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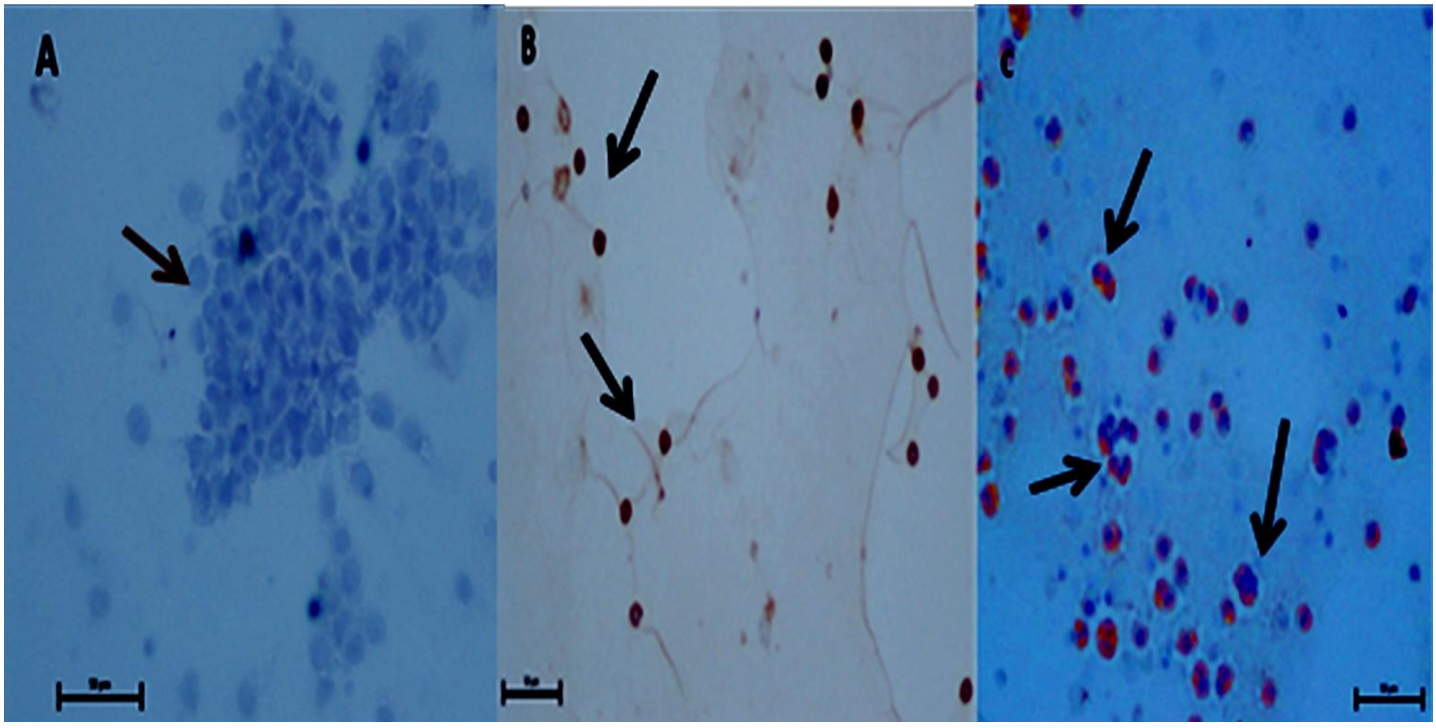

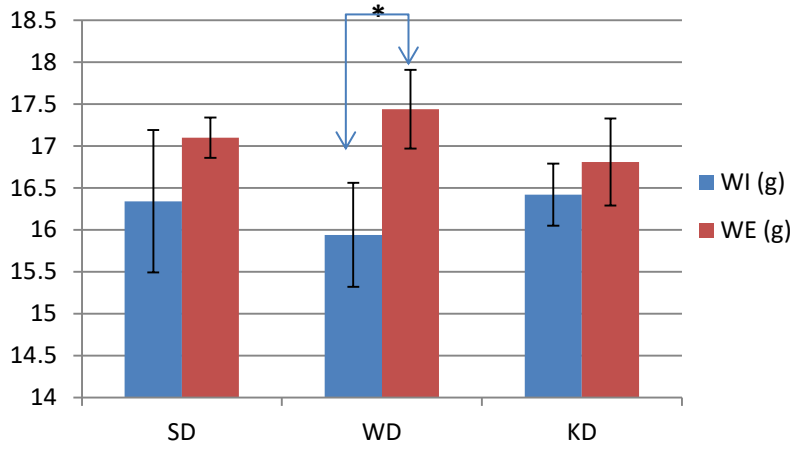

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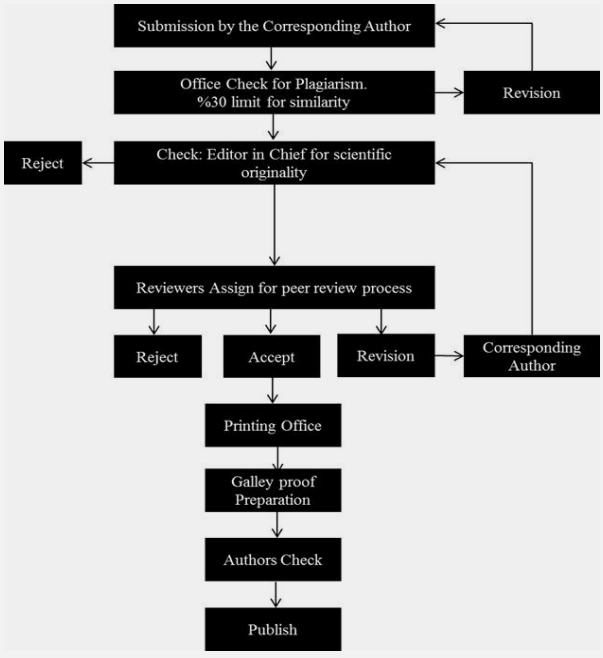

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Research Article Doi: 10.36472/msd.v7i11.432

Autoimmune thyroid disease, thyroid functions, and thyroid ultrasonography in pediatric celiac disease

Şükrü Şahin[1]*, Filiz Demir Şahin[2]

Abstract

Objective: To compare thyroid function tests, autoantibodies and ultrasound findings in pediatric celiac patients following a gluten-free diet with the non-celiac control group.

Material and Methods: The data of 64 celiac patients (median age 11 years) followed up with a gluten-free diet in the pediatric outpatient clinic and 143 control patients were retrospectively reviewed. The patient group consisted of 18 men, 46 women, and the control group 39 men and 103 women. The age range of the cases was 6-17 years. The duration of gluten-free diet was between three months and 15 years (median four years). The diagnosis of celiac disease was made according to the criteria of the European Society for Paediatric Gastroenterology, Association of Hepatology, and Nutrition. Free thyroxin, thyroid stimulating hormone (TSH), anti-thyroid peroxidase (anti-TPO), and antithyroglobulin (anti-Tg) levels were measured. In the thyroid ultrasound, gland volume, parenchymal structure, and thyroid nodules were evaluated. The positivity of thyroid autoantibodies and a heterogeneous appearance on ultrasound were assessed in favor of thyroiditis. The findings were compared between the celiac and control groups.

Results: Autoimmune thyroid disease was seen in 12.5% of celiac patients and 4.2% of the controls ($p<0.05$). The rate of abnormalities in thyroid function tests was 9.3% in the celiac group and 2.8% in the control group ($p=0.05$). The mean thyroid volume was 3.58 ml in celiac patients and 3.95 ml in controls ($p>0.05$). The parenchymal heterogeneity was 12.5% in the celiac group and 2.1% in the control group ($p<0.05$), and the incidence of thyroid nodules was 25% and 4.2%, respectively ($p<0.05$).

Conclusion: The autoimmune thyroiditis and thyroid dysfunction is more frequent in children with celiac disease. In addition, heterogeneous parenchyma and thyroid nodules are more common than the normal population on ultrasound. Celiac patients should be carefully evaluated for possible thyroid disease.

Keywords: Celiac disease, Autoimmune thyroiditis, Ultrasonography

Introduction

Celiac disease is a chronic inflammatory disease mainly affecting the small intestine caused by gluten sensitivity in patients with genetic tendencies. Increased autoimmunity has been reported in celiac disease. Celiac disease is associated with autoimmune thyroid diseases, especially Hashimoto's thyroiditis (HT) (1). The incidence of Hashimoto's thyroiditis in celiac patients is reported to be between 1.25 and 19% (2). Hashimato's thyroiditis is diagnosed with autoantibodies. In addition, thyroid dysfunction is not uncommon in celiac patients (1, 3-5). Autoimmune thyroiditis and other causes of thyroiditis can cause a heterogeneous appearance on ultrasound. It has been suggested that heterogeneous thyroid parenchyma is more useful than autoantibodies in determining the risk of developing hypothyroidism in euthyroid cases (6). However, there are only a limited number of studies on the incidence of thyroid nodules in children with celiac disease.

In this study, we aimed to evaluate the ultrasound findings, antibodies and functions of the thyroid in patients followed up for celiac disease.

Material and Methods

Patients and study design

In this study, the files of the cases followed up with celiac disease diagnosis in the Pediatrics Outpatient Clinic of Adıyaman Research and Education Hospital between September 2016 and August 2019 were evaluated retrospectively. Out of 172 cases, 108 were excluded from the study (68 of them had no ultrasound, 36 of them had missing files and 4 cases had a history of surgery).

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The diagnosis of celiac disease was made according to the diagnostic criteria of the European Society for Paediatric Gastroenterology Hepatology and Nutrition. The patients were selected from those following a gluten-free diet. The median duration of the application of gluten-free diet after diagnosis was four years (three months-15 years). Eighteen of the cases were male, 46 were female, the mean age 10.84 and the age range was 6-17 years. The control group consisted of 143 subjects (39 males and 103 females with a mean age of 10.57 years). This group comprised patients that presented to our hospital due to anemia (n = 42), palpitations (n = 38), constipation (n = 24), recent excess weight gain (n = 11), hypertension (n = 8), neurological reasons (n = 7), renal complaints (n = 7), and other reasons (n = 6). The Clinical Research Ethics Committee of Adiyaman University approved the study.

Laboratory Tests

The serum free thyroxin (fT4), thyroid stimulating hormone (TSH), antithyroid peroxidase (anti-TPO) and antithyroglobulin (anti-TG) levels were measured by chemiluminescence immunoassay using a DxI800 autoanalyzer (Beckman Coulter Inc, CA, USA). The lower and upper limit values of the measurement method used in the biochemistry laboratory were accepted as the normal reference range for thyroid function tests, anti-TPO and anti-Tg (sT4: 0.61-1.48 ng/dL, TSH: 0.34-5.6 uIU/L, anti-TPO: 0-9 IU / mL, and anti TG: 0-4 IU/mL). The cases with thyroid autoantibody levels above the specified reference value and those with heterogeneous parenchyma on ultrasound were considered to have autoimmune thyroiditis. Subclinical hypothyroidism was diagnosed based on normal sT4 despite increased TSH levels, and an overt hypothyroidism diagnosis was made if there was increased TSH and decreased sT4 levels. Increased sT4 levels and decreased TSH levels were evaluated as overt hyperthyroidism, whereas increased sT4 levels with normal TSH levels were considered as subclinical hyperthyroidism. Euthyroidism was diagnosed with normal TSH and sT4 levels. Other combinations of TSH and sT4 were excluded. A value outside the normal range in at least one of the serum TSH and sT4 values was noted as an indicator of abnormalities in thyroid functions.

Ultrasound examination

Thyroid ultrasonography was performed using a real-time Aplio 500 (Toshiba Medical Systems, Tokyo, Japan) and a 7.5 MHz transducer by the same researcher blinded to the thyroid findings of the patients. The volume of the thyroid gland was measured by ultrasonography using a special formula: transverse size x anterior posterior size x craniocaudal size x 0.479 (Figure 1). This calculation was performed for each lobe, and then the values of both lobes were added to obtain a final value in milliliters. The isthmus volume was disregarded. Thyroid parenchyma was evaluated as heterogeneous or normal. The internal structure, echogenicity, and the size and number of thyroid nodules, if any, were evaluated. Celiac patients without laboratory and ultrasound results were excluded from the study.

Statistical analysis

All analyses were performed using SPSS for Windows (version 21.0; SPSS/IBM, Chicago, IL). Normality was tested using the Kolmogrow-Smirnov test. Since the thyroid gland volume values were not normally distributed, the Mann-Whitney U test was used for the comparison of the celiac and control groups. The chi-square test was used to compare categorical data. The statistical significance level was accepted as $p < 0.05$.

Results

Celiac disease was more common in women. The incidence of autoimmune thyroiditis was significantly higher in the celiac group than in the control group. Autoimmune thyroiditis was found in 17 cases (26.6%) in the celiac group and six cases (4.2%) in the control group ($p<0.05$, Table 1). In the celiac group, nine of the autoimmune thyroiditis cases had euthyroidism, six had hypothyroidism, and two had hyperthyroidism. Of the patients with hypothyroidism, four had subclinical and two had overt hypothyroidism, while both hyperthyroidism cases were overt. There was no patient with subclinical hyperthyroidism.

There was no significant difference between the celiac group and the control group in terms of the thyroid gland volume ($p = 0.276$, Table 2). Heterogeneous parenchyma was seen on ultrasound in eight cases (12.5%) in the celiac group and three (2.1%) in the control group ($p = 0.004$). In the celiac group, only heterogeneous parenchyma was seen in four cases, while both autoantibody positivity and ultrasound heterogeneous parenchyma were observed in a further four cases. Thyroid nodules were found in 16 cases (25%) in the celiac group and six (4.2%) in the control group ($p < 0.05$). In the celiac group, only one nodule was detected in 11 cases and two nodules in five cases. There were no cases with more than two nodules. In the control group, there was one nodule in two cases, two nodules in two cases, and more than two nodules in a further two cases. Most of the detected nodules consisted of hypoechogenic, solid nodules smaller than 1 cm.

