2	41.53 ± 72.44	0.449
	0 (0 - 500)	20 (0 - 300)	

ARF: Acute renal failure, **ICU:** Intensive care unit, **ALT:** The alanine aminotransferase, **AST:** aspartate aminotransferase, **BUN:** Blood urea nitrogen, **CRP:** c - reactive protein, **WBC:** White-blood cell

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Table 5: Laboratory findings at the day before the colistin onset.

	Mean ± SD		p
	Median (Min–Max)		
ARF	**NO (20)**	**YES (17)**	
Albumin	2.77 ± 0.47	2.48 ± 0.28	**0.017**
	2.7 (1.9 - 3.8)	2.5 (2.1 - 3.2)	
ALT	93.85 ± 252.4	33.71 ± 33.75	0.217
	33 (8 - 1161)	24 (4 - 132)	
AST	87.8 ± 220.1	27.94 ± 26.22	**0.022**
	32 (10 - 1017)	19 (5 - 93)	
Daily fluid balance	1442.2 ± 1835.43	2138.29 ± 4255.79	0.726
	960 (-100 - 8500)	1100 (-674 - 18000)	
Base excess	0.18 ± 4.75	-0.96 ± 4.18	0.352
	-1.05 (-9 - 9.4)	-2.1 (-6 - 8.7)	
BUN	26.72 ± 14.16	29.22 ± 14.97	0.522
	26.35 (8 - 55)	27 (14 - 73)	
Bilirubin	0.76 ± 0.47	0.67 ± 0.37	0.444
	0.7 (0.1 - 2.4)	0.6 (0.3 - 1.4)	
CRP	112.17 ± 52.33	143.55 ± 80.27	0.345
	111.5 (27 - 242)	117 (50 - 354)	
Hemoglobin	10.67 ± 2.24	10.26 ± 1.92	0.522
	10.45 (7.8 - 18.5)	10.6 (6.7 - 15.1)	
Potassium	3.84 ± 0.59	3.8 ± 0.69	0.833
	3.9 (2.6 - 4.6)	3.8 (2.5 - 5.3)	
Creatinine	0.74 ± 0.28	1.32 ± 1.25	0.155
	0.7 (0.3 - 1.26)	0.8 (0.5 - 4.6)	
Lactate	1.38 ± 0.64	1.62 ± 0.61	0.259
	1.3 (0.3 - 2.8)	1.4 (0.6 - 2.7)	
Sodium	144.05 ± 7.26	141.76 ± 5.78	0.303
	143.5 (130 - 164)	140 (134 - 155)	
Osmolality	288.4 ± 14.63	288.47 ± 15.44	0.927
	286 (265 - 329)	288 (267 - 329)	
PCO_2	54.69 ± 75.88	36.69 ± 9.67	0.891
	36.65 (21.9 - 371)	36.6 (17.7 - 52.8)	
pH	7.45 ± 0.07	7.43 ± 0.08	0.434
	7.45 (7.34 - 7.58)	7.41 (7.28 - 7.59)	
Platelets	300640.0 ± 157330.62	215835.53 ± 113103.94	0.073
	256700 (91500 - 704000)	208000 (104 - 474000)	
PO_2	130.71 ± 69.31	114.99 ± 46.51	0.737
	109.5 (52 - 308)	111 (32 - 227)	
Troponin	0.1 ± 0.24	0.12 ± 0.28	0.218
	0.02 (0 - 0.99)	0.03 (0 - 1.14)	
WBC	13496.69 ± 6072.19	12193.63 ± 12280.58	0.106
	13815 (13.8 - 29300)	10900 (11.2 - 52740)	
Urine output	2236.05 ± 765.06	1910.0 ± 1110.07	0.300
	2165 (1110 - 3971)	1650 (0 - 4030)	
Urine density	1018.3 ± 7.69	1015.06 ± 4.62	0.124
	1015.5 (1008 - 1037)	1014 (1008 - 1025)	
Urine erythrocyte	159.72 ± 143.95	219.76 ± 125.35	0.402
	100 (0 - 330)	300 (0 - 330)	
Urine pH	6.85 ± 0.93	6.26 ± 0.83	0.054
	7 (5 - 8)	6.5 (5 - 8)	
Urine protein	38.62 ± 30.15	57.71 ± 69.27	0.556
	30 (0 - 100)	30 (0 - 300)	

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Table 6. Laboratory Findings at The First Day After The Colistin Onset.

