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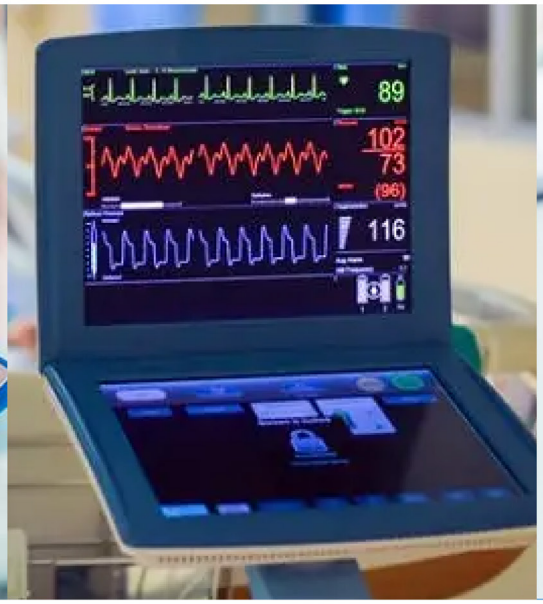

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EDITORIAL

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As of today, we are experiencing the happiness and excitement of our journal named "Intercontinental Journal of Internal Medicine". Our journal is an international peer-reviewed journal that publishes original articles, case reports, reviews, letters to the editor, short reports and original images on Internal Medicine. There are five articles in this issue of our journal. You can access all of the articles published in our journal electronically, read and download them from our website. To all our colleagues who are interested in our journal, both as writers and readers, to evaluate the publications.

I would like to thank our editors and referees for their efforts to bring the process to a conclusion as soon as possible and to raise the quality bar of our journal.

Sincerely Yours,

Alpaslan Tanoglu, MD, PhD, Associate Professor
Editor-in-Chief

The Intercontinental Journal of
Internal Medicine

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Original Article

Retrospective evaluation of platelet indices and RAS mutations in patients with colon cancer

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ABSTRACT

Aims: Colon cancers are one of the most common cancer types in the world and cause significant mortality. The presence of RAS mutation is also associated with poor prognosis in colon cancers and plays an important role in the choice of treatment. Platelet indices, which have recently been evaluated with an easy-to-reach method, have been discussed as an inflammatory marker in many studies. In this study, it was aimed to investigate the relationship between platelet distribution width (PDW), colon cancer stage and the presence of RAS mutation.

Methods: File records of 132 patients who were followed up with the diagnosis of colon cancer in Kırıkkale University Faculty of Medicine, Department of Internal Medicine and Medical Oncology Department between January 1, 2015 and January 1, 2021 were retrospectively analyzed. Patients with RAS mutations and who did not receive chemotherapy were included in the study. Patients with and without RAS mutation were divided into two groups and their platelet index (especially PDW) values were compared. PDW values were compared according to the stages of the patients. The correlation between stage and PDW was examined.

Results: Of the 132 patients included in the study, 82 (62.1%) were male and 50 (37.9%) were female. Participants; 12 (9.1%) stage 1, 23 (17.4%) stage 2, 29 (22%) stage 3, 68 (51.5%) stage 4 colon cancer patients. There were no RAS mutations in 73 (55.3%) patients, and 59 (44.7%) patients had RAS mutations. There was no significant difference in PDW between the two groups with negative and positive RAS mutations (p=0.826). According to the stages; PDW values were significantly different between the four stages (X2=9.878, p=0.020).

Conclusion: In the study, PDW increased as the stage increased, but there was no significant relationship between RAS mutation and PDW. This was attributed to the fact that as the stage increases, the level of inflammation may be associated with an increase. Larger studies on this subject are needed.

Keywords: Platelet indices, colon cancer, RAS mutation

INTRODUCTION

Cancer ranks second among the diseases that cause death, after cardiovascular diseases in Turkey as in many countries.[1] The most common malignancy among gastrointestinal tract cancers is colon cancers. Colorectal cancer (CRC) is the third most frequently diagnosed cancer in men; It is the second most common cause of cancer-related deaths in women after breast cancers, and the third most common cause of cancer-related deaths in the world.[2] According to Surveillance Epidemiology and Results (SEER) in CRC, 5-year survival rate is associated with stage and is 90% in local cancers, 72% in regional cancers and 14% in metastatic cancers.[3]

The Kirsten rat sarcoma (K-RAS) gene is a GTP-dependent membrana protein that acts as a proto-oncogen. It is one of the genetic pathways in the development of CRC.[4] Ras mutations are not only seen in CRC; they are also seen in different incidences in other types of cancer. The most common type of cancer is pancreatic cancers (90%), and this mutation is seen in the rate of lung adenocarcinomas (30%) in CRC (50%), thyroid tumors (50%) and myeloid leukemia (30%).[5] If no RAS mutation is detected in patients with metastatic CRC, it is called wild-type CRC. According to the result of Ras mutation, the chemotherapy protocols given vary. The addition of drugs such as cetuximab and panitumumab (anti-eGFR) epidermal growth factor receptor inhibitors to standard chemotherapy in wild-type CRC has been shown to prolong survival in many previous studies, and it is important for the treatment plan to perform K-RAS and N-RAS mutation analysis before the treatment plan is made.[6]

Many epidemiological studies show that inflammation is also involved in the development of cancer. Cells involved in systemic inflammation are white blood cells and platelets, which also serve as an important risk factor for cancer development. Neutrophils, monocytes, and platelets increase cancer cell proliferation, invasion, and metastasis. FAP and its variants (Gardner syndrome, Turcot syndrome, and

attenuated familial adenomatous polyposis [AFAP]) occur in less than 1% of all CRCs. Mutation in the adenomatous polyposis coli (APC) gene is responsible for the formation of FAP. Typical FAP usually begins to appear in childhood; it can present symptoms at age 16, and 90% of untreated people develop CRC by age 45. AFAP is diagnosed at a later age than FAP and is diagnosed with cancer later (mean age 44 years for FAP and 58 years for AFAP, respectively).[7] Gardner syndrome includes FAP colonic features as well as extracolonic features (desmoid tumors, epidermoid cysts, lipomas, osteomas (especially in the mandible), fibromas, excess teeth, nasopharyngeal angiofibromas).[8] Turcot syndrome is associated with familial colon cancer and brain tumors (primarily medulloblastomas and gliomas).[9]

Peutz-Jeghers Syndrome is manifested by hamartomatous polyps of the gastrointestinal tract and the classic appearance of mucocutaneous melanin pigmentation. Both the gastrointestinal tract and the extra-intestinal system may increase the risk of malignancy. Juvenile polyposis syndrome is autosomal dominant and has an increased risk of CRC. Patients with juvenile polyposis syndrome have a 39% lifetime risk of developing CRC.[10]

Inflammatory bowel diseases play a role in the development of CRC. The risk of cancer is increased in both ulcerative colitis and Crohn's patients. While the risk of CRC increases 5-15 times in ulcerative pancolitis cases, this risk is 3 times higher in ulcerative colitis cases with left colon involvement. In pancolitis cases, the duration of the disease causes a 2% increase in 10 years and an 18% increase in 30 years, in CRC development. Although there is not enough information on Crohn's patients, Crohn's pancolitis has a risk of CRC similar to ulcerative pancolitis.[11]

Smoking, high-calorie diet, excessive red meat consumption, high saturated fat, excessive alcohol consumption, obesity, and sedentary life have an important place in the risk of developing CRC. Eating foods low in fiber and consuming processed foods also increases the risk of developing CRC. High-fiber foods, aspirin, celecoxib, folic acid, increased physical activity, high-grain diet, consuming fruit and vegetable, eating foods high in calcium, selenium, some vitamins (vitamins D, A, C, E),and fish oil are protective against CRC.Long-term follow-up randomized trials have shown that daily use of 100 mg of aspirin is ineffective in preventing colon polyps, but with a delay of several years, it reduces the risk of CRC.[12]

In our study, it was aimed to compare PDW, an inflammation marker, in CRCs with and without RAS mutation. It was aimed to investigate the change in the increase of PDW with inflammation according to the presence of advanced stage and RAS mutation in CRCs.

METHODS

Study Design

This speciality thesis study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 16.06.2021, Decision No: 111). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The medical records of the patients who were followed up with the diagnosis of colon cancer between January 1, 2012 and January 1, 2021 in the Medical Oncology Polyclinic of Kırıkkale University Faculty of Medicine were examined retrospectively. The number of patients to participate in the study was determined as Clincalc.com.tr power alpha: 80%. In the study, 192 patient files were examined. A total of 132 patients were evaluated. The inclusion criteria of the patients were determined as being older than 18 years of age and being diagnosed with colon cancer, documented stage of the disease, knowledge of RAS mutation, and availability of hemogram values. The date of diagnosis was taken as the date of pathological diagnosis. By retrospectively scanning all patient records, age, gender, comorbid disease status, stage, genetic mutation analysis of the patients; K-RAS, N-RAS, neutrophil count at the time of diagnosis, lymphocyte count, platelet index parameters; platelet count, MPV, PDW, PCT, CEA values were determined. The Charlson Comorbidity Score of the patients was calculated to indicate comorbidity. Patients with and without RAS mutation were divided into two groups and hemogram parameters (especially PDW MPW values), stage, survival times were compared and the relationship between them was investigated. Survival was calculated as the time from the moment of diagnosis to death.

Statistical Analysis

Kolmogorov-Smirnov Test was used to determine the normal distribution of the independent variables obtained from the participants. ANOVA-One Way Analysis of Variance Test and Independent Samples T Test were used to analyze parametric variables ($p<0.05$). Tukey Multiple Comparison Test was applied for post hoc analysis ($p<0.05$). Kruskal-Wallis Test and Mann-Whitney U Test were used for non-parametric independent variables ($p<0.05$). Mann-Whitney U Test and Bonferroni Correction Test were used for post hoc analysis ($p<0.0083$). Pearson's Chi-square Test was used to analyze categorical data ($p<0.05$). Pearson's Rho Correlation Test was applied to test the relationship between independent variables. Overall survival was calculated as the difference between the date of diagnosis and the date of death (for any reason) or the date of the most recent information in the medical records. Survival times were estimated by the Kaplan-Meier Survival Test. Survival curves were compared with Log-rank Test, Breslow Test and Tarone-Ware Test ($p<0.05$). Cox-Regression Test was applied to determine the predictivity of independent variables affecting survival times ($p<0.05$). The ROC-Curve Test was used to test the sensitivity and specificity of independent variables that could predict mortality, and the Logistic Regression Test was applied to find the independent variable that could best predict mortality risk ($p<0.05$). The direction and strength of the relationship between study parameters and mortality risk were measured using the Odds Ratio (OR) Test and corresponding 95% confidence intervals (95% CI).

RESULTS

A total of 132 patients, 82 (62.1%) male and 50 (37.9%) female, participated in the study. 12 (9.1%) of the participants were stage 1, 23 (17.4%) of them were stage 2, 29 (22%) of them were stage 3, 68 (51.5%) of them were stage 4 colon cancer patients. 73 (55.3%) of the patients did not have a RAS mutation, and 59 (44.7%) had a RAS mutation.