Table 1: Thyroid function disorders in the celiac and control groups

	Celiac (N)	Control (N)	p
Autoimmune thyroiditis (%)	17 (26.6)	6 (4.2)	<0.05
Thyroid function			
Euthyroidism	9	2	
Overt hypothyroidism	2	2	
Subclinicalhypothyroidism	4	1	
Over hyperthyroidism	2	1	
Subclinicalhyperthyroidism	0	0	
Abnormal thyroid functions (%)	8 (12.5)	4 (2.8)	<0.05

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Table 2: Ultrasound findings in the celiac and control groups

	Celiac (n =86)	Control (n =143)	p
Thyroid gland volume (mean ± SD)	3,58±2,18	3,95±2,39	0,276
Heterogeneous parenchyma (%)	8 (12,5)	3 (2,1)	0,004
Thyroid nodule (%)	16 (25)	6 (4,2)	<0,05
Mean nodule size (SD)	4,93 (±2,95)	6,71 (±3,25)	
Nodule size range (mm)	3-12	3-15	
Number of nodules			
One	*11*	*2*	
Two	*5*	*2*	
Three or more	*0*	*2*	
Internal structure of the nodule			
Hyperechoic solid	*2*	*1*	
Hypoechoic solid	*10*	*4*	
Isoechoic solid	*2*	*1*	
Cystic	*2*	*0*	
Diabetes mellitus (%)	3 (4,7)	1 (0,7)	
Autoantibody positivity (%)	13 (20,3)	3 (2,1)	0,004
Anti-TPO (%)	12 (18,7)	2 (1,4)	
Anti-Tg (%)	2 (3,1)	1 (0,7)	

SD: Standard deviation, Anti-TPO: Anti-thyroidperoxidase, Anti-Tg: Anti-thyroglobulin.

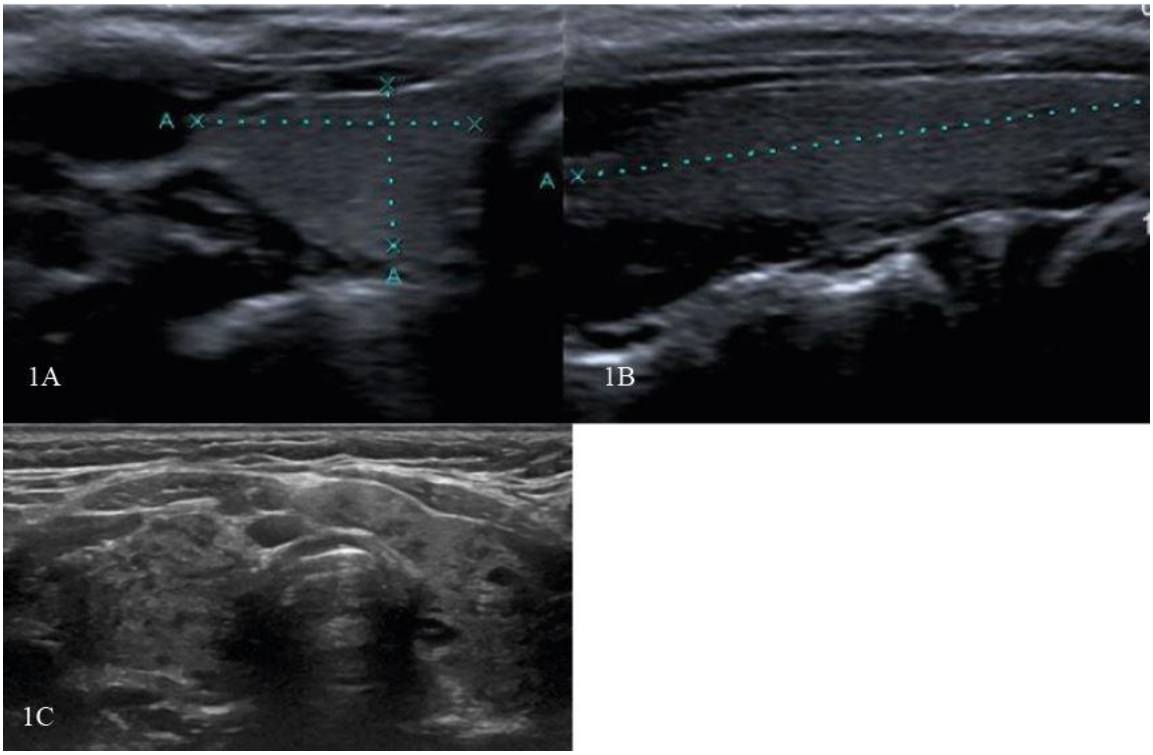

Figure 1: Ultrasound image of the right lobe of the thyroid gland on transverse scan **(1A)** and longitudinal scan **(1B)** showing the measurement planes. **(1C)** Thyroid ultrasound showing heterogeneous parenchyma with poorly defined hypoechoic areas.

Discussion

The results of this study showed; in pediatric celiac disease, the frequency of autoimmune thyroiditis and thyroid dysfunction increased ($p < 0.05$). While there was no significant increase in thyroid gland volume, the frequency of heterogeneous parenchyma and thyroid nodule increased. Most of the nodules detected are hypoechogenic solid nodules smaller than 1 cm.

The incidence of thyroiditis in celiac patients has been reported to be 5.4% to 26.2% (1, 4, 7, 8). In our study, it was found to be 26.6%. In a case-control study by Hakanen et al., the rates of anti-TPO positivity and anti-Tg positivity were reported as 11.4% and 8.8%, respectively in celiac patients (7).

In that study, while anti-TPO was significantly higher in celiac patients, there was no significant difference in anti-Tg. Although the incidence of anti-TPO was slightly higher in our study, our results were similar in statistical terms. We detected only anti-TPO positivity in 12 cases (18.8%) cases, only anti-Tg positivity in one case, and both in another case. While anti-TPO positivity was significantly higher in the celiac group, there was no significant difference in anti-Tg positivity. In a study by Çağlar et al., the incidence of anti-TPO was found to be 9.7%(9).

Some authors suggest that heterogeneous thyroid parenchyma is more sensitive than thyroid autoantibodies in predicting the development of hypothyroidism (6). Evaluating patients followed up for three years, Rago et al. reported that thyroiditis did not develop in any case with autoantibody positivity and normal ultrasound findings (6). However, hypothyroidism was observed in 58% of euthyroid cases presenting with autoantibody positivity and a heterogeneous thyroid appearance on ultrasound (6). The authors also noted that hypothyroidism developed in 13.7% of patients with autoantibody negativity and heterogeneous thyroid parenchyma (6). In our study, four patients with elevated anti-TPO also had heterogeneous parenchyma on ultrasound. In four other patients, heterogeneous parenchyma was observed without autoantibody positivity. Heterogeneous parenchyma was more common in the celiac group than in the control group ($p < 0.05$). In our study, two of the four cases with heterogeneous parenchyma alone had hyperthyroidism while both cases with heterogeneous parenchyma were euthyroid.

In our study, the incidence of hypothyroidism, hyperthyroidism, and euthyroidism coexisting with thyroid autoimmunity was found to be 9.4%, 3.1%, and 14.1%, respectively. In a study by Ansaldi et al., the incidence of hypothyroidism, hyperthyroidism, and euthyroidism was reported as 8.1%, 1.2%, and 15.7%, respectively (1). In another study, Meloni et al. determined the rate of hypothyroidism as 1.8% and that of euthyroidism as 8.6% (4). The authors noted that there were no cases of hyperthyroidism. Midhagen et al., who evaluated adult celiac patients, detected hypothyroidism at a rate of 5.8% and hyperthyroidism at 5% (3). After a follow-up of 5.5 years, Wessels et al. found the incidence of hypothyroidism as 3.2% and that of hyperthyroidism as 0.5 (5).

In the current study, the number of patients with thyroid nodules was 16 (25%) in the celiac group and six (4.2%) in the control group, indicating a significantly higher incidence of thyroid nodules in the former ($p < 0.05$). In their adult celiac study, Hakanen et al. found thyroid nodules in 34% of cases(7).

The limitation of our retrospective study was that, some cases had to be excluded due to the lack of laboratory or ultrasound results. Therefore, our patient group was not as large as we wished to evaluate.

Conclusion

The results of this study showed that the incidence of autoimmune thyroiditis, thyroid dysfunction, heterogeneous parenchyma, and thyroid nodule was increased in celiac cases. Celiac patients should be evaluated in detail for the possibility of thyroid diseases.

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Evaluation of telomere length in granulosa cells; effects of ketogenic and western diet

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Abstract

Objective: In this study, it was aimed to research the effects of nutrition with different diets on the telomere length of granulosa cells.

Material and Methods: In the study, 21 BALB C female rats (in each group; n = 7) were divided into three groups as the standard diet group (SD), the ketogenic diet group (KD) and the western diet group. The animals in the SD group were fed with standard mouse feed, while the KD and WD groups were fed with specially prepared diets. At the end of the experiment, a controlled superovulation stimulation protocol was applied to both ovaries. Smear preparations were ready for telomere length (GLT) and apoptotic (caspase-3) evaluations from granulosa cells taken from oocytes collected from ovaries.

Results: There was a negative correlation between GLT and body weight (r = -0.424; p = 0.056). However, this correlate was not statistically significant. At the end of the experiment, body weight was significantly higher in the WD group compared to the KD group ($p<0.05$). GTL value of KD group was higher compared to WD and SD groups (p = 0.000). There was a significant negative correlation between Caspase 3 activity and GLT (r = -0.594; p = 0.004). Caspase-3 H score was statistically significantly lower in the KD group compared to SD and WD groups ($p <0.05$).

Conclusion: As a result of the study, it was observed that diet types cause changes in GTL, and there was a negative relationship between ¨body weight and caspase-3 activity¨ and GTL. It has also been observed that low KD prevents cells from undergoing apoptosis by increasing GTL and decreasing caspase 3 activity.

Keywords: Granulosa telomere length, infertility, ketogenic diet, western die

Introduction

Telomeres which are hexameric cascading repeats at the ends of chromosomes maintain chromosome stability and genome integrity. Telomere lengths are preserved during growth, and their role depends on their length and structure. In somatic cells, telomere shortening occurs with each successive round of replication inducing ageing in vitro and in vivo. Short telomeres result in meiotic arrest, dissociation and separation abnormalities leading to an increased incidence of aneuploid germ cells. Also, shortened telomeres in men cause apoptosis of germ cells, while in women, it causes meiotic arrest (1). Telomere length may be an indicator of replicative ageing in somatic cells as well as reproductive ageing. In cultured cells, reducing telomere length to a critical threshold halts cell division triggering replicative ageing and can also cells may undergo apoptosis. Cells that do not have a certain length of telomerase are not involved in mitosis; they enter apoptosis in S phase.

Therefore, telomere length can be viewed as a mitotic clock indicating the remaining replicative life of a cell (2). Telomere length differs from cell to cell. Telomeres are longer in spermatogonia and stem cells. (3).

One of the most common causes of infertility in women is problems with ovulation and oocyte quality Pre-ovulation follicles contain granulosa cells and cumulus cells (4). Granulosa cells play a regulatory role during oxide maturation by promoting or delaying oocyte maturation (5). The function of granulosa cells is closely related to oocyte maturation. Granulosa cells have important functions such as preserving the integrity of the oocyte at the beginning and after ovulation, feeding oocyte and producing steroid hormones, mainly progesterone under the influence of estrogen of the theca lutein cells during developing follicle after ovulation (6). The presence of estrogen stimulates the production of telomerase and causes a decrease in reactive oxygen radicals.

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By affecting the number of possible cell divisions, telomere length may in part determine the number of primordial follicles and thus the overall reproductive potential of women. It has been reported that decreasing estrogen levels and slowing down cell renewal in postmenopausal women are effective in accelerating the shortening of telomere length (7).

There are many different genetic and physiological factors that affect telomere length. Exercise, the Mediterranean diet, and a low-calorie diet, antioxidants, and a healthy social / spiritual life have positive effects on telomere length (8). Considering the relationship between telomere length and nutrition, it has been found that a healthy diet with a Mediterranean diet contributes to telomere length, and consumption of foods with antioxidant and anti-inflammatory properties is associated with longer telomere length. Telomere length and attrition of telomeric repeats are affected by nutrition in human and animal models (4, 9). Most of the studies investigating the relationship between telomere length and nutrition in the literature were conducted with leukocytes. Some studies provide information on human ovarian cell telomere length. However, no studies are investigating the relationship between granulosa cell telomere length and diet types. Therefore, in this study, we aimed to examine the relationship between granulosa cell telomere length, which has important effects on oocyte and embryo development using different diet models.

Material and Methods

Approval was obtained from Sakarya University Ethics Committee for Animal Care and Use for the experimental protocols. (Approvel date and decision no: 01/07/2020-33) All applications on animals were carried out in Sakarya University Animal Laboratory following international guidelines. At the end of the experiment, animals were sacrificed by cervical dislocation after the surgical procedures were completed under general anesthesia with 65 mg/kg (i.p.) ketamine and 7 mg/kg xylazine (i.p.) injection.

Study Design and Creating Groups

In the study, 21 BALB C female rats weighing 15-17 grams of 10-12 weeks of age were used. The animals were kept in wire cages under standard laboratory conditions with a 12/12 hour light / dark-light cycle, a temperature of 22 ° C and a humidity of 50-60% during the experiment. All rats were fed tap water and specially prepared adlibutum diets. The rats were randomly divided into 3 groups, with 7 animals in each group

Standard Diet (SD) Group; The rats in this group were fed a regular standard diet for 4 weeks. (77.3% of calories consisting of carbohydrates, 2.7% fat and 20% protein).

Western Type Diet (WD) Group; The rats in this group were fed a western diet consisting of 39.70% of calories from carbohydrates, 39.51% from fat, 19.53% from proteins and 1.26% of other ingredients for 4 weeks.

Ketogenic Diet (KD) Group; In this group, the rats were fed a ketogenic diet consisting of 4.95% of calories from carbohydrates, 74.24% from fat, 19.53% from protein and 1.28% from other components for 4 weeks.

Controlled Ovulation Stimulation and Collection of Oocytes

Both ovaries of female rats were stimulated intraperitoneally (i.p). First injection, 15 internal units (IU) Pregnant Mare Serum Gonadotropin (PMSG) was applied. The superovulation protocol was completed by performing the second injection with 15 IU Human Chorionic Gonadotropin (HCG) hormone 48 hours after the first injection. 15 hours after the second injection, the rats were sacrificed and oocytes collected. For the incubation of the oocytes, Human Tubal Fluid (HTF) medium with 4 mg / ml Human Serum Albumin (HSA) was cultured in an incubator at 37˚C, 5% CO2 concentration from one day before. Culture drops were prepared in the form of group cultures on the culture tooth under mineral oil

Preparation of Granulosa Cell Preparations

After the collected oocytes were incubated for at least 2 hours, they were treated with Irvine Scientific TM Hyaluronidase solution to separate them from cumulus cells. Smear preparations were prepared by taking 2 separate polylyzed slides of cumulus cells purified from the enzyme. The smear preparations were fixed in the fixation solution at -20 °C for 30 minutes and kept at +4 °C for a maximum of 1 week until immunohistochemical staining.