Mean ± SD Median (Min–Max)			
ARF	**NO (20)**	**YES (17)**	**p**
Albumin	2.5 ± 0.32	2.29 ± 0.32	0.066
	2.5 (1.6 - 3.2)	2.2 (1.8 - 2.9)	
ALT	54.05 ± 62.79	25.06 ± 20.02	**0.038**
	37 (10 - 289)	23 (2.1 - 76)	
AST	36.45 ± 27.93	28.14 ± 23.44	0.165
	27.5 (14 - 131)	21 (0.3 - 93)	
Daily fluid balance	961.9 ± 981.29	922.29 ± 824.15	0.896
	820 (-1150 - 3488)	800 (-364 - 2125)	
Base excess	-0.56 ± 5.15	-2.51 ± 5.47	0.279
	-2.05 (-7.9 - 12.3)	-3.5 (-12.6 - 7.6)	
BUN	23.89 ± 11.38	53.41 ± 34.01	**0.001**
	24 (9 - 55.1)	45 (19 - 124)	
Bilirubine	1.07 ± 1.35	0.76 ± 0.4	0.939
	0.65 (0.2 - 6)	0.6 (0.3 - 1.6)	
CRP	128.34 ± 48.11	169.98 ± 115.75	0.314
	128.5 (11.9 - 197)	156 (60.9 - 521)	
Hemoglobin	10.35 ± 2.37	9.68 ± 1.07	0.410
	9.88 (7.2 - 17.6)	9.68 (7.7 - 11.8)	
Potassium	3.94 ± 0.51	4.21 ± 0.99	0.625
	4.05 (2.9 - 4.5)	3.9 (3.2 - 7.5)	
Creatinine	0.78 ± 0.48	1.62 ± 1.44	**0.004**
	0.65 (0.38 - 2.5)	1.07 (0.5 - 6.1)	
Lactate	1.44 ± 0.48	1.45 ± 0.6	0.942
	1.5 (0.7 - 2.5)	1.5 (0.1 - 2.6)	
Sodium	141.0 ± 6.28	141.47 ± 7.53	0.951
	140 (134 - 162)	141 (135 - 168)	
Osmolality	285.65 ± 14.25	286.88 ± 16.89	0.819
	283 (264 - 321)	282 (266 - 334)	
PCO_2	46.77 ± 25.31	45.76 ± 26.09	0.831
	39.5 (20.4 - 121)	40 (25.1 - 139)	
pH	7.44 ± 0.07	7.38 ± 0.1	0.051
	7.45 (7.27 - 7.54)	7.38 (7.18 - 7.51)	
Platelets	294176.22 ± 151850.66	192882.94 ± 69543.83	**0.013**
	300250 (224500 - 626600)	192500 (90410-362600)	
PO_2	122.33 ± 66.81	121.47 ± 57.07	0.726
	105.5 (41 - 296)	104 (43.1 - 261)	
Troponin	0.17 ± 0.37	0.17 ± 0.36	0.712
	0.03 (0 - 1.6)	0.03 (0 - 1.24)	
WBC	12437.66 ± 6929.69	11013.02 ± 8606.14	0.502
	12380 (9700 - 31000)	10350 (7700 - 29200)	
Urine output	2363.0 ± 755.19	1702.94 ± 688.96	**0.009**
	2300 (1350 - 3940)	1750 (300 - 2860)	
Urine dansity	1012± 2.21	1014.94 ± 6.14	0.350
	1015.5 (1011 - 1025)	1013 (1007 - 1030)	
Urine erythrocite	178.3 ± 129.44	197.41 ± 129.7	0.575
	150 (0 - 330)	300 (20 - 330)	
Urine pH	6.78 ± 0.55	6.49 ± 0.8	0.103
	7 (6 - 8)	6 (5.5 - 8.5)	
Urine protein	58.05 ± 37.69	47.12 ± 35.04	0.385
	45 (0 - 100)	30 (0 - 100)	

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Table 7. Laboratory Findings At The 3rd Day After The Colistin Onset.