Participants in the study were divided into two groups as living and exitus. When the data of the two groups were

compared, it was found that there was a difference between the groups in terms of lymph node involvement (Z=7.853, p=0.020), lymphocyte count (Z=-2.568, p=0.010), neutrophil lymphocyte ratio (Z=-2.648, p=0.008), CEA level (Z=-3.684, p <0.001) and CA 19-9 level (Z=-3.111, p=0.002). These findings revealed that lymph node involvement was higher in deceased patients (**Table 1**), lymphocyte count was lower in deceased patients, and CEA and CA 19-9 levels were higher (**Table 2**).

Table 1. Demographic characteristics of living and deceased patients

Variable	Living	Exitus	t / Z	p value
	Average±SS/ The median (min-max)/ N (%)	Average±SS/ The median (min-max)/ N (%)		
Age	62.18±12.01	66.30±14.55	-1.691*	0.093
Sex			0.384‡	0.535
Male	30 (22.7%)	52 (39.4%)		
Female	21 (15.9%)	29 (22.0%)		
Stage			7.690‡	0.053
1	8 (6.1%)	4 (3.0%)		
2	12 (9.1%)	11 (8.3%)		
3	10 (7.6%)	19 (14.4%)		
4	21 (15.9%)	47 (35.6%)		
Tumor			4.777‡	0.189
1	1 (1.4%)	0 (0.0%)		
2	3 (4.2%)	2 (2.8%)		
3	15 (21.1%)	16 (22.5%)		
4	10 (14.1%)	24 (33.8%)		
Lymph node involvement			**7.853‡**	**0.020**
0	**13 (18.8%)**	**8 (11.6%)**		
1	**10 (14.5%)**	**14 (20.3%)**		
2	**5 (7.2%)**	**19 (27.5%)**		
RAS			0.005‡	0.941
None	28 (21.2%)	45 (34.1%)		
Yes	23 (17.4%)	36 (27.3%)		
Life span (months)	58.00 (18.00-220.00)	22.30 (0.63-142.70)	-	-

(*) Independent Samples T Test; (†) Mann-Whitney U test; (‡) Pearson'S Chi-square Test; p< 0.05

Table 2. Laboratory values of living and deceased patients

Variable	Living	Exitus	t / Z	p value
	Average±SS/ The median (min-max)/ N (%)	Average±SS/ The median (min-max)/ N (%)		
WBC	8130 (3340-21730)	8470 (4300-20150)	-0.757†	0.449
HB	12.30 (8.50-15.20)	12.10 (8.00-16.20)	-0.753†	0.452
RDW	16.60 (12-26)	16.40 (13-30)	-0.435†	0.664
PNL	5120 (1100-18570)	5450 (2.84-16390)	-1.304†	0.192
Lymphocyte	**2050 (500-8300)**	**1710 (170-7200)**	**-2.568†**	**0.010**
Platelet (^10³)	304 (126-766)	301 (132-877)	-0.058†	0.953
MPW	8.80 (5.50-12.70)	8.90 (7.10-13.10)	-0.544†	0.587
PCT	0.29 (0.12-228.00)	0.28 (0.11-0.60)	-0.890†	0.373
Neutrophil-lymphocyte ratio (NLR)	**2.35 (0.50-19.33)**	**2.93 (0.00-82.12)**	**-2.648**	**0.008**
Platelet-lymphocyte Ratio	147.79 (32.40-391.34)	168.28 (48.71-1647.06)	-1.458†	0.145
CEA	**3.08 (0.72-141.70)**	**5.99 (1.03-1000.00)**	**-3.684†**	**<0.001**
CA19_9	**11.99 (0.60-1000.00)**	**20.63 (0.60-1998.00)**	**-3.111†**	**0.002**

(*) Independent Samples t test; (†) Mann Whitney U test; (‡) Pearson Chi-square test; p< 0.05

At the end of the correlation analysis applied to all data of all participants, it was observed that there was a negative correlation between life expectancy and age (r=-0.219, p=0.012), tumor stage (r=-0.201, p=0.021), MPW level (r=-0.224, p=0.010), mortality level (r=-0.585, p <0.001), CEA (r=-0.340, p <0.001) and CA 19-9 level (r=-0.228, p=0.009). With these findings, it was thought that when the patient is of advanced age, the life expectancy of the patients may be shortened as the tumor stage, MPW level, CEA, and CA 19-9 levels increase. In addition, it was thought that age, tumor stage, MPW, CEA and CA 19-9 levels could be predictors of life expectancy. In addition, there was a positive correlation between mortality rate and age (r=0.201, p=0.021), tumor stage (r=0.214, p=0.014), tumor invasion (r=-0.243, p=0.041), lymph node involvement (r=0.337, p=0.005) and NLR (r=0.231, p=0.008), CEA (r=0.323, p <0.001) and CA 19-9 (r=0.273, p=0.002) levels and a negative correlation with lymphocyte count (r=-0.224, p=0.010).

On the other hand, a positive correlation was found between WBC and CEA (r=0.252, p=0.004), CA 19-9 (r=-0.202, p=0.021) and NLR (r=-0.494, p<0.001) levels. A negative correlation was found between RDW level and hemoglobin (r=-0.521, p<0.001), MPW (r=-0.186, p=0.032) and NLR (r=-0.175, p=0.044) levels. A positive correlation was found between tumor invasion and lymph node involvement (r=0.425, p<0.001), RAS positivity (r=0.247, p=0.038) and mortality rate (r=0.243, p=0.041). In addition, a positive correlation was found between lymph node involvement and mortality rate (r=0.337, p=0.005). A positive correlation was found between smoking and the presence of metastasis (r=0.250, p=0.004), PCT level (r=0.190, p=0.029), CEA (r=0.199, p=0.023) and CA 19-9 (r=0.298, p=0.001) levels.

In addition, when correlation analysis was applied to the data of deceased people, the following results were reached. A negative correlation was found between life expectancy and age (r=-0.235, p=0.035) and CEA level (r=-0.270, p=0.016). In addition, a negative correlation was found between age and lymphocyte count (r=-0.228, p=0.041) and the presence of metastasis (r=-0.238, p=0.033). A positive correlation was observed between tumor stage and CEA (r=0.267, p=0.017) and CA 19-9 (r=0.317, p=0.004) levels. A positive correlation was observed between WBC number and CEA (r=0.241, p=0.031) and CA 19-9 (r=0.281, p=0.012) levels. There was a positive correlation between the presence of metastasis and smoking (r=0.315, p=0.004) and NLR level (r=0.226, p=0.043). Finally, there was a positive correlation between RAS positivity and CEA level (r=0.267, p=0.017).

At the end of the ROC-Curve test, which was applied to determine which of the study parameters were sensitive and specific in determining the mortality levels, it was found that age (threshold value >65 years, p=0.015), lymphocyte count (threshold value <1885, p=0.008), CEA (threshold value >3.42, p <0.001), CA 19-9 (threshold value >15.80, p=0.002) and NLR (threshold value >2.45, p=0.007) levels and lymph node involvement (threshold value > 4 and above lymph node involvement, p=0.007) may be sensitive and specific determinants in predicting the mortality rate.

At the end of The Logistic Regression Test, it was found that lymph node involvement (B=-1.821, Wald=7.291, p=0.007) and CEA level (B=0.014, Wald=4.761, p=0.029) may be the best parameters in predicting mortality rates.

On the other hand, Odds Ratio Test was applied for the effects of the parameters obtained at the end of the ROC-Curve Test on the mortality risk.At the end of this test, if the age was older than 65 years (OR=2.64, 95% confidence interval 1.28-5.42, p=0.008), if the CA 19-9 level was found to be more than 15.80 (OR=2.37, 95% confidence interval 1.16-4.86, p=0.018), and the NLR value was found to be more than 2.45 (OR=2.93, 95% confidence interval 1.42-6.06, p=0.004), the risk of mortality in these individuals was more than doubled.

When the participants were divided into four groups according to the stage of the tumor, tumor invasion level (X2=54.780, p<0.001), lymph node involvement (X2=47.200, p<0.001), presence of metastasis (X2=54.045, p<0.001) and PDW (X2=9.878, p=0.020), CEA (X2=12.447, p=0.006) and Ca 19-9 (X2=14.826, p=0.002) levels were found to be different between the groups.

As a result of the paired group comparisons, it was observed that PDW (Z=-2.634, p=0.008), CEA (Z=-2.740, p=0.006) and CA 19-9 (Z=-2.813, p=0.005) levels were different in people with stage 2 and stage 4 tumors. In addition, CA 19-9 values were found to be different in patients with stage 3 and stage 4 tumors (Z=-2.894, p=0.004).

In addition, when the participants were divided into two groups as RAS positive and RAS negative, only CEA levels were found to be different between the groups in terms of study data (Z=-2.356, p=0.018) (**Figure 1, Table 3**).

Metastasis was found to be higher in patients with stage 4 tumors and CEA and CA 19-9 levels were found to be high in these patients (**Figure 2, 3**).