Immunohistochemical Staining Protocol

The preparations were fixed in the fixation solution at -20 °C for 30 minutes. The fixed preparations were washed with Phosphate Buffered Saline (PBS) 3 times for 5 minutes. Triton X-100 was dropped and kept for 10 minutes for permeabilization. They were treated with 3% H2O2 (Hydrogen Peroxide) solution for 20 minutes; and were held at 37 ° C for 1 hour, covered with Primary Antibody (Thermo Fisher Scientific; cat no: PA5-16335). They were washed 3 times for 5 minutes with PBS. They were kept covered for 10 minutes with secondary antibody. Then they were washed 3 times for 5 minutes with PBS. Streptavidin Peroxidase was dropped and kept for 10 minutes covered. They were washed 3 times for 5 minutes with PBS. They were treated with DAB for 2 minutes and washed in running water. Counter-staining was applied with hematoxylin for 1 minute. Then they were washed in distilled water for 5 minutes. After drying, Mounting Medium was dropped on them and covered with a coverslip. For negative control, Antibody Diluent was dropped instead of Primer Antibody. The stained preparations were examined with an Olympus BX53 model light microscope, and counting operation was performed.

Immunohistochemical Evaluations

For immunohistochemical evaluation, each prepared preparation was examined with Olympus BX53 model light microscopy at 100, 200 and 400x magnification in different areas, at least 200 cells per preparation were counted. Morphologically, good and bad cells were detected in the groups. In the cell count, the areas of the cells showing uniform distribution in the preparation were evaluated, as

the areas where the cells cluster were not. Results were calculated semi-quantitatively by determining the H score (10).

Chromosomal Preparation Procedure

In this study, the method was used as a basis for chromosomal preparation. Accordingly, all steps are optimized step by step. In all stages, the metaphase spread was photographed under 1000X (oil immersion) using an Olympus BX53 microscope. Slides were incubated in freshly prepared 4% (for 6 min.) and 10% Giemsa (for 15 min) solution. The optimum concentration and duration of Giemsa stain were chosen based on the visibility of chromosomes, background clarity, and distinction between chromatids within a single chromosome. Chromosome spread counts were arcsin-square-root transformed to improve normality and homogeneity of variance. Prior to running ChAS 4.1 analysis, normality and homogeneity of data had been tested. ChAS enables you to view and summarize chromosomal aberrations across the genome. Chromosomal abnormalities may include copy number gain or loss, mosaicism, or loss/absence of heterozygosity. All values comparisons have been made through the program. In the program, using the normalization workflow for chromosome sequences, the samples were manually re-centred when necessary by automatically correcting the non-diploid status. By comparing the chromosomes with the program, measuring their lengths and comparing the standard lengths and samples, the shortenings were calculated.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago. USA). Numerical data were given as mean ± standard deviation (SD). Normal distribution of data was performed using the Shapiro Wilk test. Pearson's correlation test was used to compare body weights and telomere length, and Spearman correlation test was used for caspase-3 H score comparison. One-way ANOVA and Kruskal Wallis test (caspase-3) were used to compare more than two variables (telomere length, body weights). TUKEY HSD test was used for variables with homogenous variances of significance within the group. Significance limit was accepted as $P < 0.05$.

Results

Average body weights of the groups at the beginning and end of the experiment are given in Figure 1. Before starting the study, there was no significant difference between all groups in terms of body weight ($p > 0.05$).

At the end of the experiment, it was observed that the highest body weight was in the WD group (17.44 ± 0.47) and the lowest in the KD group (16.81 ± 0.52). When compared with the KD group, a statistically significant increase was found in the WD group ($p = 0.036$).

There was a moderately negative correlation between telomere length and body weight (Figure 2A). However, this correlation was not statistically significant ($r = -0, 424$, $p = 0.056$). Mean GTL was calculated as 12.75 ± 0.32 kb in the SD group, 12.70 ± 0.31 kb in the WD group and 13.60 ± 0.34 kb in the KD group (Table 1).

In the comparisons between the groups, the telomere length of the KD group was found to be statistically significantly higher compared to the WD and SD groups ($p = 0.000$).

Mean Caspas 3 H score was 21.54 ± 1.72 in the SD group and 25.18 ± 4.32 in the WD group and 16.38 ± 1.28 in the KD group. In the comparisons between groups, a statistically significant decrease was found in the KD group compared to the SD and WD groups ($p < 0.05$) (Table 1).

It was found that there was a moderate negative correlation between telomere length and caspase-3 H score, and this relationship was statistically significant ($r = 0, -594$; $p = 0.004$) (Figure 2B).

Caspase-3 positivity of the groups is presented in Figure 3. As a result of the immunohistochemical evaluation, it was seen that the highest Caspase-3 activity was in the WD group cells (Figure 3C) and the lowest in the KD (Figure 3A) group cells.

Table 1. Comparison of the Caspase-3 H score and the telomere length of Granulosa cells in experimental groups.

Groups (Each group n=7)	GTL kb	Caspas 3 H score
SD (G1)	12.75±0.32	21.54±1.72
WD (G2)	12.70±0.31	25.18±4.32
KD (G3)	13.60±0.34	16.38±1.28
P value	0.000 (G1-G2)	0.021 (G1-G2)
	0.000 (G1-G3)	0.000 (G1-G3)
		0.000 (G2-G3)

GTL: Granulosa cell telomere length; kb: kilobase pair. The mean difference is significant at the $p < 0.05$ level.

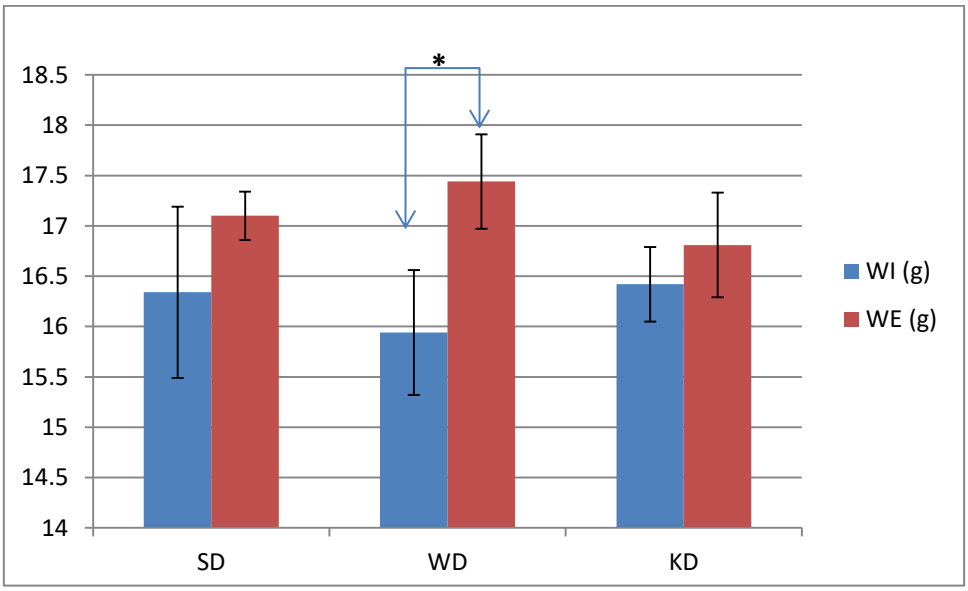

Figure 1: Body weights of all groups at the beginning and the end of the experiment (gram). WI: Initial of experiment body weight; WE: Bodyweight at the end of the experiment; SD: standard diet; WD: Western diet; KD: Ketogenic diet; values are Mean±SD. The mean difference is significant at the p <0.05 level. * Compared to the KD group; p = 0.036

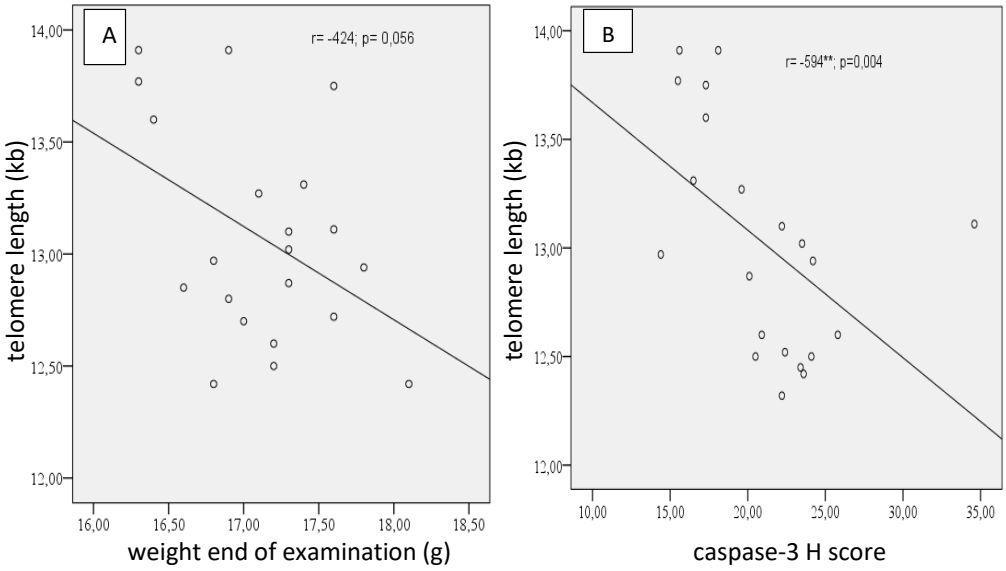

Figure 2. A: Correlation between telomere length and body weight after a 4-week diet; **B:** Correlation between telomere length and caspase 3 H score. ** Significant negative correlation between Caspase 3 activity and telomere length.

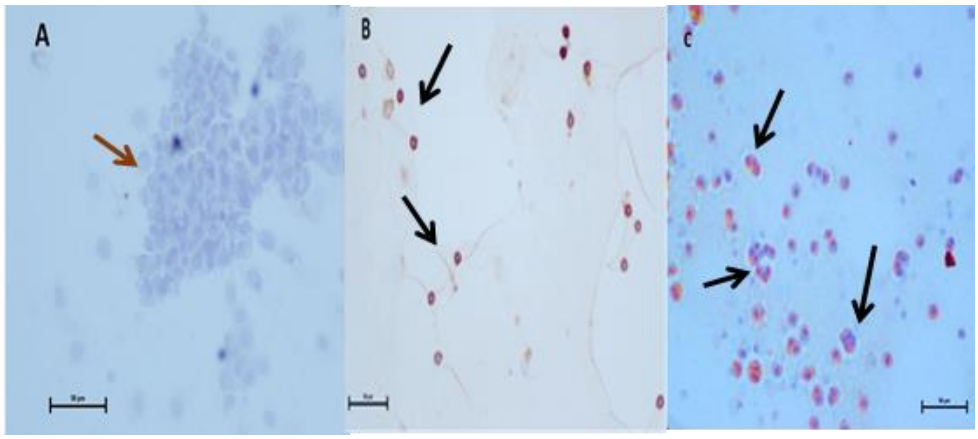

Figure 3. Immunohistochemical Caspase-3 staining of granulosa cells, at X400 magnification, 50 scale bar. Caspase-3 positivity indicates apoptosis of granulosa cells. **Figure 3A-** Low Caspas-3 activity granulosa cells of the KD group (brown arrow). **Figure 3B-** Brown stained Caspas-3 positive cells are seen around the blue granulosa cells of the SD group (black arrow). **Figure 3C-** Granulosa cells of the WD group with intense Caspas-3 positivity are seen (black arrows).

Discussion

Of interest to nutritionists, it has been shown that telomere length is correlated with nutritional status in human and animal studies. Healthy lifestyles and diets are positively associated with telomere length. Changes in diet and lifestyle may regulate telomerase activity in peripheral blood mononuclear cells, but whether this reflects changes in telomere length remains unclear (11). Studies investigating the relationship between nutrition and infertility have greatly increased in recent years. Nutrition with healthy diets provides increased fertility in women and higher quality sperm formation in men (12). A ketogenic diet is a diet consisting of high fat, sufficient protein and low carbohydrate that mimics the metabolic changes of hunger in the body. This diet has been reported to have various health effects (13). There are multiple studies in the literature investigating to what extent KDs can affect fertility outcomes. All of these studies reported a significant improvement in the menstrual cycle and / or ovulation rates with a low-carbohydrate diet (14).

In our study, we found that the telomere length of granulosa cells is affected by feeding with different diet types. At the study results, KD-fed rats had longer telomere lengths than SD and WD-fed rats. Various studies are investigating deeply the relationship between female infertility and telomere length. In addition to natural, chronological ageing; telomere shortening can be affected by genetic factors, physical activity, body mass index, smoking, chronic inflammation, hormone replacement therapy, oxidative stress, antioxidant foods and vitamins (15). Since obesity and smoking are important risk factors for age-related diseases, Valdes et al. investigated the leukocyte telomere lengths of 1122 white women. As a result of the study, they reported that obese and smoker women had significantly shorter telomeres than those of the lean and non-smokers group. This study shows that telomere length and possibly longevity can be affected by environmental factors (16). In previous studies, it was found that women with a healthy lifestyle had longer telomeres (17). In our study, where we performed a four-week diet program, we found a moderate positive correlation between weight and telomere length. There are pieces of evidence from large-scale observational studies that weight loss leads to ovulatory infertility improvement in obese patients. A prospective study of infertile obese women showed that weight loss resulted in the resumption of ovulation in 90% of women, whereas the rate of spontaneous pregnancy was 25%. In fact, it has been shown that a weight loss of only 5% of the body improves fertility in obese women (18). Researchers have reported a relationship between the amount of carbohydrate consumed and the risk of ovulatory infertility. Specifically, they reported a 78% higher risk of ovulatory infertility in women consuming high levels of carbohydrates and 20% higher in those consuming animal protein;with 43% lower risk in those consuming vegetable protein (19). Another study has shown that women who consume vegetable protein as a protein source have a lower rate of infertility compared tothose consuming animal protein.

The type of protein in the diet has been clearly shown in the study results to influence the risk of ovulatory infertility (20).

In the present study, we evaluated the effect of nutrition type on apoptotic activity in cells with caspase 3 positivity. Caspase 3 activity was highest in WD group granulosa cells. There was a negative correlation between telomere length and Caspase-3 activity. This situation was compatible with the telomere length. This result suggests that shortening telomere length and reaching the critical threshold may slow down cell division or even halt it, leading cells to apoptosis. Caspase is cysteine proteases involved in apoptotic cell death. While caspase-3 was observed in atretic granulosa cells in the ovary, no caspase-3 activity was observed in the granulosa cells of healthy follicles. Apoptosis is genetically programmed cell death. Granulosa cell apoptosis is an active cellular event dependent on transcription and protein synthesis (21). Although a study reported that caspase activation causes telomere erosion and caspase inhibitors to reduce telomere loss, it has not been explained how this happens (22).