	Mean ± SD		p
	Median (Min–Max)		
ARF	NO (20)	YES (17)	
Albumin	2.53 ± 0.3	2.3 ± 0.45	**0.024**
	2.5 (2 - 3.1)	2.3 (1.7 - 3.5)	
ALT	39.0 ± 29.66	23.0 ± 13.75	0.091
	31.5 (5 - 109)	22 (2 - 44)	
AST	47.85 ± 67.71	21.53 ± 10.98	**0.024**
	23.5 (16 - 313)	20 (7 - 56)	
Daily fluid balance	1038.2 ± 946.31	946.41 ± 1335.19	0.809
	765 (-80 - 3170)	934 (-1275 - 3860)	
Base excess	-0.2 ± 3.29	-2.76 ± 5.07	0.074
	-0.15 (-6.3 - 6.1)	-3.1 (-11.4 - 6.8)	
BUN	28.56 ± 21.28	67.19 ± 41.04	**0.000**
	22 (9 - 103.7)	51 (20 - 139)	
Bilirubin	1.3 ± 2.34	0.86 ± 0.43	0.507
	0.6 (0.4 - 11)	0.8 (0.4 - 1.7)	
CRP	137.34 ± 89.41	182.2 ± 103.27	0.170
	130.5 (28 - 378)	164 (68.8 - 438)	
Hemoglobin	10.04 ± 2.0	9.88 ± 1.01	0.636
	9.85 (6.9 - 16.1)	10.1 (7.2 - 11.5)	
Potassium	3.9 ± 0.51	4.11 ± 0.63	0.270
	3.85 (3.1 - 4.7)	4.1 (2.7 - 5.2)	
Creatinine	0.77 ± 0.4	2.36 ± 2.17	**0.000**
	0.65 (0.4 - 2.05)	1.2 (0.67 - 7.9)	
Lactate	1.41 ± 0.56	1.6 ± 0.66	0.363
	1.45 (0.6 - 3.1)	1.4 (0.6 - 3)	
Sodium	140.5 ± 6.01	141.47 ± 4.8	0.335
	139.5 (133 - 157)	142 (135 - 150)	
Osmolarity	281.35 ± 12.11	287.88 ± 14.61	0.206
	279.5 (261 - 307)	283 (272 - 314)	
PCO_2	43.12 ± 21.54	48.71 ± 39.1	0.670
	37.55 (24.6 - 105)	38.3 (20.3 - 193)	
pH	7.46 ± 0.04	7.39 ± 0.11	**0.034**
	7.46 (7.38 - 7.55)	7.41 (7.13 - 7.54)	
Platelets	273714.55 ± 157199.22	183129.41 ± 57896.17	**0.025**
	268350 (257 - 579800)	191000 (72600 - 277000)	
PO_2	133.0 ± 70.86	126.6 ± 54.62	0.763
	128 (32.6 - 269)	126 (33.9 - 208)	
Troponin	0.24 ± 0.54	0.21 ± 0.38	0.084
	0.03 (0 - 1.8)	0.04 (0 - 1.17)	
WBC	15220.05 ± 7574.56	24928.51 ± 57890.91	0.170
	12195 (10.9 - 34890)	11400 (6.34 - 248000)	
Urine output	2599.0 ± 892.81	1622.35 ± 1293.21	**0.010**
	2455 (1250 - 4080)	1400 (0 - 4160)	
Urine density	1014.7 ± 4.51	1014.06 ± 5.23	0.691
	1015 (1004 - 1021)	1013 (1007 - 1024)	
Urine erythrocyte	164.34 ± 138.71	178.59 ± 139.52	0.804
	100 (0 - 330)	100 (0 - 330)	
Urine pH	6.85 ± 0.95	6.29 ± 0.94	0.085
	6.7 (5 - 8.5)	6 (5 - 8.5)	
Urine protein	72.25 ± 103.94	85.94 ± 88.82	0.173
	25 (0 - 300)	66 (0 - 300)	

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Table 8. Laboratory Findings at The 5th Day After The Colistin Onset.