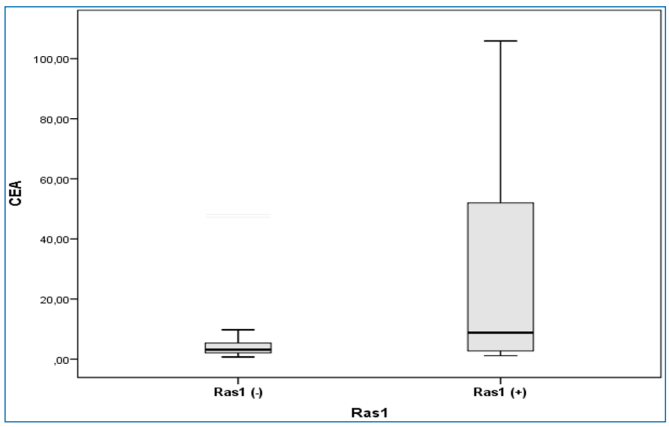

Figure 1. RAS mutation and CEA level comparison

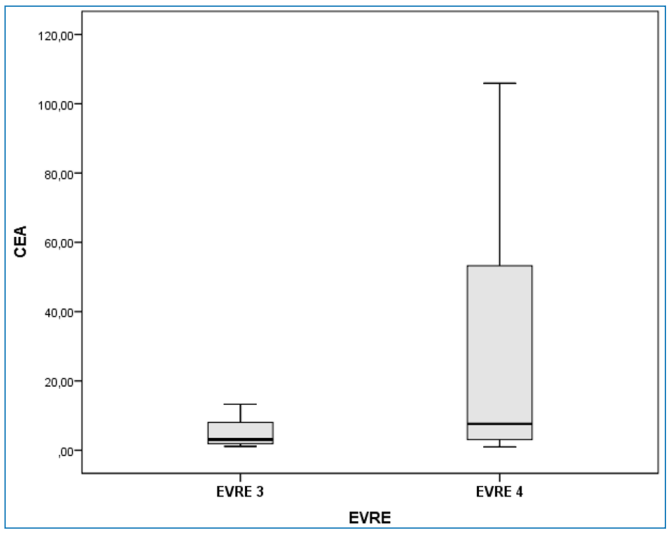

Figure 2. Comparison of Stage 3 and Stage 4 CEA level

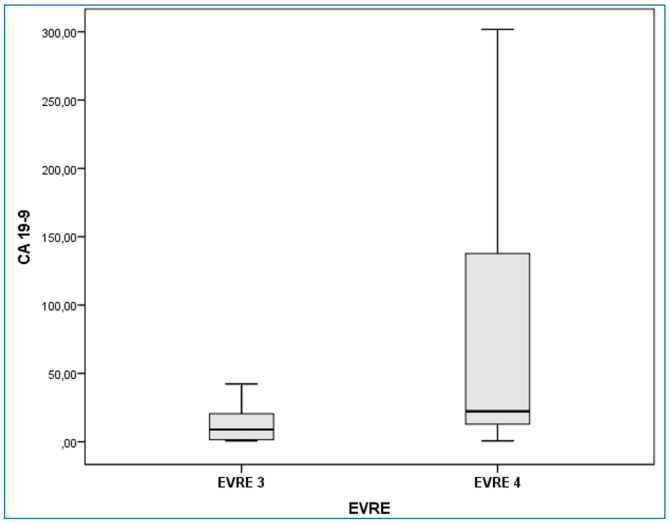

Figure 3. Comparison of Stage 3 and Stage 4 CA19-9 levels

Table 3. Evaluation of variables according to RAS status

Variable	RAS (-) Average±SS/ The median (min-max)/ N (%)	RAS (+) Average±SS/ The median (min-max)/ N (%)	t / Z	p value
Age	65.11±13.53	64.20±14.07	0.376*	0.708
Gender				
Male	46 (34.8%)	36 (27.3%)	0.055 ‡	0.814
Female	27 (20.5%)	23 (17.4%)		
Stage				
1	9 (6.8%)	3 (2.3%)	3.563‡	0.313
2	14 (10.6%)	9 (6.8%)		
3	17 (12.9%)	12 (9.1%)		
4	33 (25.0%)	35 (26.5%)		
Tumor				
1	1 (1.4%)	0 (0.0%)	4.435 ‡	0.218
2	4 (5.6%)	1 (1.4%)		
3	22 (31.0%)	9 (12.7)		
4	17 (23.9%)	17 (23.9%)		
Lymph node involvement				
0	15 (21.7%)	6 (8.7%)	1.578 ‡	0.454
1	16 (23.2%)	8 (11.6%)		
2	13 (18.8%)	11 (15.9%)		
Metastasis				
None	27 (30.3%)	13 (14.6%)	2.462 ‡	0.117
Yes	25 (28.1%)	24 (27.0%)		
Survey				
Living	28 (21.2%)	23 (17.4%)	0.005 ‡	0.941
Exitus	45 (34.1%)	36 (27.3%)		
WBC	8850 (3340-21730)	8130 (4300-20150)	-0.927†	0.354
HB	12.30 (8.50-16.20)	12.10 (8.00-15.80)	-0.222†	0.824
RDW	16.40 (12-30)	16.80 (13-28)	-1.485 †	0.137
PNL	5450 (1500-18570)	5160 (1100-16380)	-0.989†	0.323
Lymphocyte	1960 (500-8300)	1870 (170-3990)	-1.213 †	0.225
Platelet (^10³)	316 (126-877)	289 (132-666)	-1.014 †	0.311
MPW	8.75 (5.50-13.10)	9.20 (7.20-12.70)	-0.679 †	0.497
PDW	15.90 (14.00-18.20)	15.90 (11.40-17.60)	-0.220†	0.826
Neutrophil-lymphocyte ratio (NLR)	2.46 (0.88-19.33)	2.89 (0.00-82.12)	-0.428†	0.669
Platelet-lymphocyte Ratio	163.24 (32.40-712.87)	158.91 (54.10-1647.06)	-0.563 †	0.573
PCT	0Ç30 (0.11-228.00)	0.28 (0.11-0.60)	-1.087 †	0.277
CEA	3.33 (0.72-1000.00)	8.81 (1.13-1000.00)	-2.356 †	0.018
CA19_9	14.90 (0.60-1000.00)	18.27 (0.60-1998.00)	-0.684†	0.494

DISCUSSION

As it is known, CRC ranks third among the most common cancer types in the world. As with all types of cancer, inflammation causes cancer cells to spread and grow in CRCs. Inflammatory cells around the tumor have significant effects on tumor growth and spread, and it has been hypothesized that systemic inflammation markers may be useful to indicate prognosis.[13] In the studies conducted by Bambace and Holmes in 2011, and Goubran et al. in 2014, platelet cells are also involved in inflammation and have been shown to cause cancer cells to grow, angiogenesis and progress. In the studies conducted by Chadha et al. in 2015; Feng et al. in 2016, Heng and Benjapibal in 2014, Josa et al. in 2015, and Li et al. in 2014, found that platelets are an indicator of poor prognosis for many types of cancer.[14] In our study, the number of platelets did not change between stages and according to the presence of RAS mutation. This can be attributed to the disproportionate distribution of the number of patients between stages.

Lin et al.[15] found that PDW value was high in CRC patients in their study conducted in 2018.

To the best of our knowledge, no study has been conducted in the literature to determine whether there is a relationship between RAS-positive colon cancer and PDW. In our study, we aimed to show whether there is a relationship between RAS mutation and PDW in patients with colon cancer. In our study, 73 (55.3%) of the patients did not have a RAS mutation, 59 (44.7%) had a RAS mutation, and no parameters other than CEA level were found to be different between individuals, and only CEA levels of RAS positive individuals were found to be higher than RAS negative individuals. In addition, the level of PDW was found to be high in people with advanced stage tumors. In our study, it was found that the PDW value increased significantly as the stage progressed in CRC. This supports that PDW elevation can be used to indicate prognosis. It is predicted that the high PDW value may reflect the activated platelet level associated with tumor invasion and metastasis.

VEGF is associated with prognosis in many types of cancer, and circulating VEGF is mainly found in platelets and neutrophils.[16] Circulating active platelets release VEGF, which can trigger cancer invasion and metastasis. This condition can be associated with increased inflammation and, as a result, increased platelet count. The increase in inflammation and platelet count increases with stage and may cause an increase in PDW. The presence of inter-stage metastasis and increased PDW can also be attributed to VEGF release from activated platelets and secondary tumor invasion and metastatic effect. RAS mutation is a mutation in the EGFR pathway. In our study, the absence of PDW difference between RAS mutant and non-RAS mutant may suggest that thrombocytosis and PDW increase are independent of the EGFR pathway and effective on VEGF.

In addition, many studies have found a significant relationship between stage and MPV level increase in lung cancer, gastric cancer, hepatocellular cancer, endometrial cancer and colon cancers.[17]

White blood cell (WBC) count and neutrophil/lymphocyte ratio (NLR) have been investigated for their prognostic properties in some types of cancer. Walsh et al.[18] showed that NLR ≥5 was a marker of survival in colorectal cancer patients. In many studies, NLR increase has been associated with poor prognosis in cancer types.[19] In our study, it was found that if the age was older than 65, the CA 19-9 levels were found to be more than 15.80, and the NLR value was found to be more than 2.45 (OR=2.93, 95%, confidence interval 1.42-6.06, p=0.004), the risk of mortality in these individuals could be more than doubled. In addition, the increase in NLR in our study was positively correlated with the mortality rate, which was attributed to the low lymphocyte count in CRC patients with increased mortality. RAS mutations, which are an indicator of a poor prognostic factor in CRCs, were found to be higher in patients with NLR above 3. However, in our study, no significant difference was found between NLR value in RAS positive and RAS negative patients (p=0.669). This result may be due to the retrospective nature of our study and the limited number of our patient population.

CEA is a tumor marker commonly used to indicate prognosis in CRC patients.[20] In a study conducted by Li et al.[21] in 2013, it was revealed that the CEA level was high in patients with high NLR. In a study conducted in Thailand, a statistically significant difference was found in 5-year survival between low CEA level cancer and high CEA level cancer.[22] Similarly, our study shows that high NLR value and high CEA and CA 19-9 levels shorten life expectancy (p=0.008, p=<0.001, p=0.002). Instead of using tumor markers alone in long-term follow-up, the use of CEA and CA19-9 together has proven to have stronger results in showing the prognosis.[23] In our study, both CEA and CA19-9 were found to be high in patients who died and were shown to be associated with mortality. This shows us that CEA and CA19-9 tumor markers can be used not only in treatment follow-up but also to indicate the prognosis of the disease.

RAS mutations are more aggressive than other mutations and are associated with an increased risk of recurrence and death in patients with CRC.[24] In our study, a positive correlation was observed between RAS positivity and CEA level (r=0.267, p=0.017). Thus, it was thought that the life span of RAS positive individuals may have been shortened.

Tumor stage is the most important prognosis indicator in CRCs.[25] Moghimi-Dehkordi et al.[26] and Chapius et al.[27] stated that the universe should be considered as a major prognostic factor in their studies examining the prognostic factors of CRC. In a retrospective study conducted by Küçüköner et al.[28] with 767 patient data, 5-year disease-free survival rates decreased as the stage progressed. In our study, there was a negative correlation between life expectancy and age (r=-0.219, p=0.012), tumor stage (r=-0.201, p=0.021), MPW level (r=-0.224, p=0.010), mortality level (r=-0.585, p<0.001), CEA (r=-0.340, p < 0.001) and CA 19-9 level (r=-0.228, p=0.009). With these findings, it was thought that the patient's life expectancy could be shortened as the patient's advanced age, tumor stage, MPW level, CEA, and CA 19-9 levels increased. In addition, it was observed that the level of tumor invasion, involvement of more than 4 lymph nodes, and the presence of metastasis were higher in advanced stage tumor patients. In addition, PDW, CEA and CA19-9 levels were found to be higher in people with advanced stage tumors.

Smoking is a risk factor in CRCs as in many cancers. In the study of Kutlu et al. investigating the relationship between smoking and cancer, the incidence of CRC was found to be 1.11 times higher in smokers. There was a positive correlation between the presence of metastasis and smoking

(r=0.315, p=0.004) and NLR level (r=0.226, p=0.043). Thus, it was predicted that the likelihood of metastasis may increase in people who smoke, and in this case, the life span of the person may be shortened indirectly.