In the last few years, telomere biology has become an important issue in the reproduction field. Despite many hypotheses, there is little direct evidence about telomere dynamics in human gametes and embryos. Increasing evidence of the role of telomeres and telomere length in human reproduction has indeed broadened the historical view of seeing them simply as an indicator of ageing. Telomere length has been studied more frequently, especially in women with recurrent miscarriages or in-vitro fertilization (IVF). The mean oocyte telomere length was found to be longer in women who became pregnant (23). It has also been reported that the granulose telomere length is shorter in women with ovarian insufficiency of unknown origin compared to those with tubal factor infertility (24). In a study by Czamanski-Cohen et al., It was found that lymphocytes of women who underwent in vitro fertilization (IVF) due to infertility had statistically significantly shorter telomeres at various stages of the menstrual cycle compared to healthy controls (25). Barha et al. found that estradiol, a potent antioxidant with increased levels during pregnancy, preserves telomere length (26). The presence of estrogen stimulates the production of telomerase and causes a decrease in reactive oxygen radicals. It has been reported that decreasing estrogen levels and slowing down cell regeneration are effective in accelerating telomere length shortening in postmenopausal women (27). Cumulus cell telomere length has been reported to be longer in mature oocyte cells than immature oocytes. When the obtained embryo quality and telomere length were compared, it was observed that there was a positive correlation with the embryo quality (28). In another study, it is found that granulosa cell telomere length increased during the follicular development process (29). In particular, a positive correlation has been observed between short telomere length in deficient quality oocytes and oocyte maturation and interrupted gap-junctions in stromal cells (30). These remarkable findings suggest that obesity alone may not be responsible for sub-fertility and that specific dietary components may increase or impair reproductive potential.

Conclusion

In our study results, we have shown that diet types cause changes in granulosa cell telomere lengths, albeit for a short time; and there is a negative correlation between telomere length and weight gain. With recent advances in reproductive technology, it is clear that powerful predictive biomarkers are needed to improve the clinical strategy in infertile individuals. We think that telomere length can be a marker for defining reproductive capacity; nutrition and lifestyle changes with healthy diets can be effective in increasing reproductive functions, and thus infertility can be prevented. However, in order for such methods to be used efficiently in a clinical setting, it is critical to answer many questions first and to conduct further studies to understand the relationships of telomere and related structures with biological ageing and reproduction.

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Dose-Adjusted R-EPOCH Therapy for Aggressive Lymphoma: A single center experience

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Abstract

Objective: In this study, Dose-Adjusted R-EPOCH Therapy for Aggressive Lymphoma has been investigated. According to our study, when we look at diffuse large B-cell lymphomas, 5-7% are double hit lymphoma, 30-40% are double expressor lymphoma and 5-10% are triple hit lymphomas and they have an aggressive course. In our study, the efficacy of the dose-adjusted-R-EPOCH treatment regimen in the treatment of double-hit lymphoma, double expressor lymphoma, triple hit lymphoma; triple expressor lymphoma and primary large B cell lymphomas of the mediastinum are presented.

Materials and Methods: Thirty-six patients diagnosed with B-cell lymphoma who received dose-adjusted R-EPOCH treatment were included in the study. The patients were grouped cytogenetically according to the Bcl-2 and Bcl-6 rearrangement by MYC translocation. Response assessment with PET-CT was performed in patients whose planned therapy reached the number of cycles.

Results: At the end of the treatment, 61% of the patients had a complete response, 3% had a partial response, and 8% had no response. During the follow-up, 9 of the patients died and while the treatment of 2 patients was still ongoing, the treatment regimen of 1 patient was changed. When the patients were evaluated, 2 had double-hit lymphoma (complete response in 2 patients), 9 had double expressive lymphoma (5 patients had a complete response, 2 patients had progressed disease, 1 patient died and 1 patient had a change in treatment), and 8 had triple expressor lymphoma (Complete response in 5 patients, death in 2 patients, and progressive disease in 1 patient).

Conclusion: The dose-adjusted-R-EPOCH treatment regimen can also provide a high response rate in patients with lymphoma with a triple expressor cytogenetic subtype.

Keywords: Aggressive lymphoma, DA-R-EPOCH, double expressor lymphoma, double hit lymphoma, triple expressor lymphoma.

Introduction

Diffuse large B-cell lymphoma (DLBCL) accounts for approximately 30% of patients diagnosed with Non-Hodgkin lymphoma (NHL) and is the most common histological subtype of NHL. It is an aggressive NHL (1). Limited stage disease (stage I or stage II according to Ann Arbor staging system) constitutes 30-40% of DLBCL and its treatment is combined therapy consisting of chemotherapy, recombinant anti-CD20 antibody rituximab and radiotherapy in case of a bulky mass. Advanced stage disease (stage III or IV according to Ann Arbor staging system) accounts for 60-70%. Advanced stage DLBCL should first be treated with chemotherapy and rituximab (2). In order to treat patients with a diagnosis of NHL in the best way, it is necessary to know the exact histological subtype, the extent of the disease, as well as its molecular cytogenetic characteristics.

In patients diagnosed with DLBCL, MYC translocation, B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma 6 (Bcl-6) rearrangement evaluates by fluorescent in situ hybridization (FISH) or immunohistochemistry (IHC). In the World Health Organization's 2016 classification of mature B-cell neoplasms, high-grade B-cell lymphoma was defined as the accompanying Bcl-2 and / or Bcl-6 rearrangement in addition to MYC translocation. Double hit lymphoma (DHL); it has been defined as the presence of Bcl-2 or Bcl-6 rearrangement in addition to MYC translocation by FISH method (3). The presence of Bcl-2 and Bcl-6 rearrangement in addition to MYC translocation was defined as triple hit lymphoma (THL) (4). Although the frequency of DHL is stated between 3-32% in case series, its actual frequency is between 5-10%.

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Compared to other DLBCL subtypes, patients with DHL have a much higher rate of lactate dehydrogenase (LDH) levels, central nervous system involvement, high international prognostic index (IPI) values, and extra nodal involvement (5). If the standard R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone) treatment regimen is administered to patients with DHL, it will result in higher than expected relapse, worse progression-free survival, and lower response rates. Therefore, patients with DHL are candidates for a more aggressive treatment regimen, DA-R-EPOCH regimen (dose adjusted Rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin). Primary large B-cell lymphoma of the mediastinum (PMLBCL) is a rare NHL subtype. PMLBCL is an aggressive tumor originating from thymic-medullary B cell in the mediastinum. Patients were presented with a locally invasive anterior mediastinal mass that often spreads to local structures. The DA-R-EPOCH treatment regimen is recommended in the first step in the treatment of PMLBCL (6). Double expressor lymphoma (DEL) is defined as the detection of both MYC translocation and Bcl-2 or Bcl-6 rearrangement by the IHC method. It has been reported to constitute approximately 30-40% of all DLBCL patients (3). Triple expressor lymphoma (TEL) is defined as the demonstration of Bcl-2 and Bcl-6 rearrangement in addition to MYC translocation by the IHC method (7). DA-R-EPOCH is recommended for first-line therapy in patients with DHL, THL, and PMLBCL. However, there is no consensus on the use of DA-R-EPOCH in patients with DEL and TEL. In this study, we aimed to share the single-center experience of our patients who received the DA-R-EPOCH regimen.

Material and Methods

Study design and population

A total of 36 patients with a diagnosis of NHL who received a DA-R-EPOCH treatment regimen in the Department of Medical Oncology of Cukurova University Faculty of Medicine Balcalı Hospital between 2016 and 2018, which was showed in Table 1.

Demographic data of 36 patients included in the study were analyzed. In addition, NHL subtypes, beta-2 microglobulin levels, presence of B symptoms, lactate dehydrogenase (LDH) levels, ki67 proliferation index, National comprehensive cancer network-international prognostic index (NCCN-IPI) score, Eastern Cooperative Oncology Group Performance Status (ECOG PS), according to extra-nodal organ involvement, bone marrow involvement, stages according to Ann-Arbor staging system, sedimentation levels, development of neutropenia during treatment, MYC translocation, Bcl-2 and Bcl-6 rearrangement status, patients were recorded as DHL, THL, DEL and TEL. Patients who completed the planned number of treatment cycles were evaluated by PET-CT at the end of the treatment to determine the treatment response. Patients were recorded according to treatment response as partial response (PR), complete response (CR), and no treatment response. Response evaluation was not performed in patients who could not complete the planned number of treatment cycles, who died, and whose treatment was changed.

Statistical analysis

SPSS package program compatible with Microsoft Windows was used in the statistical analysis of the data. Descriptive statistics were expressed as numbers and percentages (%) for categorical measurements; and as mean and standard deviation (median and minimum-maximum where necessary) for continuous measurements. One-way Anova test was used for parameters showing normal distribution in the comparison of continuous measurements between groups. Chi-square tests were used to compare categorical variables.

Results

Of the total 36 patients participating in the study, 75% (n: 27) were male and 25% (n: 9) were female. The average age of all patients was 54.36 years. The average Ki67 proliferation index of the patients included in the study was 79.4%. When the diagnostic distribution of the patients according to the NHL subtypes was examined, 72% (n: 26) of 36 patients had DLBCL, 14% (n: 5) had high-grade B cell lymphoma, 11% (n: 4) had PMLBCL and 3% (n: 1) had Burkitt's lymphoma (BL). One patient developed DLBCL from chronic lymphocytic leukemia as a result of Richter transformation. In the response evaluation performed with PET-CT at the end of treatment has shown that 61% (n: 22) of the patients attained CR, 3% (n: 1) attained PR, and 8% (n: 3) had no response. CR was attained in 61% (n: 16) of 26 patients diagnosed with DLBCL at the end of the treatment, while there was no response in 8% (n: 2) of the patients. The characteristics of the patients are shown in Table 2.

Table 1. Dose adjusted R-EPOCH chemotherapy administration scheme

Drug	Dose and route	Given on days
Rituximab	375 mg/m^2-IV	Day 0 or 1
Etoposide	50 mg/m^2-IV per day	Days 1 to 4 (96 hours)
Doxorubicin	10 mg/m^2-IV per day	Days 1 to 4 (96 hours)
Vincristine	0.4 mg/m^2-IV per day (dose not capped)	Days 1 to 4 (96 hours)
Cyclophosphamide	750 mg/m^2-IV	Day 5
Prednisone	60 mg/m^2-orally twice daily	Day 1 to 5
Granulocyte colony stimulating factor		Start day 6-10

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Table 2. Characteristics of all patients (n:36 patients)

	% (n) patients		% (n) patients
Age (>60 years)	61 (22)	Bone marrow involvement (yes)	52 (19)
Gender (Male)	75 (27)	Extranodular involvement (yes)	8 (3)
NHL subtype		Beta-2 microglobulin (>ULN)	77 (28)
DLBCL	72 (26)	Lactate dehydrogenase (>2XULN)	86 (31)
Gigh grade B cell lymphoma	14 (5)	Uric acid (>ULN)	63 (23)
PMLBCL	11 (4)	ESR (>ULN)	80 (29)
Burkitt lymphoma	3 (1)	Ki67 proliferaton index (>%70)	88 (32)
Stage		Cytogenetic subtype	
I-II	39 (14)	DHL	5 (2)
III-IV	61 (22)	DEL	25 (9)
NCCN-IPI		TEL	22 (8)
Low-Low intermediate	30 (11)	Response status	
High intermediate-High	70 (25)	CR	61 (22)
ECOG PS		PR	3 (1)
0-1	45 (16)	No response	8 (3)
2-4	55 (20)		
B symtpom (yes)	69 (25)		

NHL: Non-hodgkin lymphoma; DLBCL: Diffuse large B cell lymphoma; PMLBCL: Primary mediastinal large B-cell lymphoma; NCCN-IPI: National comprehensive cancer network-international prognostic index; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ULN: Upper limit normal; ESR: Erythrocyte sedimentation rate; DHL: Double hit lymphoma; THL: Triple hit lymphoma; DEL: Double expressor lymphoma; TEL: Triple expressor lymphoma; CR: Complet response; PR: Partial response

27% (n: 7) of the patients diagnosed with DLBCL had deceased and the treatment regimen was changed in 4% (n: 1) of the patients. CR was attained in 40% (n: 2) of the patients diagnosed with high-grade B cell lymphoma at the end of the treatment, while 20% (n: 1) did not respond, and 40% (n: 2) of the patients decreased.

At the end of the treatment, CR was attained in 50% (n: 2) of the patients diagnosed with PMLBCL, while PR was attained in 25% (n: 1) of the patients, and the treatment process of 25% (n: 1) of the patients was ongoing.

CR was attained in the response evaluation performed at the end of two cycles of the patient whose treatment process was ongoing. In one patient with a diagnosis of BL, the treatment process was ongoing and CR was detected in the response evaluation performed after two cycles.

While 25% (n: 9) of the patients died during the follow-up period, 6% (n: 2) were continued their therapy, and 3% (n: 1) had their treatment regimen changed. When the cytogenetic subtypes of all patients in the study were examined, it was found that two patients diagnosed with DLBCL and high-grade B-cell lymphoma had a DHL cytogenetic subtype with Bcl-6 rearrangement.

CR was attained in both cases with DHL cytogenetic subtype. 33% (n: 3) of nine patients with DEL cytogenetic subtype had Bcl-2 and 66% (n: 6) had Bcl-6 rearrangement. While 55% (n: 5) of the patients with DEL cytogenetic subtype attained CR, 22% (n: 2) had no treatment response.

In two patients with a DEL cytogenetic subtype and a diagnosis of DLBCL, the treatment regiment was changed in one of the patients and the other patient had deceased.

While 22% (n: 2) of the patients with DEL cytogenetic subtype had a diagnosis of high-grade B cell lymphoma, 78% (n: 7) had a diagnosis of DLBCL. Bcl-2 rearrangement was present in both patients with DEL cytogenetic subtype who diagnosed with high-grade B-cell lymphoma.

One of the patients with a diagnosis of DLBCL had Bcl-2 rearrangement, while 6 patients had Bcl-6 rearrangement. All eight patients with TEL cytogenetic subtype had a diagnosis of DLBCL. While 62% (n: 5) of the patients with TEL cytogenetic subtype attained CR at the end of the treatment, 12% (n: 1) had no response and 25% (n: 2) of the patients had deceased.

Discussion

In our study, we aimed to share the single center experience of 36 NHL patients who received DA-R-EPOCH treatment.

As a result of the study, we concluded that DA-R-EPOCH treatment regimen, which is accepted as the standard treatment regimen in lymphomas with DHL cytogenetic subtype and PMLBCL, is also an effective treatment regimen for lymphomas with DEL cytogenetic subtype and TEL cytogenetic subtype. In studies involving 132 patients with a diagnosis of PMLBCL in eleven centers and a study involving 39 DLBCL patients receiving DA-R-EPOCH therapy, the CR rates were 70% and 66%, respectively (9,8).