	Mean ± SD Median (Min–Max)		p
ARF	NO (20)	YES (17)	
Albumin	2.52 ± 0.35	2.27 ± 0.4	0.051
	2.55 (2 - 3.2)	2.4 (1.5 - 2.9)	
ALT	59.75 ± 48.12	29.65 ± 28.9	**0.043**
	44.96 (8 - 176)	22 (2 - 118)	
AST	65.52 ± 57.44	47.65 ± 83.36	**0.026**
	39.5 (19 - 212)	24 (6 - 361)	
Daily fluid balance	1008.93 ± 1014.52	1469.82 ± 1354.9	0.437
	870 (-680 - 3100)	950 (135 - 5670)	
Base excess	-0.75 ± 3.46	-4.14 ± 5.52	**0.029**
	-1.55 (-4.7 - 7.7)	-4.1 (-13.1 - 8.2)	
BUN	27.87 ± 23.36	77.44 ± 31.66	**0.000**
	21.5 (7.9 - 111)	75 (42 - 151)	
Bilirubine	1.38 ± 2.37	1.02 ± 0.54	0.306
	0.7 (0.2 - 11.09)	0.9 (0.4 - 2.3)	
CRP	117.8 ± 66.45	180.78 ± 86.98	**0.017**
	124 (16 - 264)	171 (62.2 - 378)	
Hemoglobin	9.78 ± 1.5	9.54 ± 1.07	0.784
	9.4 (7.42 - 14.1)	9.6 (6.8 - 11.2)	
Potassium	3.97 ± 0.79	4.19 ± 0.81	0.423
	4.04 (2.8 - 6.1)	4.2 (2.8 - 5.7)	
Creatinine	3.8 ± 12.56	2.91 ± 1.76	**0.000**
	0.7 (0.3 - 57)	2.68 (1.15 - 7.6)	
Lactate	2.05 ± 2.22	2.25 ± 1.05	0.090
	1.5 (0.7 - 11)	2.1 (1 - 4.5)	
Sodium	139.61 ± 5.8	134.18 ± 32.53	0.160
	139.5 (131 - 156)	142 (13 - 156)	
Osmolality	281.43 ± 17.34	275.24 ± 11.45	0.217
	281 (252 - 308)	278 (247 - 293)	
PCO_2	47.48 ± 29.28	54.06 ± 52.72	0.749
	37 (27.6 - 138)	42 (20 - 252)	
pH	7.43 ± 0.05	7.36 ± 0.11	**0.013**
	7.44 (7.25 - 7.49)	7.38 (7.11 - 7.49)	
Platelets	289215.69 ± 133575.07	181041.18 ± 84565.69	**0.008**
	267400 (128900 - 626000)	174600 (65300 - 387000)	
PO_2	124.95 ± 70.85	100.29 ± 54.3	0.249
	114.31 (2 - 265)	101 (3.7 - 199)	
Troponin	0.22 ± 0.61	0.21 ± 0.42	0.075
	0.02 (0 - 2.6)	0.07 (0 - 1.8)	
WBC	12999.59 ± 8039.1	23328.26 ± 54397.01	0.411
	11560 (12.8 - 30850)	10000 (13.25 - 232000)	
Urine output	2315.11 ± 947.3	1394.71 ± 1188.46	0.014
	2210 (900 - 3680)	1080 (0 - 3800)	
Urine erythrocyte	156.37 ± 131.5	215.35 ± 143.47	0.169
	100 (0 - 330)	330 (11 - 360)	
Urine pH	6.9 ± 0.94	6.21 ± 0.79	0.021
	7 (5 - 8.5)	6.5 (5 - 7.5)	
Urine protein	31.11 ± 29.28	159.71 ± 144.4	**0.000**
	27.5 (0 - 100)	100 (15 - 500)	
Urine density	1012.55 ± 5.61	1015.65 ± 5.11	0.090
	1011.5 (1003 - 1024)	1014 (1009 - 1025)	