As it is known, the first thing to do before treatment in colon cancer is to determine the stage of the disease. Lymph node involvement is the most important parameter in determining the stage of the disease.[30] In a retrospective study conducted by Benedek et al.[31] in 2020 by prioritizing the lymph node status in Romania, it was revealed that the most important prognostic factor in 5-year survival was lymph node status. In the study conducted by Ortega et al.[32] in 2017, the 5- and 10-year disease-free survival of surgically removed lymph nodes in stage 2 CRC patients was found to be 86.5% in those with more than 12 lymph nodes resected. In many studies in the literature, it is recommended to remove at least 12 lymph nodes during surgery, especially in stage 2 patients. In our study, it was shown that lymph node involvement was sensitive with age, lymphocyte count, CEA, CA19-9 levels in predicting mortality rate. This situation supports that lymph node involvement is an indicator of prognosis with stage. In our study, it was revealed that the number of lymph nodes kept in predicting life expectancy may be an important parameter, and it was observed that the detection of lymph node involvement in our study increased the risk of mortality in CRC patients by more than 3 times. In addition, in our study, it was observed that the number of metastatic lymph nodes was higher in patients who died than in living patients (p=0.020). This situation supports that as the number of lymph node involvement increases, the stage and mortality rate increases and lymph node involvement is an important criterion in determining the prognosis.

CONCLUSION

Previously, many studies have investigated the relationship between cancer and platelet indices, but to the best of our knowledge, it has not been evaluated together with the RAS mutation. In our study, the relationship between RAS mutation and PDW was investigated, but no significant relationship was found between the two. A low level of correlation coefficient but a significant positive correlation was found between stage and PDW. This may be associated with an increase in inflammation level as the stage increases. A significant relationship was found between the presence of RAS mutation and only CEA level and mortality. This result may be due to the narrow patient population of our study and the insufficient number of patients. More studies are needed on this subject.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 16.06.2021, Decision No: 111).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

Evaluation of the effect of obesity on oxidative stress with thiol/disulphide balance

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ABSTRACT

Aims: As the body mass index (BMI) increases, the percentage of body fat increases and oxidative stress increases accordingly. This change can be determined by looking at the thiol/disulphide balance which is a biochemical test. We aim to emphasize the importance of oxidative stress due to obesity. Thus, by protecting obese individuals from oxidative stress (with antioxidant support treatments, etc.), studies to minimize the impact of obesity can be opened the front.

Methods: The study group of our study was selected from 18-55-year-old female obese or overweight female patients who applied to Kırıkkale University Medical Faculty Internal Medicine and Endocrinology (Obesity) Polyclinics for slimming. Acontrol group consisting of healthy and normal weighted women who were in the same age range and applied to our hospital for general control were included in the study. According to BMI; groups consisting of 45 women, normal weight (18,5-24,9 kg/m^2), overweight (25-29,9 kg/m^2), stage 1 (mild obese; 30-34,9 kg/m^2), stage 2 (moderately obese; 35-39,9 kg/m^2) and stage 3 (morbid obese; 40-49,9 kg/m^2), were formed. Participants were premenopausal women with no additional disease. Demographic data and routine investigations were obtained from the hospital system. Blood samples were studied with thiol/disulphide balance measurement tests.

Results: We analyzed the five groups that were graded as normal weight, overweight, obese (mild, moderate, morbid) and the positive correlation between BMI and waist circumference andbody fatpercentage was significant in all groups ($r = 0.936$, $p<0.001$; $r = 0.857$, $p<0.001$, respectively). In all groups, the levels of native thiol and total thiol were decreased as BMI increased. There is a relatively lower difference between the normal thiol and total thiol levels of the normal weight and overweight group; there was a significant decrease in these values when passing from overweight to any stage of obesity. Negative correlation between all groups with native thiol ($r = -0.473$, $p<0.001$), total thiol ($r = -0.472$, $p<0.001$) and SH/total SH values ($r = -0.296$, $p<0.001$) were significant. The positive correlation between SS/SH ($r = 0.296$, $p<0.001$) and SS/total SH ($r = 0.296$, $p<0.001$) was significant in all groups. The positive correlation between disulphide and all groups ($r = 0.103$, $p=0.25$) was not significant.

Conclusion: Increased fat tissue inflammation in obesity is associated with oxidative stress. Thiol-containing organic compounds are antioxidants for defense against oxidative stress. Measures should be taken in the early period to reduce oxidative stress in the management of obesity.

Keywords: Obesity, body mass index, oxidative stress, thiol/disulphide balance

INTRODUCTION

Obesity, one of the biggest health problems of today, is defined by the World Health Organization (WHO) as an abnormal or excessive amount of fat tissue accumulation that poses a health risk.[1] Obesity is associated with endocrinological diseases (type 2 diabetes mellitus and insulin resistance, metabolic syndrome, etc.) and cardiovascular diseases (hypertension, hyperlipidemia, etc.), cerebrovascular diseases, cholecystitis, sleep apnea, osteoarthritis, hyperuricemia and gout, endometrial, breast, gallbladder cancers in women, and colon, rectum, prostate cancer in men. Obesity was treated when it caused any disease in the past. Today, along with preventive health policies, it is aimed to reduce mortality by treating obesity without causing any chronic diseases.[2]

The mechanism of obesity associated with diet is one of the most common causes of obesity. Dietary intake of free fatty acids in excess of antioxidant capacity leads to lipid peroxidation and may cause oxidative stress.[3]

Free radicals or reactive oxygen species (ROS) are products of normal cellular metabolism and are essentials for low levels of biological functions and enzymatic reactions. Free radicals have been described as having one or more unconjugated electrons, small molecular mainly, and highly unstable in molecular structure, and tend to damage molecules such as nucleic acids, proteins, carbohydrates, and lipids.The formation of free oxygen radicals is an ongoing metabolic event. Organisms have developed antioxidant systems that

reduce the damage of free radicals or ROS.Disruption in molecular and cellular functions as a result of loss of balance between ROS production and the body's antioxidant system is defined as "oxidative stress".[4-6]

Oxidative stress causes necrosis and death of the cell as a result of the high amount of free radicals produced during metabolism and cannot be rendered harmless by antioxidants, thus causing tissue damage and chronic diseases.[5] Thiol is an organic compound containing sulfhydryl (-SH) group that has a role in preventing any oxidative stress from occurring in cells. The main intention of ROS is the thiol groups of sulfur-containing amino acids in proteins. Thiol groups turn into reversible disulfide bonds after oxidation with ROS. Thus, the dynamic thiol/disulfide balance moves towards the disulfide form.[6] This transformation is the earliest sign of radical-mediated protein oxidation. The resulting disulfide bond structures can be reduced to functional thiol groups again and thus the dynamic thiol/disulfide balance is maintained.[7]

Dynamic thiol/disulfide balance has important roles in many mechanisms such as antioxidant defense, apoptosis, enzyme regulation, and cellular signal transduction in the organism.[8] It is examined as an indicator of oxidative stress in some metabolic diseases such as obesity and cardiovascular diseases; thiol/disulfide balance cancers; neurological diseases such as Parkinson's and Alzheimer's.[9] Therefore, investigating the dynamic thiol/disulfide balance can provide valuable information about the diagnosis, treatment, and mortality/morbidity processes of some common diseases in the society.

In this study, our aim is evaluating the relationship between oxidative stressand biochemical parameters and thiol/disulfide levels in obese people. Thus, with the antioxidant support treatments etc. that can be applied, studies to minimize the effect of obesity by protecting people from oxidative stress can be paved.

As the body mass index increases, the percentage of body fat increases, and in parallel with this, oxidative stress increases. This change in oxidative stress can be determined by looking at the thiol/disulfide balance, which is a biochemical test.

METHODS

Selection of the Study Group

This speciality thesis study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 31.10.2017, Decision No: 20/02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written consent was obtained from the patient participating in this study.

The study group of our study was selected from obese or overweight female patients aged 18-55 years who applied to internal diseases and endocrinology (obesity) outpatient clinics for weight loss between 15.12.2017-15.03.2018. A control group consisting of healthy and normal weight women who were in the same age range as the study group and who applied to our hospital for general control was included in the study. Informed consent form was obtained from all of the 225 volunteers.

Inclusion Criteria for Research

- Being older than 18 years old
- Being younger than 55 years old
- The study group will be overweight or obese, and the control group will be those with a BMI of <25 kg/m²
- Persons who apply to the internal diseases and obesity outpatient clinic for slimming purposes and undergo the routine examinations
- Those without known systemic disease
- Those without malignancy
- Those who are unpregnant
- Those who do not currently have an acute pathology (such as upper respiratory tract infection)
- Agree to participate in the study

Exclusion Criteria for Research

- Being younger than 18 years old
- Being older than 55 years old
- Not agreeing to participate in the study
- Patients who take medication (oral contraceptives, vitamin supplements, antioxidant drugs, people taking medication that affects platelet function)
- Malignancy
- Patients with a history of other endocrinological diseases: Hypothyroidism, hypogonadism, Polycystic Ovary Syndrome, Cushing's syndrome
- People who are followed up with anemia, thrombocytopenia
- Being a member of another study
- People treated for obstructive sleep apnea
- Patients with previous myocardial infarction, cerebrovascular accident, peripheral artery disease
- People treated for DM
- People treated for hypertension
- People treated for dyslipidemia
- Pregnant women
- People with a history of liver and kidney failure
- History of arrhythmia
- Those who smoke and drink alcohol
- Patients treated for acute or chronic infection

Table 1. Rating of obesity in adults according to BMI (kg/m²)

Slim	<18.50
Normal-weight	18.5-24.99
Overweight	25.00–29.99
Obese	≥30.00
Slightly Obese	30.00–34.99
Moderately Obese	35.00–39.99
Morbid Obese	40.00-49.99
Super Obese	≥50.00

Population and Sample of the Research

Our study was grouped as normal weight, overweight, stage 1 (mild) obese, stage 2 (moderate) obese, stage 3 (morbid) obese, and a total of 225 female volunteers between the ages of 18-55, 45 from each group, were included in our study.

Data Collection Methods

Demographic data of the patients, results of laboratory analysis (biochemical and hormone tests) , bioelectrical impedance measurement results and native thiol (-SH), total thiol [(-SH+SS-)], disulfide (-SS-), disulfide/native thiol [-SS-/-SH)], disulfide/total thiol [-SS-/(-SH+SS-)], native thiol [-SH/(-SH+SS-)] results were used for our study. Demographic data and routine examinations of the participants were obtained from the hospital system. Demographic data and routine examinations of our study group consisting of premenopausal

women without known additional disease were taken from the hospital system. All volunteers participating in the study were physically examined in the outpatient clinic. Blood pressure was measured with a sphygmomanometer from the brachial artery for 5 minutes after resting, with the patients in the sitting position, at 2-minute intervals, and their averages were recorded. Participants with pathological ECG, blood pressure, pulse values, and smoke and/or alcohol users were not included in the study.