In our study, we administered DA-R-EPOCH treatment regimen to 36 patients with aggressive lymphomas and achieved a complete response in 61% of the patients.

On the other hand, although the number of patients was few, our patients with a diagnosis of PMLBCL had a CR rate of 75%. Compared to the literature, although the overall response rates seem to be less, 52% of the patients who participated in our study had a cytogenetically aggressive cytogenetic subtype. For this reason, the overall CR rate may have seemed less.

In the study conducted by Huang et al., the efficiency of R-CHOP and DA-R-EPOCH treatment regimens in patients with a diagnosis of DLBCL with Ki67 proliferation index greater than 80% was compared and as a result, the CR rate was found to be 83% in patients who received DA-R-EPOCH treatment regimen and it was found to be higher than patients receiving R-CHOP treatment regimen.

The same study found that overall survival and progression-free survival rates were better with the DA-R-EPOCH treatment regimen. (10). The average Ki67 proliferation index of the patients included in our study was 79.4%, and the Ki67 proliferation index of 88% of the patients was over 70%. In our study, 61% of the patients had attained complete response, and 52% of the patients included in our study had DHL, DEL or TEL cytogenic subtypes.

In the study conducted by Huang et al, cytogenetic subtypes are undefined and therefore higher response rates may have been obtained.

In a study involving a total of 394 lymphoma patients with DHL cytogenetic subtypes, DA-R-EPOCH and R-CHOP treatment regimens were compared and it was found that the risk of progression was relatively lower and the CR rate was higher in patients receiving the DA-R-EPOCH treatment regimen. However, there was no difference between the two treatment regimens in terms of overall survival (11). In our study, CR was attained in both of two patients with DHL cytogenetic subtype.

In a study conducted at MD Anderson Cancer Center with patients with the DEL cytogenetic subtype, it was observed that patients who received the DA-R-EPOCH treatment regimen had a higher rate of response than patients who received the R-CHOP treatment regimen and recurrence rates were found to be higher in patients receiving the R-CHOP treatment regimen. (12,13).

In our study, CR was attained in 55% of the patients diagnosed with DEL. In the literature review, no data could be found regarding the efficacy of DA-R-EPOCH treatment regimen in patients with TEL cytogenetic subtype. 22% of the patients participating in our study had TEL cytogenetic subtype and 62% CR rate was obtained in this patient group. With this information, it can be said that DA-R-EPOCH treatment regimen can provide a high response rate in patients with TEL cytogenetic subtype.

Conclusion

DA-R-EPOCH treatment regimen can provide a high response rate in patients with lymphoma with THL, DHL and DEL cytogenetic subtypes as well as in patients with lymphoma with TEL cytogenic subtype. However, there is no data in the literature on patients with TEL cytogenetic subtype. Multi-center studies with large patient participation are needed.

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The Role of Ganoderma Lucidum Polysaccharide Peptide in Endothelial Progenitor Cells and Circulating Endothelial Cells as anti Endothelial Dysfunction from Stable Angina Pectoris Patients

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Abstract

Objective: Endothelial dysfunction is the primary initial step for atherogenesis in cardiovascular disease. Stable angina pectoris is a stable form of cardiovascular disease that profoundly alters Endothelial Progenitor Cells (EPC) and Circulating Endothelial Cells (CEC). Both CEC and EPC have a significant role as native homeostasis biomarker of endothelial, which could initiate cytokine storm when homeostasis was altering. Ganoderma Lucidum is known for the antioxidative, anti-inflammatory, and anti-cancer properties and indirect anti endothelial dysfunction. The previous study has proven the Polysaccharide Peptide (PsP) of Ganoderma Lucidum as an effective antioxidant and anti-endothelial dysfunction in atherosclerosis rats and shows no toxicity in an animal model. This study goals to prove the effect of PsP in CEC and EPC in stable angina patients.

Methods: This is a quasi-experimental trial of 35 Stable Angina patients, determined based on ESC Stable CAD Guidelines with pre and post-test design without a control group. The parameters are CEC and EPC counts. The patients were given PsP 750mg/day in 3 divided doses for 90 days. A paired t-test perform for normally distributed data, and the Wilcoxon test for not normally distributed data, and a significant level of $p \leqslant 0,05$.

Results: CEC significantly reduced in stable angina patients, with p=0,001. EPC count significantly reduced in stable angina with p=0,001.

Conclusion: Ganoderma Lucidum PsP is a potent anti-endothelial dysfunction against atherosclerosis's pathogenesis in stable angina.

Keywords: Polysaccharide Peptide (PsP), Ganoderma Lucidum, anti-endothelial dysfunction, stable angina, CEC, EPC.

Introduction

The primary leading cause of mortality and morbidity in the western world and project to be the first killer globally in 2020 is cardiovascular disease. It is responsible for 45% of deaths or equivalent to 4 million deaths per year and remains the most common reason for Europe's mortality. The Sample Registration System (SRS) survey in Indonesia in 2014 showed that cardiovascular disease was the highest etiology of death at all ages after stroke, 12.9% (1,2).

Atherosclerosis act as a primary backbone of coronary heart disease pathomechanism, which relies on endothelial dysfunction as a significant phase of its development. Endothelial dysfunction cause by inflammation, oxidative stress, metabolic abnormality, and risk factors (hypertension, diabetes, and dyslipidemia) (3).

Risk factors like smoking, hyperlipidemia, hypertension, and high blood sugar are the source for vascular failure, which initiates direct simultaneous deterioration effect in endothelial function and exaggerates inflammation, oxidative stress, and other factors metabolic pathway. Nevertheless, Inflammation and Oxidative stress will lead to other endothelial dysfunction with dysfunction of the respiration chain inside mitochondria, causing tissue damage (4).

Injured endothelium will activate detachment sequences of endothelium cells and release circulating endothelial cells (CEC) to circulation and derived endothelial progenitor cells (EPC) subsequently to restore endothelial integrity.

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There are several potential mechanisms for detachment of endothelial lining cells, partially attenuating adhesive properties in endothelial cells because of protease and cytokine and mechanical injury (5).

EPC first describes and isolated from the peripheral circulation, which studies by Asahara and colleagues. EPC derives from bone marrow, significantly influencing tissue neovascularization in ischemic tissue and re-endothelialization of the injured vessel (6). In general, EPC's working mechanism will divide into mobilization, homing, differentiation, and survival or regeneration (7).

A Higher CEC count finds in stable CAD than in a healthy person. Contrarily, lower EPC count observes in stable CAD, cardiovascular risk factor, and a sedentary lifestyle than a healthy person (8,9). Thus, it is essential to find a potent anti-endothelial dysfunction agent that can prevent or improve atherosclerotic cardiovascular disease.

Lingzhi in China or Reishi in Japan names for Ganoderma Lucidum has antioxidative, anti-inflammatory, and anti-cancer properties (10). Scientists have studied Ganoderma Lucidum since the end of the century due to its beneficial health effects and found β-D-Glucan as the main active component in polysaccharide peptide (PsP).

The previous PSP study has proven Ganoderma Lucidum as an effective anti-inflammatory and antioxidant in atherosclerotic rats and revealed subchronic toxicity in animal doses with no toxic effect immunologic, blood test, and histopathologic (11). This study goals to prove the effect of PsP on CEC and EPC as anti-endothelial dysfunction in stable angina patients.

Material and Methods

Quasi-experimental research with pre-and post-test design, single-blinded, to know the effect of Polysaccharide Peptide (PsP) on 35 Stable Angina patients determined based on ESC Stable CAD Guidelines without a control group. The research was held at Saiful Anwar General Hospital Malang, assisted by Indonesia Heart Association (YJI), Lavalette Hospital Malang, and geriatric foundation in Malang, Indonesia, cooperations with Biomedical Laboratory and Physiology Laboratory of Faculty of Medicine, Brawijaya University Malang and Prodia and Proclinic Laboratories for the blood sampling for each patient. Participated patients in this research were patients that come to the Cardiology outpatient clinic at Saiful Anwar General Hospital Malang and Indonesian Heart Foundation Malang branch, without ischemic symptoms and classified as stable angina, and who are willing to participate in research and filled the informed consent. Criteria for stable angina patients are patients with ischemic symptoms at exercises or activities and emotional stress but resolved at rest. Patients who did not consume PSP for three months, and patients with new cardiac symptoms during the study dropped out of the research.

All of the protocols in this research has already approved through informed consents by the Ethical Committee of Saiful Anwar General Hospital Malang, Indonesia, and by the patients (no. 400/ 79/ K.3/302/ 2015)

Polysaccharide Peptide – Sahabat Lingkungan Hidup company supplied PsP that contained Ganoderma Lucidum extract. Each preparation was in the form of freeze-dried preparations in which each capsule 250 mg PSP contains about 180 mg β-D glucan. The PsP 750 mg/day in 3 divided doses for 90 days given in patients. Stable angina patients continued their previous medications besides PsP.

Flow cytometry—CECs (Circulating Endothelial Cells) assays using CD45 and CD146 antibodies and EPCs (Endothelial Progenitor Cells) tests using CD133 and CD34 antibodies Plasma was freshly collected from the blood samples and assayed using PE anti-human antibodies (BioLegend, USA). ELISA- TNF alfa, CRP, IL-6 (anti-inflammatory marker), SOD and MDA (oxidative marker), and adiponectin were collected from blood samples (Pre- and post-intervention) and then assayed with an ELISA kit (Elabscience, Wuhan)

Statistical Analysis - The Data give in mean ± SD. A paired t-test perform to see the differences between pre-test and post-test of stable angina patients. Wilcoxon would use If the normality test indicated the data was not homogeneous. The statistical calculation uses SPSS version 22 (SPSS Inc). The $p \leqslant 0.05$ were considered statistically significant.

Results

Subject characteristics-The study conduct for three months at Dr. Saiful Anwar Malang. The samples of the 45 patients with stable angina pectoris were studied. Of this amount, for three months, PsP Ganoderma Lucidum 3 x 200 mg was given periodically every one month. Of the 45 patients (ten patients excluded from the study, Two patients due to the effects of nausea due to consumption of PsP Ganoderma Lucidum, five patients due to moving domicile, one patient due to death, while two patients for no apparent reason). In total, up to the end of the study, samples of 35 patients with stable angina pectoris were studied.

Patients have an average age of 62.25 ± 9.28 years (Table 1). Patients with male gender as many as 8 (22%) patients while female sex as many as 27 (78%) patients. This group of patients had a bodyweight of 65.35 ± 10.87 kg and a BMI of 27.00 ± 4.06.

Tabel 1: Baseline characteristic

Age > 65 year	11 (31%)
Male	12 (34%)
Hypertension	16 (46%)
TDS (mmHg)	118.42 ± 47.07
TDD (mmHg)	72.85 ± 28.55
DM	18 (51%)
GDP (mg/dl)	113.09 ± 68.63
HbA1c	6.59 ± 2.00
Dyslipidemia	7 (20%)
Total Cholesterol (mg/dl)	205.49 ± 48.49
LDL (mg/dl)	126.17 ± 38.87
TG (mg/dl)	122.37 ± 62.04
HDL (mg/dl)	46.20 ± 12.55
Obesity/overweight	22 (62%)
Smoking	9 (25%)

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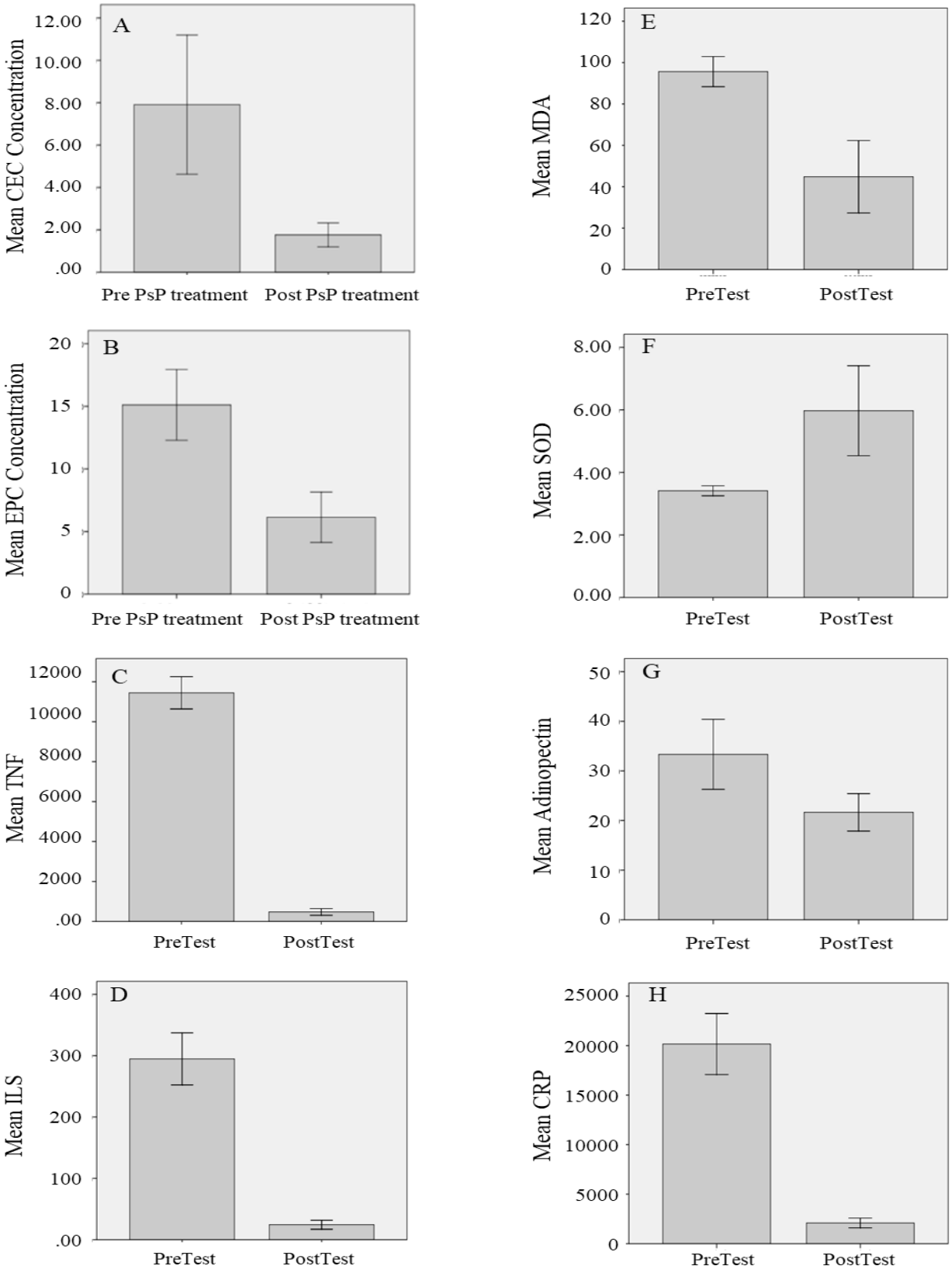