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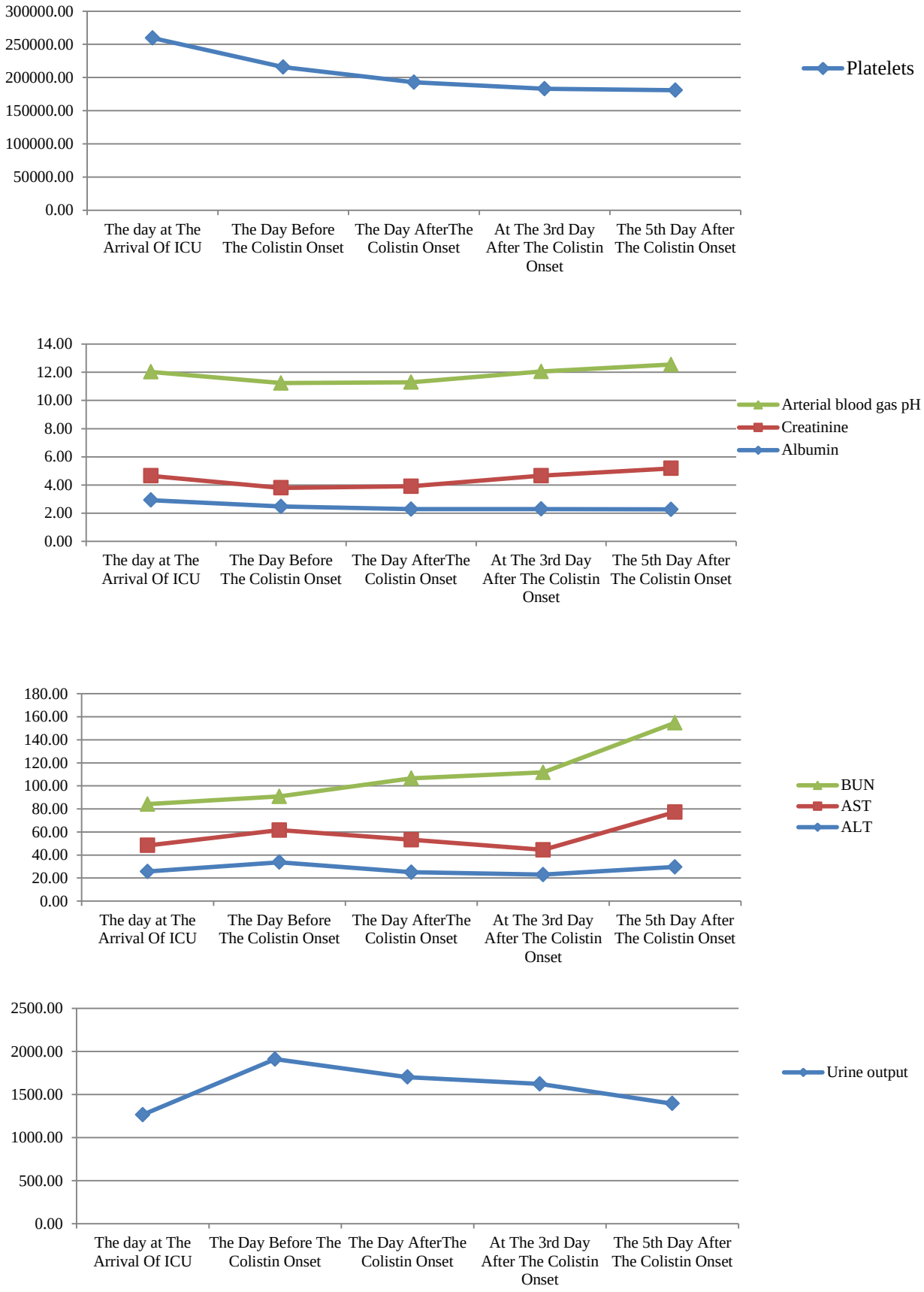

Graph 1. Changing blood parameters according to Colistin Onset. Graph produced from table (4-8)

Discussion

In this study we analyzed 37 patients treated with colistin in an intensive care unit (ICU) and risk factors of ARF after colistine treatment. Seventeen of 37 patients (%45.9) had been developed ARF.

We found that patients who developed ARF were older and colistin treatments were initiated earlier than patient who did not developed ARF. Significant changes were found in the follow-up of the parameters related to ARF. In patients with ARF; BUN, creatinine and CRP levels was found to be higher while albumin lower on the first day of hospitalization before colistin. AST was found to be higher while albumin lowers the day before colistin onset. It was seen that ALT, BUN, creatinine and urine output are significantly higher just after the day of colistin treatment. On the 3rd day, the platelet height also stands out. And on the 5th day, AST, ALT, BUN, arterial blood gas base excess levels, creatinine, urine pH, protein amount in urine and urine output amount seem to be more impaired.