Bioelectrical Impedance Analysis (BIW) and Measurement of Waist Circumference

Bioelectrical impedance analysis was performed using the "Tanita-BC 420 MA® device. Impedance is the resistance of the tissue to electric current and is inversely proportional to conductivity. The device has a weak electrical and 1.5 Ampere current feature at a frequency of 50-60 KHz. It is a method based on the difference in permittivity of tissues. The difference in impedance between lean tissue and adipose tissue is measured.[10]

Thiol /Disulfide Balance Measurement Tests (Erel Panel)

The blood samples taken from the antecubital vein to the biochemistry tube between 08:00 and 09.00 in the morning, following 8-12 hours of night fasting, were centrifuged at 1500 rpm for 10 minutes. Separated serum samples were placed in ependorphs and stored for a maximum of 3 months at-80°C to study the serum thiol/disulfide balance until the target number was reached. Serums that separated from the blood were sent to Ankara Yıldırım Beyazıt University Atatürk Training and Research Hospital Biochemistry Laboratory, where thiol/disulfide balance measurement method developed byErelet al.[9] was used and thiol/disulfide KİTs were studied. Measurements were presented using an automated clinical chemistry analyzer (Cobas 501, Roche) and results were presented in µmol/L. In this method, dynamic disulfide bonds(-SS-) are reduced to functional thiol groups (-SH) with sodium borohydriate (NaBH4). Unused NaBH4 is completely removed with formaldehyde. The total amount of thiol is calculated by spectrophotometric measurement of the chromogen compound formed by the modified Ellman's reagent at a wavelength of 415 nm. Native Thiol is measured using a modified Ellman's reagent (The Classic Ellman's reagent was modified by adding a formaldehyde solution). The Native Thiol value is subtracted from the total thiol and the amount of disulfide bond is found by dividing the difference obtained by two.This newly developed method is an easy, inexpensive, practical, automatic and manual spectrophotometric test to determine the plasma dynamic thiol/disulfide balance. With this method, native thiol (-SH), total thiol [(-SH) + (-S-S-)], and dynamic disulfide (-SS-) values were determined by measuring. With the ratios of these values, disulfide/native thiol [(-SS-/-SH)], disulfide/total thiol [-SS-/(-SH+SS-)], native thiol/total thiol [-SH/(-SH+SS-)] parameters are obtained.

Statistical Analysis

Statistical analyses of the study were performed using the "Statistical Package for the Social Sciences" (SPSS) version 20.0 package software. Descriptive statistics are summarized as number, percentage, average and standard deviation. Variables that fit the normal distribution are given with mean ±standard deviation and those that do not fit with the median (minimum-maximum) values. The suitability of the variables for normal distribution was examined using analytical methods (Shapiro-Wilk Tests). Kruskal Wallis test was used for numerical variables that did not show normal distribution in comparisons between multiple groups, and One-way Analysis of Variance (ANOVA) test was used to compare more than two independent groups for numerical variables with normal distribution. Pothoc Tukey Test was used for normally distributed groups and Kruskal Wallis Posthoc Test was used fornot normally distributed groups. In the comparisons between the two groups, Mann Whitney-U Test was used for numerical variables that did not show normal distribution, and T Test was used for numerical variables that showed normal distribution in independent samples between the two groups. In the determination of the relationships between variables, when both variables were normally distributed, correlation coefficients and statistical significance were calculated with Pearson Test, and correlation coefficients and statistical significance were calculated with Spearman Test for the relationships between variables at least one of which was not normally distributed. Results with a p value of <0.05 were considered statistically significant.

RESULTS

The study was evaluated for all parameters for the general population (225 people) who applied to internal medicine and endocrinology (obesity) outpatient clinics at Kırıkkale University Faculty of Medicine Hospital and met the inclusion criteria. 90 non-obese women (group 1 and group 2) and 135 obese women (group 3, group 4, group 5) were compared among themselves. In addition, sub-analyses were performed to determine the relationships between all groups, including 45 normal weight (group 1) and 45 overweight (group 2), 45 mildly obese (group 3), 45 moderately obese (group 4), and 45 morbid obese (group 5).

The average values of the general age and anthropometric measurement parameters of the participants are given in Table 2.

Table 2. Average anthropometric measurement and age values of all participants, p values according to the differences between groups 1, 2, 3, 4, 5 (n =225)

Age (years)	33.0 (18.0-52.0)	p =0.051*
BMI (kg/m²)	31.80 (18.50 -49.80)	p =0.08*
Waist Circumference (cm)	96.0 (62.0-134.0)	p <0.001*
Body Fat Percentage (%)	38.70 (11.30-53.90)	p <0.001*
Height (cm)	162.0±6.88	p =0.015**
Weight (kg)	82.13±20.54	p <0.001**

p <0.05 was considered statistically significant
* The significance level between the groups was given according to the K.Wallis Test.
** Significance level was given according to ANOVA Test.

The average values of the thiol/disulfide balance parameters examined in the serum plasma samples of the participants are shown in Table 3.

Table 3. Comparison of average thiol/disulfide balance variables of the all participants, and p values according to the between differences of the groups (groups 1, 2, 3, 4, 5) (n=225)

Native thiol (SH) (µmol/l)	296.60 (65.0-480.50)	p <0.001*
Disulfide (SS) (µmol/l)	13.70 (2.0-55.30)	p =0.011*
SS/SH (%)	4.60 (0.61-62.62)	p <0.001*
SS/Total SH (%)	4.21 (0.60-27.80)	p <0.001*
SH/Total SH (%)	91.57 (44.0-98.79)	p <0.001*
Total thiol (Total SH) (µmol/l)	330.01 ±67.77	p <0.001**
Albumin (g/dl)	4.45 ±0.31	p <0.001**

p <0.05 was considered statistically significant
* The significance level between the groups was given according to the K.Wallis Test.
** Significance level was given according to ANOVA Test.

In the comparison of the significance of age, anthropometric measurements, and BFP values between the paired groups, sub-analyses were performed for anthropometric measurements among the five groups we classified as normal weight, overweight, obese (mild, moderate, morbid), and it was found that weight, BMI, WCM, and BFP values showed a linear increase in all groups. All paired group comparisons were statistically significant ($p < 0.001$).

It is seen that there is a serious increase in waist circumference, especially when passing from the overweight stage to any stage of obesity. This increase was found to be statistically significant ($p<0.001$).

There was no significant difference between all groups for age ($p = 0.78$).

The level of thiol/disulfide balance parameters by groups is given in Table 4.

In all groups (as the body mass index increased), native and total thiol levels decreased.

While there is a relatively small difference between the native thiol and total thiol levels of the normal weight and overweight groups; a significant decrease was detected in these values when passing from overweight to any stage of obesity (BMI >30 kg/m^2). This relationship was statistically significant. However, no linear relationship was found between disulfide level and groups (Table 4).

There was no significant difference between all parameters of thiol/disulfide balance and albumin levels when the normal weight (group 1) and overweight group (group 2) were compared. Similar results were also valid for the comparison of moderately obese (group 4) and morbid obese (group 5) (Table 5).

In the comparison of group 1 with group 4 and group 1 with group 5, the difference between oxidant (disulfide/native thiol, disulfide/total thiol) and antioxidant (total thiol, native thiol, native thiol/total thiol) parameters was significant. However, there was variability in the analysis for disulfide level and albumin. A significant difference was found for oxidant-antioxidant parameters in between group 2 and group 4,and in between group 2 and group 5 comparisons. There was no significant difference for all ratios of balance (disulfide/native thiol, disulfide/total thiol, native thiol/total thiol) when group 1 was compared with group 2, and group 1 with group 3, and group 2 with group 3, and group 4 with group 5. A statistically significant difference was found in the comparisons of all other groups (Table 5).

Pairwise comparisons were made between non-obese groups of 90 premenopausal women formed with normal weight and overweight and obese groups (mild, moderate, morbid) of 135 premenopausal women. Both groups were similar for age factors and there was no significant difference between them ($p=0.09$). In addition, there was an increase in all anthropometric measurements in obese patients compared to non-obese patients, and this was statistically significant ($p<0.001$). Antioxidant values (native thiol, total thiol, native thiol/total thiol) and albumin values of non-obese groups were significantly higher than those of obese groups ($p<0.001$). Oxidant values (disulfide, disulfide/native thiol, disulfide/total thiol) were lower in the non-obese group compared to the obese group. However, there was no statistically significant difference in these comparisons for disulfide ($p=0.35$) (Table 6).

Table 4. Average and median values of thiol/disulfide balance variables by groups

	Normal-weight (n:45)	Overweight (n:45)	Slightly Obese (n:45)	Moderately Obese (n =45)	Morbid Obese (n:45)
Total SD (µmol/l)	379.36±76.34	362.83±59.66	320.59±52.80	307.96±60.33	289.33±60.48
SH (µmol/l)	350.70 (236.50-483.10)	327.50 (227.30-467.10)	280.80 (191.0-194.0)	276.30 (97.20-399.70)	257.0 (65.0-370.9)
SS (µmol/l)	16.50 (3.50-32.30)	13.20 (2.0-28.50)	12.60 (3.30-34.60)	15.0 (5.50-55.30)	17.45 (3.70-43.20)
SS/SH (%)	4.83 (0.73-11.21)	4.0 (0.61-10.07)	4.42 (1.02-12.89)	5.82 (1.71-56.89)	6.13 (1.63-62.62)
SH/Total SH (%)	4.40 (0.72-9.16)	3.70 (0.60-8.38)	4.06 (1.0-10.25)	5.21 (1.65-26.61)	5.46 (1.58-27.80)
SS/Total SH (%)	91.19 (81.68-98.55)	92.60 (83.24-98.79)	91.88 (79.50-98.0)	89.57 (46.78-96.69)	89.07 (44.40-96.83)
Albumin (g/dl)	4.51±0.4	4.49±0.26	4.34±0.27	4.40±0.23	4.25±0.26

Table 5. Significance comparison of thiol/disulfide balance parameters between paired groups

Case (n:225)	Significance Levels						
	Albumin (g/dl)	Total SH µmol/l)	SH (µmol/l)	SS (µmol/l)	SS/SH (%)	SH/Total SH (%)	SS/Total SH (%)
Group 1							
Group 2	p =0.99	p =0.71	p =0.97	p =0.05	p =0.09	p =0.083	p =0.091
Group 3	p =0.03	p <0.001	p =0.002	p =0.041	p =0.66	p =0.66	p =0.67
Group 4	p =0.56	p <0.001	p <0.001	p =0.76	p=0.034	p =0.04	p=0.034
Group 5	p <0.001	p <0.001	p <0.001	p =0.56	p =0.002	p =0.002	p =0.002
Group 2							
Group 3	p =0.10	p =0.01	p =0.002	p =0.94	p =0.21	p =0.199	p =0.209
Group 4	p =0.80	p <0.001	p <0.001	p =0.023	p <0.001	p <0.001	p <0.001
Group 5	p =0.002	p <0.001	p <0.001	p =0.011	p <0.001	p <0.001	p <0.001
Group 3							
Group 4	p =0.67	p =0.87	p =0.30	p =0.019	p =0.011	p =0.011	p =0.011
Group 5	p =0.65	p = 0.12	p =0.008	p =0.009	p <0.001	p <0.001	p <0.001
Group 4							
Group 5	p= 0.055	p =0.61	p =0.104	p =0.009	p =0.35	p =0.353	p =0.351

p <0.05 was considered statistically significant

Table 6. Results of thiol/disulfide parameters of obese and non-obese groups

	Non-obese (n = 90)	Obese (n=135)	p-value
Total SH µmol/l)	370.60±68.63	305.10±58.97	<0.001**
SH (µmol/l)	340.73 (227.3-483.1)	271.89 (65.0-399.7)	<0.001*
SS (µmol/l)	15.19 (2.0-32.30)	17.04 (3.30-55.30)	0.35*
SS/SH (%)	4.57 (1.0-11.0)	7.58 (1.0-63.0)	<0.001*
SH/Total SH (%)	91.76 (81.68-98.79)	88.16 (44.40-98.0)	<0.001*
SS/Total SH (%)	4.12 (0.60-9.16)	5.92 (1.0-27.80)	<0.001*
Albumin (g/dl)	4.51±0.34	4.30±0.26	<0.001**