Figure 1. Biomarkers value pre and Post-test. A) CEC, B) EPC, C) TNF-alfa, D) IL-6, E) MDA, F) SOD, G) Adiponectin, H) CRP. CEC: circulating endothelial cells, EPC: endothelial progenitor cells, TNF-alfa: tumor necrosis factor-alfa, IL-6: interleukin-6, MDA: malondialdehyde, SOD: superoxide dismutase, CRP: c-reactive protein. ‘’Error Bars %95 Cl’’

Biomarkers endothelial dysfunction such as CEC found reduced with p=0.01 from 7.91 ± 9.11 cells/µl to 1.76 ± 1.56 cells/µl. Unfortunately, EPC as restorative endothelial markers found reduced too with p=0.001 from 15.11 ± 7.44 cells/µl to 6.14 ± 5.30 cells/µl. Improvement of endothelial function also mark with significantly reduced of ant-inflammatory biomarkers such as TNF-α with p=0.001 from 2094.70 ± 123.28 U/ml to 24.41 ± 21.45 U/ml, Il-6 with p=0.001 from 11444.00 ± 123.28 U/ml to 476.13 ± 482.99 U/ml, and CRP with p=0.001 from 20158.88 ± 8969.08 U/ml to 2092.00 ± 1437.16 U/ml. The antioxidant biomarker shown better improvement as SOD increased with p=0.001 from 3.41 ± 0.46 U/ml to 5.79 ± 4.19 U/ml and MDA decreased with p =0.001 from 95.63 ± 21.27 U/mL to 44,84 ± 50.95 U/mL. Interestingly, adiponectin as glucose and lipid metabolism hormone reduced with p=0.001 from 15.01 ± 8.39 U/ml to 5.43 ± 5.36 U/ml. (Figure 1)

Discussion

β-Glucans extracted from many resources such as barley or oats and black yeast. There are differences from the type of β-Glucans where β-(1,3-1,4)-D-glucan from barley or oat and β-(1,3-1,6)-D-glucan from the black yeast. The molecular weight of β-glucan extracted from barley with warm water is 40,000–100,000 Da; the oligomer prepared from the macromolecule β-glucan by enzymatic degradation with lichenase has a molecular weight of approximately 2,000 Da. In this study, the molecular weight of immunomodulatory protein in PsP, from Ganoderma Lucidum, was between 14.000-17.000 Da through SDS PAGE method (12).

In this study, the administration of PsP Ganoderma Lucidum for three months found a significant decrease in CEC, which showed an improvement in endothelial function. CEC itself is a marker of vascular damage. The administration of beta-glucans will reduce the gene expression of CDH13, which is cadherin, a Cyclin-Dependent Kinase Inhibitor 1C as a negative regulator of cell proliferation, another name is T-Cadherin; which generally provide stimuli for the release of epithelial cells. The research conducted by O'Hara stated that beta-glucan affects cadherin expression (13-14) The mechanism for involving other cadherins, especially E-cadherin through the inactivated immune receptors in the form of CLR receptors (C-type Lectin Receptor) and signal pathways Syk (15)

Wu revealed that dectin-1 receptors located in endothelial cells exposed to beta-glucan would express CD8 + T cells, which would then express CD103, a ligand for E-cadherin (16). Upregulation of E-cadherin would increase endothelial cell adhesion, which led to a decline in the CEC. Several studies also found that activation via the MAPK pathway will increase E-cadherin and increase inter-cell adhesion and activation of growth factors (17,18)

The hypothesis that establishes for EPC is restorative/regenerative cells, which the primary function as replacing/renew the damage of endothelial cells in parallel for this significant reduction in CEC from this study that suggests improvement of endothelial function after PsP administration

Besides examining CEC as a marker of endothelial dysfunction, EPC examination also carries out. EPC itself is a potential cell source that contributes to neovascularization through postnatal vasculogenesis. Several clinical studies show evidence that EPC in the circulation regenerates damaged endothelial cells. The amount of EPC in circulation also reports being inversely proportional to the risk of coronary heart disease. It shows that EPC status in blood circulation presents endothelial dysfunction and impaired vascular health. EPC capacity in repairing vascular damage shows an essential role in maintaining endothelial homeostasis.

As stated above, the atherosclerosis risk factor plays a vital role in EPC reduction. These perspectives indicate that endothelial dysfunction may involve many components of a risk factor. So, handling coronary heart disease management must be comprehensive in all risk factors (4)

In all form cardiovascular disease either acute or chronic, damage of endothelial cells become a source for CEC (elevated) and decreasing EPC. However, it more complicated process than a usual situation. For example, acute myocardial infarction has shown increases in both CEC and EPC. Furthermore, statins and endurance training in CAD patients lead to an improvement in endothelial function. Endothelial turnover in individuals is different. The low shear stress area with high endothelial death rates could make cells need a high turnover rate for maintaining vessel homeostasis (19). This study result shows a significant reduction of CEC but also EPC. CEC reduces, so a detachment of endothelial lining cell decreases, low turnover rate and will not induce EPC mobilization. The other study shows that EPC and CEC count were higher at baseline after 7, 30, and 180 days than healthy controls in AMI events.20,21 This enhance by Lee reported a decrease in EPC inpatient CAD mainly derived from EPC's exhaustion (22). Also, based on the study population in the form of patients with stable CAD found that the chronic inflammatory process will underlie the occurrence of EPC exhaustion (23)

Alessio et al., in their study for DVT patients revealed, CEC levels were increased significantly 24 hours after induction and decreased after 72 hours. The same phenomenon occurred for EPC levels, which markedly increased on day seven and returned to baseline within 60 days in AMI (Acute Myocardial Infarction) (24). It shows a process of mobilization and homing to the injured area; Wojakowski, in his study, showed an increase of fewer than 12 hours at AMI and decreased in 7 days, which was by the decrease in reduced levels of cytokines, especially inflammatory cytokines. Mobilization in AMI conditions involves EPC and affects hematopoietic cells, non-hematopoietic cells, and mesenchymal cells (25).

Research conducted by Mikirova et al. found that beta-glucan supplementation will increase EPC levels through VEGF upregulation (23). Cramer et al. showed that beta-glucan would improve mobilization of EPC from both

hematopoietic and non-hematopoietic types through MMP-9, definitely through CR3 receptor activation (26)

Mobilization of EPC has a complex mechanism. The majority of EPC remain quiescent in the bone marrow. The sequencing process involves migration of HSC (hematopoietic stem cells), translocation of SDF1 (stromal-derived factor 1), and activates MMP 9 (matrix metalloproteinase 9) that release sKitL (soluble Kit Ligand), which allow EPC to exit. Other factors involved are VEGF (vascular endothelial growth factor), IL-8 Groβ, nitric oxide (NO), erythropoietin, and other inflammatory agents or pharmacological modulation (24).

Morrone's research showed a low EPC level in patients with stable CAD (chronic ischemic heart disease) compared with patients without CAD. Besides, the value of the EPC density is higher for patients with CAD than for non-CAD; this emphasizes that EPC's mobilization and homing occur in CAD patients (26). From a prospective study by Morrow, it is shown that the decrease of EPC increased mobilization and homing to the site of vascular injury that occurs in CAD.

As reported from the previous study, SOD activity in a patient with CAD decreased by 17% compared to healthy (27). In this study, there is a significant increase in SOD, which could derive from the effect of β-Glucan as an antioxidant, whereas the patient still on medication consumption (28). The mechanism was involved in the increased activity of SOD was modulate via MnSOD-related angiogenesis. Dectin-1 was expressed by endothelial cells engaged with β-Glucan and induce MnSOD expression via histone acetylation.29 Furthermore, common risk factors such as diabetes mellitus, hyperlipidemia, hypertension, aging, and smoking increase free radicals from endothelial cells, which trigger lipid oxidation, apoptosis of endothelial cells, expression of adhesion molecules, and alteration vasomotor activity (30). Subjects in this study may have atherosclerotic risk factors.

SOD, as an antioxidant enzyme, works to detoxify hydrogen peroxide and convert it to lipid hydro-peroxides to become non-toxic substances (31). Catalyzation was the first process in SOD, which initiates the dismutation of superoxide anion to hydrogen peroxide in the vascular wall to reduce oxidative stress damage. the Previous study has revealed β-Glucan can improve SOD and inhibits lipid peroxidation in animal models (32) and from in-vitro studies revealed a protective effect against damage induced by H2O2 and Trp-P-2 (33).

MDA was one of the lipid peroxidation products and one of the oxidative markers. Increasing MDA levels will enhance the production of free radicals and a reduction of antioxidant activity. In this study, there are significantly reduced MDA levels, following the study by Sener et al. showed that β-Glucan could lower MDA level (34). Therefore, β-Glucan could be considered therapeutic agents because they can attenuate the oxidant's deterioration effect.

The marker's inflammatory study results show a significant decrease in pre-test IL-6, TNF-α, and CRP. It is probably due to the polysaccharide peptide (PSP) originating from the mycelium Ganoderma Lucidum acting as an anti-inflammatory, inhibiting the NF-kB activation pathway. This study supported several results of previous studies that stated that polysaccharide peptides derived from Ganoderma Lucidum could be anti-inflammatory (35,36).

Previous research has shown that β-glucan has a good effect on innate and adaptive immunity — the innate immune system can quickly recognize and respond to pathogens' entry, useful for controlling the infection. Dectin-1 is a type 2 transmembrane protein receptor that binds to β-1,3 and β-1,6 glucan, which can initiate and regulate the immune response. Dectin-1 express in cells responsible for innate immune responses found in macrophages, neutrophils, and dendritic cells. The dectin-1 working mechanism through ITAM, TLR-2/6, and Syk pathway will activate T cells, which will cause the release of cytokines. Cytoplasmic. In this study, it proved that the PSP extract of Ganoderma Lucidum through the active compound β-glucan was able to inhibit Dectin-1 in macrophages and thus inhibit activation of the Nuclear Factor kappa B (NF-kB) transcription factor, which can then inhibit activation of inflammatory cytokines such as IL-6, TNF-α, and hs-CRP. Overall, by inhibiting NF-ƙB and the inflammatory process, administration of PsP can ultimately prevent and slow down the process of atherogenesis (36).

In general, beta-glucan activates via the NF-kB pathway, JNK-MAPK, PI3K / Akt, JAK-STAT, TLR 2/6, and ITAM, while from the receptor side are dectin-1, CR-3, lactoseril ceramide, and Langerin receptor (37)

Research conducted by Verma et al. Shows that increasing CRP (C Reactive Protein) direct reduces differentiation, function, and survival of EPC, which is a critical component of angiogenesis and the response to chronic ischemia by significantly increasing EPC apoptosis and disrupting EPC induces angiogenesis. It also inhibits the expression of specific endothelial markers such as Tie-2, EC-lectin, and VE-Cadherin. This mechanism of CRP through CRP decreases eNOS expression by EPC and interferes with EPC antioxidant defense, antioxidant sensitivity EPC, and telomerase inactivation (38,39). Contrary to this, a study conducted by Fasing et al. found that there was no relationship between changes in CRP levels in EPC dysfunction in healthy people.40 What is interesting is that EPC levels increased in patients with unstable angina, but there is no adhesion disorder compared to patients with stable coronary heart disease (Stable CAD) (41).

Witztum et al., In their study, demonstrated that CRP increases the uptake of ox-LDL and not LDL native (42). Correlated with this, especially in LDL, from several studies it has shown that LDL native will bind to T-cadherin protein and activate intracellular pathways via ca (2 +) - tyrosine kinase-ERK1 / 2 and activation of small G-proteins by further reorganizing actin and affecting the interaction of epithelial cell attachments. T cadherin finds to increase atherosclerotic lesions and post-angioplasty restenosis associated with pathological angiogenesis (43).

Interestingly, LDL binds to T-cadherin and adiponectin, which activates NF-kappa B through AdipoR1 and AdipoR2 receptor paths (44).

This study revealed better improvement in endothelial function, which decreased CEC, which was strengthened by a better profile of anti-inflammatory and antioxidant properties, either adiponectin but unfortunately, EPC decreased.

Conclusion

Polysaccharide Peptide of Ganoderma Lucidum acts as a potent anti-endothelial dysfunction against atherosclerosis's pathogenesis in stable angina with proof from this study showed decreased CEC value, but unfortunately, EPC decreased too.

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Author's contributions: NU, DS, SA; Literature search, study design and patient selection, data collection and analyzes, **NU;** Article preparation and revisions

Ethical issues: All authors declare originality and ethical approval of research. The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Prediction of prognosis with neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, and pathological parameters in operated gastric cancer

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Abstract

Objective: Inflammatory markers are of prognostic importance in many malignancies. This study aimed to examine the effects of neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and pathological parameters on survival in preoperative complete blood counts in patients with operated gastric cancer.

Material and Methods: Between 2012 and 2017, 281 patients were analyzed after total/subtotal gastrectomy. According to the ROC curve, we determined the cut-off values for NLR as 2.5 and PLR as 158. Overall survival (OS) was calculated from surgery to the last interview or to death.

Results: In univariate analysis age ⩾ 55 (p = 0.028), non-adeno hystology (p = 0.003), lenfovascular invasion (LVI) positivity (p = 0.003), perineural invasion (PNI) positivity (p < 0.001), T 3-4 stage (p = 0.006), lymph node involvement (LN) 2-3 (p < 0.001), metastatic stage (p < 0.001), NLR ⩾ 2.5 (p<0.001) and PLR ⩾ 158 (p < 0.001) were statistically significant for OS. In multivariate analysis age (HR 0.652, 95% CI: 0.475-0.895; p = 0.008), PNI positivity (HR 0.493, 95% CI: 0.337-0.720; p<0.001), more lymph node involvement (HR: 0.608, 95% CI:0.412-0.896, p = 0.012), metastatic stage (HR 0.377, 95% CI: 0.265-0.537; p < 0.001) and PLR ⩾ 158 (HR: 0.610; 95% CI: 0.433-0.859; p = 0.005) were found to be independent prognostic factors affecting OS.

Conclusion: Age ⩾ 55, PNI positivity, more lymph node involvement, metastatic stage, and PLR ⩾ 158 are independent prognostic factors for shorter overall survival. Given the high morbidity and mortality of gastric cancer, besides classical known prognostic factors, parameters such as preoperative PLR may have benefits for forecast the prognosis of gastric cancer.