Comparing the groups before colistin, it may be considered that older age and early initiation of colistin treatment are risky for ARF development. Köksal et al. [14] showed that older age, presence of COPD, and DM increased the risk of nephrotoxicity. In our study, similarly older ages were related to ARF. There are several studies that revealed the association between COPD and renal failure. Mapel et al. [15] found that COPD patients have a substantially increased prevalence of renal diseases as well as abnormal renal and hepatic laboratory values. Similarly in our study, COPD was higher in ARF group but was not statistically significant.

Evaluation of nephrotoxicity by blood tests includes the measurements of blood urea nitrogen (BUN), glomerular filtration rate (GFR), concentration of serum creatinine (SCr) and creatinine clearance (CrCl). However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged [16, 17]. Studies found that kidney injury molecule 1 (KIM-1), Cystatin C and urinary NGAL might be more reliable parameters than plasma creatinine levels to supervene renal functions during colistin medication [18,19]. But those are costly biomarkers. In this study we aimed to evaluate the routine laboratory findings can predict ARF during colistin treatment. In our study in all times most related biomarker was albumin. Similarly, previous studies showed that albumin is a good predictor for ARF (18, 20)

In a study, high APACHE II score and CRAB infection were significantly associated with 30-day mortality in ARF patients (21). But in our study APACHE II score was higher in ARF group but was not statistically significant.

Hemodiafiltration treatment ratio at first day of ICU was higher in patients with ARF. This treatment may seem more likely due to early onset of renal failure symptoms.

Clinical signs of colistin nephrotoxicity are decreased creatinine clearance and probable potential oliguria (low output of urine) or proteinuria (22). Similarly in our study, creatinine increase on the first day (end of 24 hours) of colistin treatment and additionally proteinuria on the 5th day are noteworthy in patients who developed ARF.

Studies that colistin exposure causes oxidative stress in proximal tubule cells suggest that an antioxidant strategy may be beneficial (23, 24). One of these anti-oxidant strategies is NAC usage. In a study, NAC was used at a dose of 150 mg/kg/day given to rats intraperitoneally and they reported that NAC prevented colistin-induced nephrotoxicity (25). In our study in 21 patients we used 25 mg/kg/day NAC but we did not find relationship between NAC and ARF. This can be due to low dose of NAC administration in our patients.

The retrospective design of the study and the low number of patients are limitations of this study. Patient group were not a homogenous group in terms of diagnosis. Comparisons are needed in the same diagnostic groups. Another limitation of this current study is the lack of control groups with a similar number of subjects.

Conclusion

In this study older age and early initiation of colistin treatment in the ICU should be consider before colistine treatment for possible ARF development. Before colistin treatment BUN, creatinine, CRP, albumin and AST levels should be consider to be risky for ARF development. After colistin treatment ALT, BUN, creatinine, urine output, platelet, AST, arterial blood gas base excess levels, urine pH, protein amount in urine and urine output amount should be consider to be risky for ARF development. Also on all follow up albumin may be a good predictor for ARF. Whatever happens, Acinetobacter baumannii or colistin treatments both still have high mortality.

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Author's Contributions: AZŞ, KŞB; Research concept and design, Patient examinations, Biochemical Analyzes, Research the literature, preparation of the article **AZŞ;** Revision of the article.

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Homozygous SCN2A gene mutation causing early infantile epileptic encephalopathy: The second case in literature

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Abstract

Objective: Early infantile epileptic encephalopathy type11 (EIEE) generally known as an autosomal dominant inherited disease caused by the voltage-gated sodium channel neuronal type 2 alpha subunit (Navα1.2) encoded by the SCN2A gene mutations. The clinic of the disease is variable. Herein we report the second case with a homozygous missense mutation of the SCN2A gene (c.1588 G>T).

Material and methods: NGS gene panel including the SCN2A gene from genomic DNA extracted from peripheral blood using a commercially available kit and quantified using standard methods. Illumina miseq analysis platform was used for this purpose, we performed analysis of coding regions and exon-intron boundaries and the data was analyzed by IGV.