Non-obese: It consists of normal weight and overweight (BMI = 18.5-29.99 kg/m²) groups.
Obese: It consists of mildly obese, moderately obese, and morbid obese groups (BMI ≥30 kg/m²).
*The significance level between the groups was given according to the Mann-Whitney U-Test.
* * Significance level was given according to T Test in independent groups.
p <0.05 was considered statistically significant

The correlation relationships between BMI, WCM, BFP parameters and each other, all groups, and thiol/disulfide balance were examined. The positive correlation between BMI and waist circumference (WCM) (r =0.936, p < 0.001) and between BMI and body fat percentage (BFP) (r =0.857, p < 0.001) was significant (**Figure 1**).

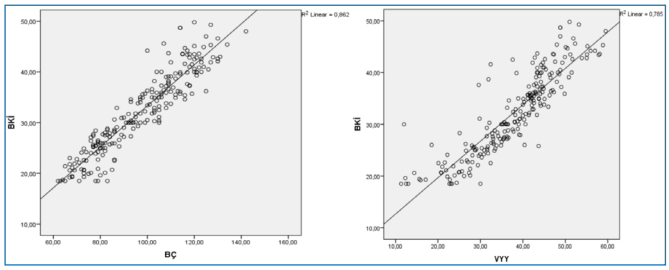

Figure 1. The relationship between BMI and WCM, BMI and BFP

The positive correlation relationship between all groups and BMI, WCM and BFP was significant (((r =0.980, p < 0.001), (r =0.932, p < 0.001), (r =0.887, p < 0.001), respectively) (**Figure 1**).

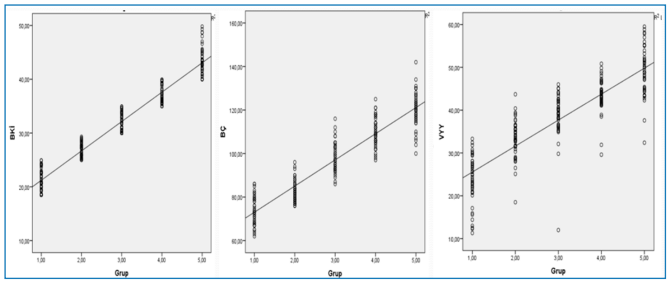

Figure 2. Relationship between all groups and BMI, WCM and BFP

A negative correlation was found between BMI and native thiol (r = -0.479, p<0.001), total thiol (r=-0.450, p<0.001), SH/Total SH values (r=-0.280, p<0.001), and the analysis was significant. A positive correlation was found between BMI and SS/SH (r =0.280, p< 0.001), SS/Total SH (r =0.280, p<0.001), and the analysis was significant. A positive correlation was also found between disulfide and BMI (r=0.08, p=0.25); however, no significant difference was found. A negative correlation was found between all groups and native thiol (r=-0.473, p<0.001), total thiol (r=-0.472, p<0.001), SH/Total SH values (r = -0.296, p<0.001), and this relationship was significant.

A positive correlation was found between all groups and SS/SH (r=0.296, p<0.001), SS/Total SH (r=0.296, p<0.001), and this relationship was significant. There was also a positive correlation between disulfide and all groups (r=0.103, p=0.25); but no significant difference was found. A negative correlation was found between WCM and native thiol (r = -0.456, p=0.002), total thiol (r = -0.429, p<0.001), SH/Total SH values (r=-0.240, p<0.001), and the analysis was significant. A positive correlation was found between WCM and SS/SH (r=0.280, p<0.001), SS/Total SH (r=0.280, p<0.001), and the analysis was significant.A positive correlation was also found between disulfide and WCM(r=0.08, p=0.25); however, no significant difference was found.

A negative correlation was found between BFP and native thiol (r=-0.479, p<0.001), total thiol (r= -0.450, p<0.001), SH/Total SH values (r=-0.280, p<0.001), and the analysis was significant. A positive correlation was found between BFP and SS/SH (r =0.280, p<0.001), SS/Total SH (r =0.280, p<0.001), and the analysis was significant.A positive correlation was also found between disulfide and BFP(r=0.08, p=0.25); however, no significant difference was found.

In the comparison with albumin level, the negative correlation relationship between BMI (r=-0.297, p<0.001), WCM (r=-0.208, p<0.001), BFP (r=-0.298, p<0.001), and all groups (r=-0.269, p<0.001) was significant, respectively. There was a negative correlation between non-obese and obese groups and albumin and it was statistically significant (r=-0.273, p<0.001). The negative correlation between all groups and albumin was also statistically significant (r=-0.264, p<0.001).

DISCUSSION

Elmas et al.[11] found that thiol/disulfide balance deteriorated as BMI increased in their study with 75 obese and 64 normal weight children who were similar in terms of age and gender and did not have any endocrinological risk factors.

In all of our sub-analyses, we found that thiol/disulfide balance deteriorated as BMI, WCM, and BFP increased. A negative correlation was found between BMI, WCM, BFP and antioxidant parameters (native thiol, total thiol, native thiol/total thiol) and the analysis was significant.

Waist circumference measurement is associated with the amount of intraabdominal (visceral) luposis.[11] In our study, it is seen that there is a significant and serious increase in waist circumference as BMI increases, especially when passing from overweight to any stage of obesity.

While there is a relatively small difference between the native thiol and total thiol levels of the normal weight and overweight groups; a significant decrease was detected in these values when passing from overweight to any stage of obesity (BMI >30 kg/m²). In our study, when BMI was >30 kg/m², we think that this significant change in WCM and the increase in visceral adipose tissue were strong factors in disrupting the thiol/disulfide balance.

We found that the disulfide level, another oxidant parameter, was also higher in obese patients. However, this correlation did not make sense. In the study of Elmaset al.[11] the disulfide level was higher in obese children and the relationship was not significant, which is similar to our study. Since plasma thiols are composed of many molecules such as albumin and glutathione, this can be explained by the presence of other unmeasured parameters and enzymatic/

nonenzymatic activities while looking at components such as native thiol, total thiol, and disulfide.

In the study of Du Nan et al.[12] showed that the risk of urinary albumin excretion increased in individuals with increased BMI and WCM, and stated that weight loss may be beneficial for reducing excretion.

According to our results, the albumin level decreased with the increase in BMI. Since plasma thiols are mostly composed of albumin, one of the reasons for the deterioration in the thiol/disulfide balance in obesity may be the decrease in albumin. We predict that the level of native thiol and total thiol may also have decreased due to this decrease.

Similar to our study, Jankovic et al.[13] conducted a study with premenopausal normal weight and obese women. It was found that glutathione (GSH) levels, an intracellular thiol, were significantly reduced in human visceral and subcutaneous adipose tissues in obese patients. They explained this with NADPH oxidase enzyme production and ROS variables in increased adipose tissue. They stated that antioxidant intracellular thiols are oxidized by activating to reduce reactive oxygen derivatives, thus measuring GSH level less in adipose tissue.

Brown et al.[14] similar toour study, investigated the relationship between BMI, WCM and BFP and some antioxidant parameters (lipid hydroperoxide, total antioxidant status, superoxide dismutase, and reduced glutathione) in groups classified as normal-weight, overweight and obese adults. In the sub-analyses, they showed differences between the groups in terms of obesity degrees and suggested that these differences in their study were related to the degree and duration of obesity development.

Wong et al.[15] in their study on obese and middle-aged mice, they found that β-mercaptoethanol (BME), which is a thiol antioxidant, had less increase in fat masses than those who did not. They suggested that the use of β-mercaptoethanol reduced inflammation through insulin resistance and lipid peroxidation.

Şimşek et al.[16] showed that thiol/disulfide balance was impaired regardless of age, gender and BMI in patients with familial hypercholesterolemia. They explained this situation with oxidized LDL, endothelial dysfunction and atherosclerosis.

Özler et al.[17] reported that thiol/disulfide balance was impaired in overweight adolescents with PCOS compared to normal weight patients with PCOS. Although this deterioration is associated with hyperandrogenism and insulin resistance, they stated that more weight and BMI increase are at the forefront.

In obesity, the release of free fatty acids from adipose tissue increases, causing insulin resistance by being stored in the liver and muscles.Metabolic dysfunction, which starts with insulin resistance, progresses to prediabetes and all these conditions are associated with oxidative stress. Obesity can be associated with many conditions related to increased oxidative stress such as insulin resistance, diabetes, dyslipidemia, atherosclerosis.[18,19]

In our study, we examined obese individuals without known comorbidities such as diabetes and dyslipidemia. Thus, we found that the balance was impaired in obese people regardless of other mechanisms. This only emphasizes the importance of increased BMI and the inflammatory effect of increased adipose tissue.

Limitations of the study: Thiol/disulfide balance is affected by many other factors such as dietary pattern and physical activity. Our study is a cross-sectional and descriptive study. Current thiol/disulfide values and anthropometric measurements of the individuals were used. Although many factors were tried to be excluded in our study, not all causes could be eliminated. Therefore, the cause-effect relationship has not been fully established. While the consumption of foods with antioxidant properties provides positive effects in antioxidant defense such as thiol/disulfide balance, high-calorie diets have the opposite effect. In our study, it is one of our important deficiencies not to take a story about the lifestyle of the participants such as nutritional characteristics and diet type.

One of our other limitations is that the participants' data on education, physical activity, income level, marriage, number of births, sleep patterns, and family history were not included in the study. In addition, the duration of the occurrence of obesity was not questioned by questioning the process of being obese or overweight throughout the life of the individuals (infancy, childhood, adolescence, etc.). Another limitation is that factors other than the parameters of native thiol, total thiol, disulfide, SH/total SH, SS/SH, SS/Total SH cannot be measured. In our study evaluating the thiol/disulfide balance; Obese and non-obese individuals according to BMI; multiple subgroups analyzes were performed for the first time, grading according to BMI as normal weight, overweight, mild, moderate, and morbid obese.