Keywords: Gastric cancer, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, prognosis

Introduction

Gastric cancer is the fifth most common malignancy worldwide (1). Surgery is considered the main curative treatment for gastric cancer, but survival is fairly low (2). Even if gastric surgery is performed, distant metastasis or local recurrence will be seen in approximately 35-70% of patients within 5 years (3). The TNM stage is the gold standard for predicting the prognosis of patients with gastric cancer, still. However, the TNM stage is determined postoperatively. Laboratory parameters showing systemic inflammation were researched as prognostic biomarkers in many cancers (4-6). Compared to other factors, NLR and PLR are easily, routinely, and almost cheaply achieved. Some indexes of inflammatory cells, such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), have become prognostic factors and are used to predict survival (7).

With the reduction of vascular perfusion, the endothelium is activated, which causes inflammation. Pro-inflammatory factors are secrete from platelets, which also contributes to inflammation and tumor progression (8). Neutrophils have different duties in the process from the onset of the tumor to its growth and metastasis. Tumor manufactures granulocyte colony-stimulating factor (G-CSF) and the number of neutrophils in the peripheral blood increases (9). One of the highest tumor burden sicknesses is gastric cancer. Blood count parameters such as PLR can be used to predict survival along with known prognostic factors in patients with operated gastric cancer. We aimed to be able to forecast the prognosis with simple assays that can be performed before surgery and with clinicopathological parameters after surgery in this study.

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Material and Methods

The study was approved by the Ethical Committee of the our hospital and complied with the standards of the Declaration of Helsinki (1993/2019). This was a retrospective study. The inclusion criteria as follows: (1) Karnofsky performance status being ⩾ 80; (2) not in the metastatic stage. The exclusion criteria as follows: (1) another synchronous or metachronous malignity (2) chronic inflammatory disease (3) blood transfusion before surgery (4) receive neoadjuvant chemotherapy. Between 2012 and 2017, 281 patients were included in this retrospective study after total/subtotal gastrectomy. The patients were followed at the Department of Radiation Oncology at the University of Health Sciences, Istanbul Training, and Research Hospital. Radiotherapy was planned three-dimensional conformal radiotherapy (3D-CRT) or intensity-modulated radiotherapy (IMRT). After gastric surgery for radiotherapy, tumor bed and nodal volumes were structured with preoperative/postoperative imaging and surgical clip location. If lymph nodes were involved, for all resection and anastomosis fields, and nodal drainage areas uses to 45 Gy radiotherapy doses. If surgical margins positive, the dose is raised to 50.4 Gy to the surgical bed or at risk areas. Systemic chemotherapy as infusional 5-fluorouracil and orally capecitabine were administered. In the first two years, patients were watched over with three monthly periods and then each six months in the third year and annually afterward. For complete blood counts was used XN-900 hematology analyzer (Symex, Japan). The normal reference range for neutrophils 1,56-6,13 × 109 / L, for lymphocytes to 1.18–3,57 × 109 / L and platelets for 142-424 × 109 / L. The NLR and PLR respectively were found as follows: NLR = Neutrophil / Lymphocyte; and PLR = Platelet / Lymphocyte. Complete blood count was performed before surgery.

Statistical analysis

Categorical variables were analyzed using the Pearson Chi-square test, and non-categorical variables were analyzed using Fisher's exact test. The relationship between NLR, PLR, and pathological parameters were evaluated by ROC curves, Kaplan-Meier analyses, and Cox regression survival analyses and used to calculate overall survival (OS) characteristics. OS was calculated from surgery to the last interview or to death. The SPSS 22.0 (Chicago, IL, USA) program was used in all analyses. A p-value of <0.05 was considered statistically significant.

Results

According to the ROC curve, we determined the cut-off values for NLR 2.5 (AUC: 0.687; 95% CI: 625-749; p<0.001) and the cut-off values for PLR 158 (AUC:0.648; 95% CI: 583-714; p<0.001). The median age was 57 (range:22-80). Female/male ratio of the patients was:78/203, approximately 1/3. There were 178 (63%) patients diagnosed with adenocancer and 103 (rignet cell:84, mucinous type:19) patients of other hystological subtypes. The location of tumor was antrum (n=80; 29%), small curvature (n=76; 27%), corpus (n=49; 17%), cardia (n=45; 16%), pilor (n=19; 7%) and big curvature (n=12;4%), respectively. Two hundred fourty-eight (78 %) patients had total gastrectomy and 63 (22 %) patients had subtotal gastrectomy. Postoperative lenfovascular invasion (LVI) positive patients were 210 (75 %) and perineural invasion (PNI) positive patients were 193 (69 %). There were patients at the T1-2 stage (n=36; 13%) and T3-4 stage (n=245; 87%); patients at the N0-1 stage (n =96; 34%) and N2-3 stage (n=185; 66%). Metastasis present patients were 119 (42%) and metastasis absent were 162 (58%). There were TNM stage 2 (n=87; 31%) and TNM stage 3 (n=194; 69%) patients. The radiation was applied by 1.8 Gy fractions/day 45 Gy in 25 fractions in 269 (96%) patients and 1.8 Gy fractions/day 50.4 Gy in 28 fractions in 12 (4%) patients. All patients were completed radiotherapy. FUFA (5-Fluorourasil and calcium folinate) regimen was administred 222 (79%) patients, infusional 5-fluorouracil was administered to 32 (12%) patients and orally capecitabine in 21 (7%) patients as a cycle before and after radiotherapy (RT). There were 6 (2%) patients have not receiving (because of advanced age) concomitant chemoradiotherapy (CCRT). And all advanced age patients were performed radiotherapy. The most common metastasis was to the peritoneum (n=37), liver (n=36) and lung (n=16), bone (n=11), lymph nodes (n=10) and others (n=9) respectively. Patients overall survival rate was 54 % for 2-years and 28 % in 5-years.

When the patients were evaluated according to NLR ⩾ 2.5 and NLR < 2.5 levels; LVI positivity (p=0.014), advanced T stages (p=0.004), LN 2-3 (p < 0.001), metastatic stage (p<0.001) were statistically significant.

When the patients were evaluated according to PLR ⩾ 158 and PLR < 158 levels; the metastatic stage (p=0.001) were statistically significant. There was no statistical significance found between other parameters and PLR. Age, gender, histology, tumor differentiation, and PNI were not statistically significant in both NLR and PLR groups. The general demographic comparisons of patients according to NLR and PLR values are seen in Table-1.

In univariate analysis; age ⩾ 55 (p = 0.028), other hystology (p = 0.003), LVI positivity (p = 0.003), PNI positivity (p < 0.001), T 3-4 stage (p = 0.006), LN 2-3 (p < 0.001), metastatic stage (p < 0.001), NLR ⩾ 2.5 (p < 0.001) and PLR ⩾ 158 (p < 0.001) were statistically significant for OS.

In multivariate analysis, age (HR: 0.652, 95% CI: 0.475-0.895; p = 0.008), PNI positivity (HR: 0.493, 95 % CI: 0.337-0.720 p< 0.001), more lymph node involvement (HR 0.608, 95 % CI: 0.412-0.896; p= 0.012), metastatic stage (HR: 0.377, 95 % CI : 0.265-0.537 p <0.001) and PLR ⩾ 158 (HR: 0.610, 95%CI 0.433-0.859; p = 0.005) were found to be independent prognostic factors affecting OS (Table-2).

For patients with PLR ⩾ 158; 2-year survival was 45%; 5-year survival was 16% (Figure-1).

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Table 1. The general demographic comparisons of the patients according to NLR and PLR values. (LVI: Lenfovascular invasion, PNI: Perineural invasion, T: Tumor, N: Lymph node)

		NLR<2,5 (n %)	NLR≥2,5 (n %)	p value	PLR<158 (n %)	PLR≥158 (n %)	p value
Age	< 55	49 (40)	70 (44)	0.452	56 (42)	63 (43)	0.777
	≥ 55	74 (60)	88 (56)		79 (58)	83 (57)	
Gender	Female	32 (26)	46 (29)	0.565	36 (27)	42 (29)	0.694
	Male	91 (74)	112 (71)		99 (73)	104 (71)	
Hystology	Adeno	79 (64)	99 (63)	0.786	83 (62)	95 (65)	0.533
	Others	44 (36)	59 (37)		52 (38)	51 (35)	
Tumor differantiation	Well	14 (11)	12 (8)		15 (11)	11 (8)	
	Moderately	54 (44)	56 (35)	0.114	52 (39)	58 (39)	0.585
	Poorly	55 (45)	90 (57)		68 (50)	77 (53)	
LVI	negative	40 (33)	31 (20)	**0.014**	41 (30)	30 (21)	0.058
	positive	83 (67)	127 (80)		94 (70)	116 (79)	
PNI	negative	46 (37)	42 (27)	0.052	46 (34)	42 (29)	0.338
	positive	77 (63)	116 (73)		89 (66)	104 (71)	
T stage	1	4 (3)	4 (3)		5 (4)	3 (2)	
	2	21 (17)	7 (4)	**0.004**	18 (13)	10 (7)	0.184
	3	52 (42)	70 (44)		59 (44)	63 (43)	
	4	46 (38)	77 (49)		53 (39)	70 (48)	
N stage	0	33 (27)	14 (9)		28 (21)	19 (13)	
	1	27 (22)	22 (14)	**<0.001**	23 (17)	26 (18)	0.316
	2	29 (23)	42 (27)		30 (22)	41 (28)	
	3	34 (28)	79 (50)		53 (40)	60 (41)	
M stage	absent	99 (81)	63 (40)	**<0.001**	91 (67)	71 (49)	**0.001**
	present	24 (19)	95 (60)		44 (33)	75 (51)	

Table 2. Univariate and multivariate analyses of factors for the prediction of overall survival. (LVI: Lenfovascular invasion, PNI: Perineural invasion, T: Tumor, N: Lymph node, NI: Not included)

		Univariate HR (95% CI)	p value	Multivariate HR (95% CI)	p value
Age	<55	1		1	
	≥55	0.711(0.524-0.963)	**0.028**	0.652(0.475-0.895)	**0.008**
Gender	Female	1			
	Male	0.933(0.669-1.302)	0.684		NI
Hystology	Adeno	1		1	
	Others	0.635(0.471-0.854)	**0.003**	0.772(0.561-1.062)	0.112
Tumor differantiation	Well	1			
	Moderately	1.102(0.605-2.005)	0.751		
	Poorly	1.732(0.972-3.084)	0.062		NI
LVI	negative	1		1	
	positive	0.578(0.400-0.834)	**0.003**	0.881(0.593-1.307)	0.528
PNI	negative	1		1	
	positive	0.451(0.316-0.642)	**<0.001**	0.493(0.337-0.720)	**<0.001**
T stage	1-2	1		1	
	3-4	0.478(0.281-0.811)	**0.006**	1.053(0.594-1.866)	0.861
N stage	N0-1	1		1	
	N2-3	0.428(0.302-0.606)	**<0.001**	0.608(0.412-0.896)	**0.012**
M stage	absent	1		1	
	present	0.288(0.211-0.394)	**<0.001**	0.377(0.265-0.537)	**<0.001**
NLR	NLR<2.5	1		1	
	NLR≥2.5	0.502(0.367-0.687)	**<0.001**	0.971(0.669-1.410)	0.879
PLR	PLR<158	1		1	
	PLR≥158	0.549(0.406-0.741)	**<0.001**	0.610(0.433-0.859)	**0.005**

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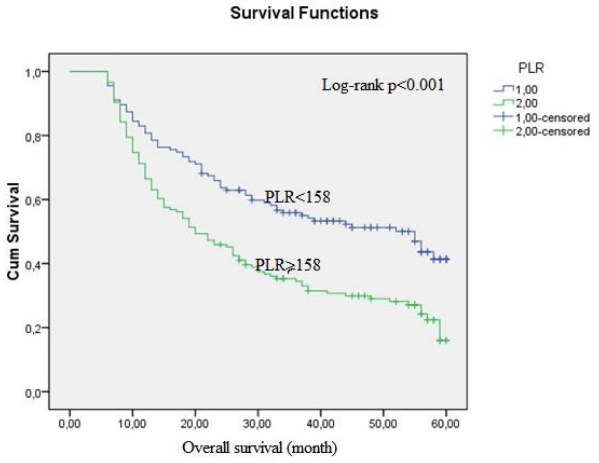

Figure-1. OS for PLR < 158 and PLR ⩾158.

Discussion

The idea that age is a prognostic factor for gastric cancer is contentious. It is most commonly seen in the 50-70 age range (10). In a study of 7762 diseases showing the effect of age on the prognosis of patients with operable gastric cancer, the prognosis was shown to be better in patients diagnosed between 56 and 65 years of age (11). Age was found as a prognostic factor in our study too.

We know that prevalence of gastric cancer is higher among the male than female(12). In our patients, the ratio was in favor of males, too. The WHO classification (13) was used for histopathological classification. And we found 63 % of patients were in adenocarcinoma histology. And when subtypes were evaluated, we did not achieve a statistically significant result that would affect the prognosis.

In Feng et al's study with 3090 patients who treated surgery with a diagnosis of gastric cancer, it was shown that the differentiation status had no prognostic value (14). In this study, we were not found an association with differentiation status and prognosis.

Despite adjuvant treatments administered after surgical treatment, it has not been reported long survival in gastric cancer. This is because most patients have lymph node metastasis or micrometastases during diagnosis. LVI has been described as a beneficial predictor for lymph node involvement or distant metastasis. Lymphovascular invasion was determined to be an independent prognostic factor in many of the studies (15-17). In our study, in univariate analysis lymphovascular invasion was statistically significant, but not prognostic.

In patients with gastric cancer, the stage T, PNI and positive lymph nodes are independent indicators of poor prognosis. More aggressive postoperative treatments should be recommended in PNI-positive patients after surgery (18,19). In our research advanced stage (T3/T4) and the positivity of PNI was statistically important in univariate analysis. But only PNI positivity was found prognostic.

Jiao et al's research, the involvement of lymph nodes in patients with gastric cancer is one of the principal risk factors for poor prognosis. And mostly recognized which is related to recurrence of tumors (20). The involvement of the lymph nodes has been found to be an independent prognostic factor for OS, in our research too.

A systemic inflammatory response is known to be associated with poor outcomes in many types of cancer. It's not too clear to forecast the recurrence and prognosis of gastric cancer in gastrectomy patients. The mechanisms caused by the inflammatory response are still not absolutely known. Inflammation and cancer are connected each other (21). The tumor microenvironment is extremely significant in carcinogenesis and supports tumor proliferation and dissemination (22,23). Tumor cells and tumor-associated leukocytes can produce various inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin-6, and vascular endothelial growth factor. These have strong effects on cancer growth, invasion, and metastasis (24). Cancer-related inflammation can activate regulatory T cells and chemokines that suppress antitumor immunity (25,26). Inflammation is related to, lymphocytopenia, neutrophilia, thrombocytosis, and leukocytosis (27,28).