Results: The results confirmed by sanger sequencing show us an SCN2A (NM_001040142) c.1588 G>T homozygote mutation.

Conclusion: This shows us more clinical and molecular studies need for SCN2A associated disease pathogenesis

Keywords: SCN2A, Heretozygote mutation, infantile epilepsy

Introduction

The benign familial infantile seizures type 3 (BFIS3; OMIM: 607745) is the most common clinical presentation of SCN2A defect which is an autosomal dominant neurological disorder. In this syndrome, apnea, cyanosis, and cluster seizures that occur over one or several days can be seen(1). These seizures usually disappeared by the end of the 1 year of life but some patients continue to have seizures through adulthood without neurological abnormality(2, 3). Early infantile epileptic encephalopathy type 11 (EIEE11; OMIM: 61372) is another phenotype associated with SCN2A pathogenesis. This syndrome compromise more severe neurological manifestation than BFIS3 but has a similar inheritance pattern, autosomal dominant. Early-onset of infantile refractory seizures cause an eventual delay in intellectual and motor development in patients with EIEE(4, 5). Patients may firstly present neonatal hypotonia that proceeds to partial and generalized refractory tonic-clonic seizures. Additionally, dysphagia, dysarthria, excessive daytime sleepiness, disturbed visual contact, paralysis can be seen among patients(5-7) Although brain MRI findings of these patients can vary, brain atrophy commonly reported(4, 7, 8)

Case

A five-year-old case came to our clinic because of refractory seizures. She was born as the fifth child of a first cousin parent (Mother age: 37, father age: 40) with an uneventful pregnancy(Figure 1). At birth, she was 50 cm (10th-25th centile), 3.3 kg (10th-25th centile), head circumference (HC) of 35 cm (10th-25th centile). After birth, she had stayed in an intensive care unit because of respiratory distress in a period of 12 days. Her head-neck control started during the third month of her life. When she was six months old, her afebrile myoclonic seizures started.

On physical examination, She was 94 cm(97th centile) and 15 kg (10th-25th centile). Her head circumstance was 50 cm. She has a flat nose, upslanting palpebral fissure, high palate, bilateral epicanthal folds, rotatuar nystagmus (Figure 2). Pes equinovarus, joint laxity, negative Babinski and clonus reflexes are the other findings (could not taken a photo because of agitation of the case). She was hypotonic, she can not walk and speak. Her mental cooperation was negative. Her eye and hearing examination was normal. In laboratory findings there in not any abnormalities and her metabolic scannings were normal. Her abdominal USG result was normal.

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Her MR result showed mild vermis hypoplasia, corpus callosum hypoplasia and right temporal region cortical thickness.

Her peripheral blood chromosome analysis result was normal also the array-CGH result was normal. Because of her clinical presentation, we studied an early infantile epilepsy custom NGS gene panel including the SCN2A gene from genomic DNA extracted from peripheral blood using a commercially available kit and quantified using standard methods.

Illumina miseq analysis platform was used for this purpose, we performed analysis of coding regions and exon-intron boundaries and the data was analyzed by IGV.

The results confirmed by sanger sequencing show us an SCN2A (NM_001040142) c.1588 G>T homozygote mutation. After that, we performed carrier analysis to parents. Both parents were carriers in terms of SCN2A c.1588 G>T. This change has not been shown before

Discussion

We describe the second documented homozygous SCN2A mutation causing EIEE in autosomal recessive inheritance, the first case was published three months before(9). All previous patients were reported having a heterozygous SCN2A variant in either dominant or de novo fashion. The variant was evaluated by MutationTaster and Varsome databases as pathogenic. Epileptic phenotypes seen in patients with de novo or dominant heterozygous SCN2A gene mutations so, it is not surprising that homozygous SCN2A mutation carriers also have similar epileptic phenotype. There are two main facts that should take into account by clinicians in terms of SCN2A mutations after our case. First, SCN2A gene defects in any patient presenting with seizures without family history may be an indicator of an autosomal recessive or autosomal dominant pattern. Another fact is that autosomal recessive patterns may lead to more severe clinical presentation, such as early neonatal epileptic encephalopathy rather than other phenotypes (ataxia or autism) in heterozygous mutation carriers.