There are studies with fewer people investigating the thiol/disulfide balance in premenopausal adult women. In our study, it is our strength to create a large sample with more participants. Apart from obesity that may cause oxidative stress, factors such as additional diseases (CVD, DM, dyslipidemia, HT, OSAS, acute-chronic infections, etc.), menopause, drugs, smoking and alcohol use were excluded and only BMI and thiol/disulfide balance parameters were compared.

CONCLUSION

With weight gain, there is an increase in BMI, WCM, and BFP, and the formation of a subclinical inflammation with the effect of cytokines released from fat cells affects thiols. In order to prevent obesity-related morbidity and mortality, our main treatment goal should be to control weight and prevent abdominal obesity in people who are overweight. Therefore, we believe that the thiol/disulfide balance may be a guide for oxidant-antioxidant status in obese adults.

We think that thiol antioxidant treatments such as BME and NAC can increase adipose tissue and anti-inflammatory effect at the systemic level in obese and overweight patients.

We think that by looking at waist circumference, body mass index, body fat percentage measurements, and albumin levels, we can have an idea about the thiol/disulfide balance, which is related to oxidative stress. Thus, studies to minimize the effect of obesity by protecting people from oxidative stress with antioxidant support treatments, etc. that can be applied in addition to weight control can be paved. However, large-scale studies are needed to make more comments about these parameters.

ETHICAL DECLARATIONS

Ethics Committee Approval: This speciality thesis study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 31.10.2017, Decision No: 20/02).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

The impact of *Helicobacter pylori* eradication on the oxidative stress index

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ABSTRACT

Aims: The oxidative stress index is the ratio between total antioxidant status (TAS) and total oxidant status (TOS). In this study, we aim to assess the pre and post-eradication oxidative stress status of patients with *Helicobacter pylori* infection.

Methods: A total of 100 patients with *H. pylori*-positive were included in the study. Pre and post-eradication TAS, TOS, and oxidative stress index (OSI) of patients were compared. P<0.05 was assumed statistically significant.

Results: TAS, TOS, and OSI levels did not change significantly following *H. pylori* eradication (p>0.05). 57 of 100 patients infected with *H. pylori* achieved eradication.

Conclusion: This study demonstrates TAS, TOS, and OSI levels may not change following *H. pylori* eradication.

Keywords: *Helicobacter pylori*, oxidative stress, eradication treatment

INTRODUCTION

Helicobacter pylori (*H. pylori*) is an active, urease positive, and (-) gram bacterium curved or thin spiral-shaped, with 4-6 flagella at one end.[1] Approximately half of the World's population is infected with this bacterium. In general, *H. pylori* infection is often seen in socio-economically undeveloped societies. The prevalence of *H. pylori* infection in Turkey has been reported up to 70-80% in adults and 64% in children. *H. pylori* is found in 70% of patients with a stomach ulcer, 90-95 % of patients with a duodenal ulcer, and 90 % of patients with stomach cancer.[2,3]

H. pylorus produces free oxygen radicals in the organism by targeting the mitochondria in the cell and causes oxidative stress.[4] It is thought that *H. pylori* infection has a key role in gastric cancer development as it creates stress by increasing the production of free oxygen radicals, which in turn causes DNA damage.[5,6] It is thought that oxidative stress has also a role in the pathogenesis of various diseases.[7]

Oxidative stress index (OSI) is defined as the ratio of the TOS to TAS level. The organism tries to deal with the reactive oxygen metabolites due to metabolic and physiological processes with enzymatic and/or non-enzymatic reactions. Therefore, it is aimed that the organism does not get harmed by these reactive oxygen metabolites. An increase in the oxidant and/or decrease in the antioxidant status leave the organism vulnerable to reactive oxygen radicals and cause cell damage.[8] Antioxidant molecules inhibit these harmful reactions and protect the organism from oxidative stress.[9,10]

In this study, we aim to investigate whether *H. pylori* eradication leads to a change in TAS, TOS, and OSI in patients infected with *H. pylori*.

METHODS

The study was approved by the Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date:07.04.2009, Decision No: 2009/04/31). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This is a single-center, prospective, and self-controlled study. A total of 100 patients who applied to internal medicine and gastroenterology polyclinics with dyspeptic complaints between April and November 2009 were enrolled in the study.

The diagnosis of *H. pylori* infection depended on the finding of a biopsy taken from the gastric antrum and/or corpus.

Patients were informed about the study and written consent was obtained from those who accepted to participate in the study. Criteria to include in the study were:

1. To be between 30-60 years old
2. Absent of hypertension, heart failure, diabetes, chronic obstructive pulmonary disease, hyperlipidemia, malignancy, liver and kidney failure, cerebrovascular disease,
3. No addiction such as drugs, smoking, and alcohol,
4. Absence of anemia (Hb should be above 12 g/dl), absence of abnormalities in kidney and liver tests, and total bilirubin value below 2 mg/dl
5. Not to be pregnant
6. Not to be on therapy of a proton pump inhibitor, H_2 receptor blocker, antibiotic, or nonsteroidal anti-inflammatory drug in the last 1 month

Patients received 2×30 mg lansoprazole, 2×500 mg clarithromycin, and 2×1000 mg amoxicillin for *H. pylori* treatment for 14 days. 6- 8 weeks following this therapy, a 14C urea-breath test (Heliprobe, Kibion AB Uppsala, Sweden) was applied as eradication control. Urea-breath test was used in a way by collecting breath samples using a dry cartridge system 10 minutes after the capsule form containing 14C urea/citric acid mixture was given with 250 ml of water after 1 night of fasting and following that by evaluating with Geiger-Müller counter.

Following the urea-breath test, blood was taken from the patients and centrifuged at 4000 rpm for 10 minutes in the biochemistry laboratory; plasma was taken in the Eppendorf tube and kept at -86°C. Plasma kept was taken out when the work was completed and made dissolved. Total oxidant status (TOS) and total antioxidant status (TAS) were studied using the Total Oxidant Status Assay Kit and Total Antioxidant Status Assay Kit (Rel Assay Diagnostics®, Gaziantep, Turkey) on the SIEMENS ADVIA 2400 spectrophotometric autoanalyzer device. The following formula was used to calculate the oxidative stress index.

$$OSİ = \frac{\text{TOS, µmol } H_2C_2 \text{ equivalent/L}}{\text{TAS,µmol Trolcx equivalent/L}} \text{ X } 100$$

Statistical Analysis

Data were analyzed by using Statistical Package for Social Sciences (SPSS) 15.0 package program (SPSS Inc. USA). The normal distribution of the data was analyzed with the Kolmogorov-Smirnov test. In the analysis of normally distributed numerical data independent sample t-test; in the analysis of data that were not normally distributed Mann-Whitney U test was used. The chi-square test was used in order to analyze whether there was a difference in terms of gender distribution among the groups. p value less than 0.05 ($p<0.05$) was considered statistically significant in statistical comparisons.

RESULTS

A total of 100 patients with *H. pylori*-positive and who were given eradication treatment were included in the study; a total of 46 men 29 of whom with *H. pylori* eradication and 17 without; a total of 54 women 28 of whom with eradication and 26 without. Demographic and laboratory data of the patient groups are given altogether in the **Table 1**. No significant difference was found in oxidative stress levels between eradicated and non-eradicated groups. Because all the patients included in the study were *H. pylori* positive, no comparison could be made with those with *H. pylori* negative.

Table 1. Demographic and laboratory data of the study groups

	H. pylori eradication (fail), n=43	*H. pylori* eradication (succes), n= 57	P value
Gender, female/male	26/17	28/29	>0.05
Age, years	42.7±7.9	46.0±8.5	>0.05
TAS	2.5±0.4	2.5±0.3	>0.05
TOS	14.6±5.5	15.5±7.9	>0.05
OSI	0.6±0.20	0.6±0.25	>0.05

DISCUSSION

This study investigated the impact of *H. pylori* eradication on the oxidative stress status of patients with *H. pylori* infection. Since the previous studies demonstrated the increased oxidative stress in patients with *H. pylori* infection, whether *H. pylori* eradication is related to recovery in oxidative stress status is not clear. However, this study failed to reveal the impact of *H. pylori* eradication on oxidative status in those patients.

Oxidative stress, named as the imbalance between the production of free oxygen radicals and other oxidants and anti-oxidant defense, is the damage that is caused by reactive oxygen products on biological structures such as proteins, lipids, carbohydrates, and DNA.

Antioxidant products' levels decrease during infection and disrupting this balance causes various pathological changes. When this happens, either the anti-oxidant defense system has weakened or the production of free oxygen radicals has increased or both effects are seen at the same time.[11,12,15] An increase in free oxygen radicals and deficiency in antioxidant systems are accepted to have a role in the etiopathogenesis of gastroduodenal diseases.[16] An important factor for the increase of free oxygen radical levels, which are chemically highly toxic, is also *H. pylori* besides factors such as insufficient antioxidants in the diet and smoking. In the study carried by Davies et al., it is found out that the production of free oxygen radicals has increased in *H. pylori*-positive chronic antral gastritis and there is a correlation between *H. pylori* density in the antral mucosa and free oxygen radicals.[17] One of the characteristic cases of inflammation is the infiltration of the tissue affected by the neutrophils causing the production of ROS in huge amounts in the affected gastric mucosa. It is shown in the studies carried out that the factor causing increased oxidative DNA damage as a result of neutrophil infiltration of the gastric mucosa of *H. pylori* infection is associated with increased ROS production.[18] Moreover, it is known that *H. pylori* infection is associated with increased oxidative stress both in the tissue and the blood.[19,20]

At the same time, it is known that increased oxidative stress plays an important role in gastroduodenal mucosal inflammation observed in the course of *H. pylori* infection, and in the pathogenesis of peptic ulcer and gastric cancer.[21] Gastric epithelium and *H. pylori* also cause the production of IL-8, which contributes to the formation of ROS and at the same time the emergence of IL-1 β, IL-6, IL-8, IL-12, TNF-αand IFN-gamma.[22-26] In general, the production of TNF- α, IL-1 β, IL-6, and IL-8 is correlated with the degree of inflammation, however Bauditz et al., and Tanahashi et al., shown in the studies they carried out that there was no difference between *H. pylori* positive and negative patients. On the other hand, it is shown in several studies that the production of IL-10 and IL-12 is associated with the presence of *H. pylori*.[24-27] Studies have shown that oxidative damage increases in gastric inflammatory diseases (149-151) and that accumulation of oxidative DNA damage in tumor suppressor genes like p53 can have an important role in the formation of gastric cancer.[28-31] DNA damage index has to be determined primarily in most of the methods used to measure oxidative DNA damage. This index can be determined through urinalysis. Although

there are many measurable base damage products in the urine, 8-oxo-dG, which is the most sensitive to oxidation and is easily measured, is preferred.