Yu et al's research of 291 patients, in univariate analysis, showed that T-stage, N-stage, TNM stages, and high NLR ⩾ 3.5 were statistically significant. NLR was identified as an independent prognostic factor in multivariate analysis (29). And we also identified these three factors statistically significant in univariate analysis. In multivariate analysis, we found advanced N stage as independent prognostic factors.

Deng et al's study involving 389 patients who underwent gastric surgery found that preoperative NLR ⩾ 2.3 and PLR ⩾ 132 were associated with poor prognosis in a significant (30).

Jiang et al's study of 377 non-advanced resectable gastric cancer patients; NLR ⩾ 1.4 and PLR ⩾ 184 values are analyzed. The NLR was found to be the prognostic factor for predicting overall survival (31).

Zhang et al's recent study involving 182 patients showed that NLR ⩾ 2.8 is a prognostic factor in overall survival and PLR ⩾ 172 also has predictive value (32). In our study, only PLR was independent prognostic factor for OS.

Cao et al., in gastric cancer meta analysis PLR correlated meaningful with prognosis, but there was no statistical difference between NLR and prognosis (33). But they noted that the study design, the country in which the study was conducted, sample size, treatment methods, and cut values of PLR can be affected by the quality of the study.

Gu et al., they noted that high PLR ⩾154 level is an independent risk factor for poor prognosis in patients with gastric cancer, and that PLR can predict the response of adjuvant chemotherapy (oxaliplatin / 5-fluorouracil combination) in patients with gastric cancer after surgery (34).

In the meta-analysis, which included 49 studies (51 cohorts) with 28,929 gastric cancer patients, they not only investigated the prognostic value of PLR ⩾ 160 for OS and DFS, but also investigated the relationships between PLR

and gastric cancer clinicopathological characteristics. This analysis showed that high PLR led to a higher risk of lymph node metastasis, increased risk of T stage, and advanced TNM stage risk in patients with gastric cancer (35).

In a study conducted by Zhou and colleagues for 451 patients with operated gastric cancer, preoperative PLR ⩾ 167 was identified as an independent prognostic factor, as in our study(36).

The 5-year survival rate of patients is less than 20-30%. Our patient's survival rate was 28 % in 5-years. The estimated survival time was 29 months (95% CI:21.90-36.09).

The main constraints of our study were that it was retrospective, small sample size and single-centered.

Conclusion

Age, PNI positivity, more lymph node involvement, metastasis present and PLR ⩾ 158 are independent prognostic factors for shorter overall survival. Our findings demonstrated that preoperative higher PLR values may be predictors for gastric cancer patients. Given the high morbidity and mortality of gastric cancer, besides classical known prognostic factors, parameters such as preoperative PLR may have benefits for forecast the prognosis of gastric cancer.

Ethical approval: The study was approved by the University of Health Sciences, Istanbul Training and Research Hospital, Ethics Committee.

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Conflict of interest and financial disclosure: The authors declare that there is no conflict of interest and financial relationships.

Author's contributions: OM: Study design, **BI:** Literatur search, **OM,BI:** Material preparation, OM,BI: Data collection, **BI:** Statistics, **OM:** Manuscript preparation and revisions.

Ethical issues: All authors declare originality and ethical approval of research. The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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A rare, but to be considered disease, in the differential diagnosis of abdominal pain: Mesenteric Panniculitis

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Abstract

Abdominal pains are one of the major topics of emergency clinics. Mesenteric panniculitis (MP) is one of the rarely encountered causes of abdominal pain and is known as an idiopathic disease with a good prognosis. MP may cause abdominal pain, abdominal mass and intestinal obstruction. Infections, autoimmune diseases and abdominal traumas may trigger MP. The disease may coexist with malignancy and has a high probability of developing malignancy in MP patients. In this case report, a 59-year-old female patient, who came to our Internal Medicine Outpatient Clinic with abdominal pain and who was diagnosed with MP, is presented.

Keywords: Mesenteric Panniculitis, Mesenteric Weber-Christian disease, Abdominal pain, Mesenteric lipodystrophy

Introduction

Mesenteric panniculitis (MP) is a non-specific inflammatory disease of the mesenteric fatty tissue (1). Its prevalence was reported to be at a rate of 0.16-3.3% (2). It is mostly seen in men and the ratio of men to women is 2-3/1. The disease is frequently seen in patients at an age of about 50-60 years. The clinical picture varies widely, from incidentally caught asymptomatic ones to cases with mass(es) detected in the abdomen (3). Initially, it was defined by Jura in 1924 and is known by the names mesenteric lipodystrophy, retractile mesenteritis, liposclerotic mesenteritis, or mesenteric Weber-Christian disease (4).

Material and method

A 59-year-old female patient presented to our Internal Medicine Outpatient Clinic with blunt abdominal pain with pain scale severity between 6 and 7. It had been happening for three days and the severity of the pain was gradually increasing, comprising the upper quadrants and the periumbilical area, and with a dull feeling of nausea. She denied having any other complaints, such as lack of appetite, fatigue, vomiting, fever, diarrhea, constipation, or distention.

The patient, who stated that she was defecating regularly every day, did not have any urinary symptoms. She had been diagnosed with Hashimoto thyroiditis, hypertension, hypercholesterolemia, and psoriatic arthritis in her history and she had undergone cholecystectomy 6 years ago.

The patient stated that she had given a normal, healthy birth 25 years ago and that she has not undergone any gynecological disease.

There was no history of alcohol consumption and smoking. There was no severe disease in her family history. She was on the following medications: Levothyroxine 100 mg qd, amlodipine 10 mg qd, nebivolol 5 mg qd, atorvastatin 10 mg 1x1, methotrexate 10 mg per week.

On physical examination, blood pressure was 110/70 mm Hg, heart rate was 84/min and fever was 37.2 °C. The epigastrium and umbilical area were sensitive on abdominal examination. However, there was no defense or rebound and bowel sounds were hyperactive. There were no pathological findings.

As a first step blood and urine tests were done. The patients laboratory test results are shown in Table 1. Urinalysis was normal.

The abdominal computed tomography (CT) scan showed hypodense nodules (cyst) in the liver, the largest of which was measured 7 mm.

CT findings showed the mesenteric fatty planes with paramedian localization at the abdominal midline showing signs of panniculitis, and atherosclerotic changes were determined at the main vascular structures (Image 1).

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Table 1: Laboratory findings of the Patient

	Patient's results	Normal range
ALT	25 U/L	20-40 U/L
AST	17U/L	20-40 U/L
GGT	18 U/L	0-45 U/L
Pancreatic Amylase	24 U/L	20-80 U/L
Lipase	31 U/L	10-140 U/L
C-Reactive Protein	8.4 mg/L	0,5 mg/L
Leucocyte	4.47 x10^3/uL	4,1-11,1 x10^3/uL
Haemoglobin	13.1 g/dL	11,7-15,5g/dL
Haematocrit	39%	35-45%
Thrombocyte:	240,000 /microliter	150,000-400,000/microliter

U/L:unit/liter , mg/L:milligram/liter , g/L:gram/liter , g/dL:gram/deciliter, Alanine Aminotransferase (ALT), Aspartate Aminotransferase: (AST), Gamma Glutamyl Transferase (GGT)

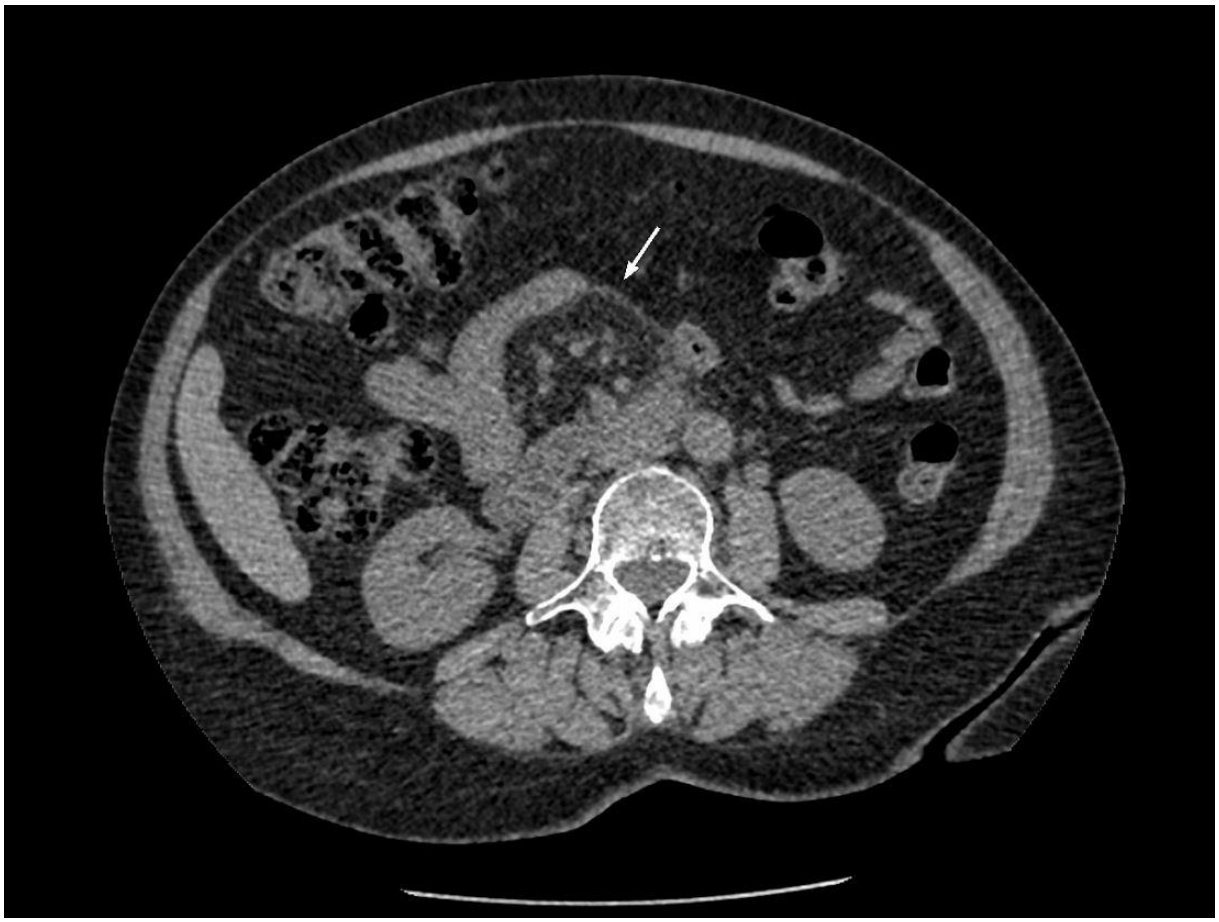

Figure 1: View of Mesenteric Panniculitis in Patient's Computed Tomography. (Arrow shows 'Fat Ring Sign')

Discussion

Anatomically, the mesenterium is a structure extending from the duodenojejunal flexura to the mesorectum. MP is an IgG4-related auto-immune disease progressing with the inflammation of the fatty tissue (5).

The pathogenetic mechanism of the disease could not be fully understood. However, it is observed together with an agent that it is triggering non-specific inflammation in the adipose tissue. These triggers may be rheumatoid and auto-immune diseases such as primary sclerosing cholangitis, rheumatoid arthritis, Sjogren's syndrome.

Other causes are infections such as typhoid fever, tuberculosis; peptic ulcer, gallbladder stone, abdominal trauma, abdominal surgery, and malignancies such as lymphoma and mesenteric tumors. Malignant diseases which cause MP most commonly are breast cancer, non-Hodgkin lymphoma, colon cancer, and lung cancer (4,5).

The findings of the patients are variable. The patients may be asymptomatic or may show findings such as abdominal pain, nausea, vomiting, constipation, diarrhea, intestinal obstruction and palpable abdominal mass.

The findings of the disease may present within a few days or may progress as a chronic disease for several years. In different clinical series, abdominal pain (70%), distention and gas (26%), and diarrhea (25%) are the most common symptoms and 10-27% of the patients were reported to be asymptomatic (1).

In our case, the main symptom was an abdominal pain and slight nausea. The patient had auto-immune diseases, such as Hashimoto thyroiditis and psoriatic arthritis. In previous cases, auto-immune diseases such as Riedel thyroiditis, Sjögren's syndrome, and rheumatoid arthritis was reported to coexist with the disease. In the laboratory tests, the C-Reactive Protein (CRP) result was slightly higher than its normal value. Thereforer, we can differentiate her condition from a severe acute abdomen. The most common positive laboratory finding observed in MP is high sedimentation rate and a high CRP (4).

For the diagnosis of the disease, ultrasonography is generally not sufficient. Thickening of the mesenteric root and a slight decrease in echogenicity may be determined. The diagnosis is generally established by means of CT. Mesenteric involvement is its typical symptom and further signs of MP are an increase in the density of the mesentery with intercalated nodules, a "fat-ring sign" around vessels in the form of a halo, the formation of a pseudocapsule and the displacement of bowel loops. Magnetic resonance imaging (MRI) findings are similar to those of CT (6). In our case, the diagnosis was reached by CT. The patient's pain declined with symptomatic therapy. Although the pain in this disease responds well to corticosteroid treatment in a short time, this was not necessary for our patient. Apart from the treatment of the accompanying diseases, surgical intervention may be necessary in cases of obstruction. However, even after surgical interventions, there is a probability of recurrence of panniculitis (5).

In MP, it is important to exclude malignancy. Along with the suggestions to handle MP in patients like a paraneoplastic syndrome, there are also publications asserting that the probability of the development of malignancy increases about 5 times (7).

Differential diagnosis is very important. Since the presenting symptoms of the disease are not specific, it can be misdiagnosed. MP may present itself with intestinal obstruction or abdominal mass. If the patient is misdiagnosed, it may result in a surgical operation. Although rarely, in some MP patients presented with intestinal obstruction ; a surgery intervention is necessary. But it should also be noted that only a small portion of these patients needed no additional treatment (8).

Therefore, after the regression of the patient's acute condition, tests were planned in order to investigate underlying diseases or malignancies. No pathological finding was observed on autoimmune markers, gynecologic tests, mammography, positron emission tomography (PET) scan, gastroscopic and colonoscopic examinations.

Conclusion

Although MP is generally a chronic mesenteric inflammation, the patients may present with an acute condition such as abdominal pain, nausea, vomiting, diarrhea, abdominal mass, and bowel obstruction. Since it is a rarely encountered disease, it may initially not come to mind in the differential diagnoses. When there is no guiding symptom in the abdominal ultrasonography, which is the method of analysis most frequently used by us at the emergency station as first step, it should be kept in mind that in the evaluation of these patients CT is especially of significance and CT scan should be made as soon as possible for the necessary cases.

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