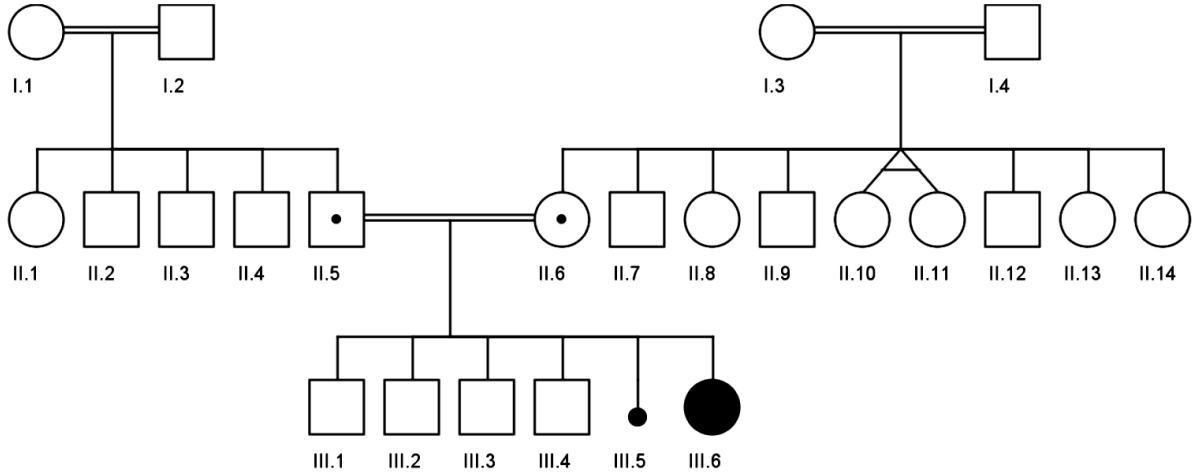

Figure 1: Pedigree of the family (III.6 is the case, II.6 is the mother and II.5 is the father)

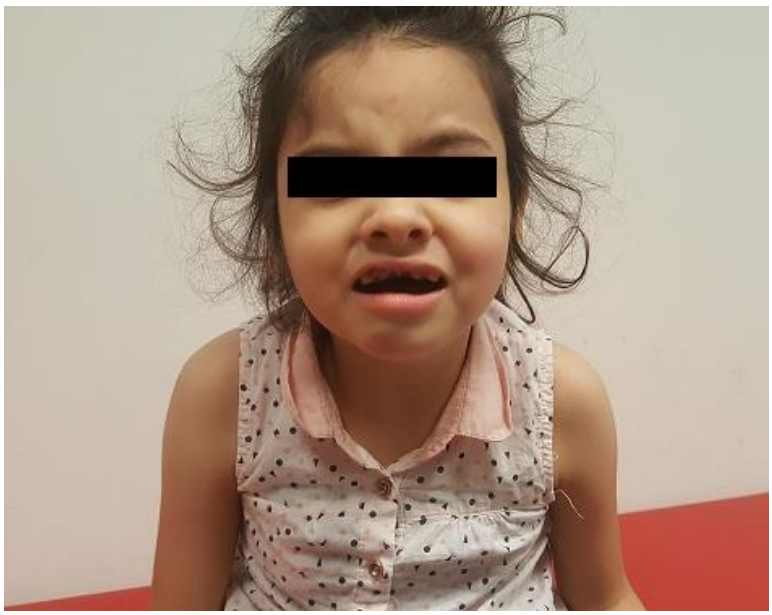

Figure 2: The case.

Conclusion

Being the second case described in her family and literature is the main limitation of the current study. Therefore, further homozygous reported cases with similar phenotypes are necessary to confirm such a conclusion. We report the second case of a homozygous SCN2A gene mutation in a female patient from Turkey. In addition, we describe a novel mutation that can help to increase the mutational spectrum of SCN2A-associated disease pathogenesis. We hope our findings will open new insights for the molecular and inheritance spectrum of SCN2A gene defects.

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Author's Contributions: HÖY; Research concept and design, Patient examinations, Genetic Analyzes, Research the literature, preparation of the article **HÖY;** Revision of the article.

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