In studies performed both by direct measurement of 8-hydroxydeoxyguanosine (8-OHdG) residues on DNA and by identifying DNA strand breaks and fpg-sensitive sites, DNA damage was found to be higher in *H. pylori*-infected gastric mucosa than in uninfected normal mucosa.[32-38] Moreover, Laderia et al., in the studies they carried out, found out that oxidative DNA damage was directly associated with the intensity of gastritis in patients with *H. pylori* (+)gastritis and that DNA damage was higher in cases over 50 years of age than in young people.[36] On the other hand, they mentioned in the same study that DNA damage in people infected with *H. pylori* was not only higher in the gastric mucosa but also higher in peripheral leukocytes than in uninfected individuals.[37] Normally, if *H. pylori* infection is increasing oxidative DNA damage, DNA damage is expected to decrease after *H. pylori* eradication treatment.

It has been determined that 8-OHdG level, which has an oxidative DNA stress marker, in the antral mucosa of patients with extensive gastritis is higher than in patients with uninfected gastritis, and that after eradication treatment both 8-OHdG levels in the antral mucosa and gastric juice mutagenicity decrease.[33] Similarly, Hahm et al, reported that 8-OHdG level in biopsy samples taken from gastric mucosa following the eradication treatment in *H. pylori*-positive patients decreased compared to the pre-treatment level.[34] However, in the study Everett et al., carried out to find out DNA damage in epithelial cells obtained from antral biopsy samples in *H. pylori*-positive people, it was reported that DNA damage in normal mucosa is higher than in the area with gastritis and they showed that in biopsy samples taken after 6 weeks of eradication, DNA damage was increased in the gastritis area, but still at a lower level than in the normal mucosa; and they suggested that low DNA damage in gastric epithelial cells infected with *H. pylori* might be due to increased cell transformation in gastritis.[35] Again similarly in the study Farinati et al., carried out they found out that 8- OHdG level increased after *H. pylori* eradication in biopsy samples of antrum in *H. pylori*-infected cases and concluded that eradication treatment would not reduce the damage already occurred.[32] In another study, it was stated that there was no significant difference in urinary 8- OHdGlevels after and before the eradication treatment of children infected with *H. pylori*.[39]

Yoshida et al created *H. pylori* infection empirically in the study they carried out. They reported that neutrophil infiltration increased clearly in the area where infection occurred and in connection with this, lipid peroxidation and hemorrhagic erosions occurred in the gastric mucosa.[40] Malondialdehyde (MDA) level reflects the damage of free oxygen radicals on lipids and the damage they do on the cell membrane and is accepted as an indicator of oxidative damage.

In the study Drake et al., carried out, MDA concentration in *H. pylori* gastritis was found significantly higher than in those who had normal histology.[41] The 8-OHdG level which is accepted as an indicator of oxidative damage in *H. pylori* gastritis was found to be high by Hahmet. al.[34]

Moreover, in a study by Dinçer et al., carried out in Turkey, the effect of eradication treatment performed on *H. pylori*-infected patients on oxidative DNA damage was searched by comet method and they found that DNA damage decreased following the eradication.[42] According to the findings in our study eradication treatment on *H. pylori*-infected persons does not have an effect on TOS and OSI levels, which are oxidative stress markers.

Antioxidants detoxifying free oxygen radicals in the organism are the substances that prevent or delay oxidative damage in the targeted molecule or even repair the occurred damage, and endogenous or exogenous antioxidants can be effective at different stages of an oxidative case. Enzymes taking place in this system are superoxide dismutase, catalase, and glutathione peroxidase.[43,44] It is thought that in defense against free oxygen radicals, the superoxide dismutase (SOD) enzyme could be the first step among endogenous antioxidant enzymes. In a study SOD and glutathione peroxidase (GPX) activities in the mucosa of patients with gastric ulcers are reported to be decreased compared to normal tissue.[45] In our study, no significant change was detected at TAS levels between *H. pylori* positive and negative patients after eradication treatment was given. It is shown that, C vitamin, which is known as an antioxidant, acts as a free radical scavenger in gastric mucosa and that ascorbyl radical level in *H. pylori* gastritis has increased. Again in the same study, following *H. pylori* eradication with combined treatment, the MDA level was found to be lower compared to before the treatment.[41]

In a study realized in Turkey, a significant decrease in free radical levels was found in the *H. pylori* (+) group with single and combined treatments.[46] In the study, Tanyalçın et al. carried out reduced glutathione levels increased after triple eradication treatment.[47] Again in Turkey, in a study carried out by Bahçecoğlu et al., after eradication treatment of *H. pylori*-infected people, MDA, and glutathione peroxidase activity was found significantly to be lower than in the beginning. Thus, they showed in this study that the eradication of *H. pylori* could eliminate the oxidative stress caused by this microorganism.[48] Oxidative stress is associated with many diseases, including gastric diseases such as chronic gastritis, peptic ulcer, gastric cancer, and MALT lymphoma.[49-51] These gastric diseases are a result of *H. pylori* infection, which is believed to be a major etiologic agent.[50-52]In the presence of *H. pylori* infection; gastric mucosa directly encounters the metabolic products of the bacteria. Rapid regeneration in damaged epithelium cells increases the risk of DNA damage.[53] In our study, after eradication treatment was given in patients infected with *H. pylori*, no significant difference was found between the *H. pylori* positive and negative groups in both antioxidant and oxidative stress levels.

CONCLUSION

Although most of the studies have demonstrated antioxidant and oxidant levels can change following treatment of any infection, studies reporting adverse outcomes similar to our study are quite rare. However, it is thought that oxidative stress would be decreased if antioxidant vitamins are given together with eradication treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date: 07/04/2009, Decision No: 2009/04/31).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

A new hope in the management of hematologic malignancy: immunotherapy

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ABSTRACT

In recent years, Hodgkin's and Non-Hodgkin's lymphoma incidence is increasing all over the world. In the United States, lymphomas are the fourth most common malignancies among all. Lymphoid malignancies have a broad spectrum from mild indolent types, such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL), follicular lymphoma (FL), marginal zone lymphoma, and cutaneous T-cell lymphoma to aggressive types such as diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma, Burkitt's lymphoma, peripheral T-cell lymphoma. With better response rates to chemotherapy, longer disease-free survival and overall survival rates, lymphomas have satisfactory treatment results rather than solid organ tumors for oncologists. Throughout history, conventional chemotherapy agents, radiotherapy and their combinations have been used for the treatment of lymphoma. Especially, in the early stages of Hodgkin's lymphoma, a rate of 85-90% complete remission and high rate long-term remission can be achieved. However, despite this high response rate, 15-30% of patients are resistant to treatment. With current therapies response rate and persistent long-term remission rate in Non-Hodgkin's lymphomas are around 50%. Therefore, new therapeutic approaches such as immunotherapy and the using of cytotoxic properties of T cells against tumor has been developed and used in recent years for the treatment of refractory lymphoma.

Keywords: Hematologic malignancy, immunotherapy, new target

INTRODUCTION

Success of imatinib mesylate treatment abolished the need of allogeneic stem cell transplantation in chronic myeloid leukemia (CML) and also has guided the treatment of other hematologic malignancies. Especially the usage of Bruton's kinase inhibitors in CLL and indolent non-Hodgkin's lymphoma treatment has increased the response rates.[1]

T/B lymphocyte, macrophage, natural killer cell and cytokine mediated tumor immunology is a complex system that forms the basis of response against cancer. Primarily, perception of tumor cells as a foreigner and then proliferation of regulatory T lymphocytes to create a destroying microenvironment for tumor cells are necessary to create an initial response against cancer cells.[2]

Transfer of The Adaptive Immune Response and Chimeric Antigen Receptor (CAR) T Cell

One of the most effective treatment approaches is usage of CD19 specific, potential cytotoxic T cells which are programmed and created by genetic engineering, especially for the treatment of B-cell NHL and CLL. Mononuclear cells derived from peripheral blood accompanied by leukapheresis are collected and CD 19-specific T cells are isolated.[3] Several studies related to CAR T cell treatment has been conducted for the treatment of DLBCL and FL. In refractory FL patients, partial response achieved up to 32 weeks, by National Cancer Institute (NCI) researchers. In another study, 4 complete responses, 2 partial responses, and 1 stable disease symptoms achieved in 7 patients with DLBCL.[3,4]

CAR T cell treatment related side effects has been examined and there was no correlation between the rate of the infused T cells and the side-effect profile. Nevertheless, most important problems encountered was encephalopathy and B-cell aplasia. B cell recovery can occur in 6 months after infusion of T cells. Intravenous immunoglobulin support might be protective against infection, especially for the patients with B-cell aplasia.

Immunotherapy Aimed for Eliminating Immune Tolerance

For a long time, oncologists have dreaming to use the power of immune system to fight malignancies. Initial usage of immunotherapy for lymphomas began with thesis of graft versus tumor effect of T cells which were collected from the donor during allogeneic stem cell transplantation. After the discovery of rituximab treatment's efficacy, which acts against CD20 positive B cells, the idea of using immune agents in lymphoma treatment beside conventional chemotherapy has emerged, especially in non-Hodgkin's lymphomas.

Immunotherapy agents shows a wide variability from monoclonal antibody-based agents to transfer of adaptive

immune response in cytotoxic T lymphocytes or inhibition of PD-1 (programmed cell death) receptors that prevents T cell activation.[1]

Immune checkpoint system leads to protection from self-tolerance and autoimmunity. Chronic antigenic stimulation of T cells via signaling through the PD-1 pathway is substantial. Once PD-1 stimulated by its ligands, T-cell proliferation, cytotoxicity, and cytokine production is decreased and apoptosis is increased. So, this interaction leads to generation and maintenance of peripheral tolerance.

In normal conditions, interaction of T cell receptors with antigen-presenting cells is necessary for immune system activation. In cancer patients, synthesis of proteins from cancer and non-cancer cells in microenvironment that suppresses T cell functions are increased, such as cytotoxic T Lymphocyte associated protein 4 (CTLA 4) and programmed cell death protein-1 (PD-1). The effect of PD-1 for the inhibition of T-cell activity seems to be stronger than by CTLA-4.[5]

Immune Checkpoint Inhibitors; Hodgkin's Lymphoma and Non-Hodgkin's Lymphomas

Although both CTLA 4 and PD-1 has an inhibitory effect on tumor immunity, they act with different mechanisms. CTLA 4 is particularly expressed in T cells in the lymph nodes. Mobilization of CTLA 4 is increased by CD28 stimulation via T-cell receptor signalization. Once CTLA 4 binds to CD80 and CD86 ligands, it blocks the communication between CD28 and T cell, thus inhibiting the T cell activation.[6]

PD-1 is particularly activated in extra nodal/ peripheral T cells and also their ligands on tumor cells. After PD-1 is activated and bounded to PD-L1 and PD-L2 receptors, phosphatidylinositol 3-kinase (PI3K) pathway is antagonized and T cell activation is blocked. The idea of increasing T cell activation by inhibiting PD-1 activity and decreasing the self-tolerance at lymphoid malignancies leads us to think that expression levels of PD-L1 and PD-L2 receptors may be effective in terms of response to treatment. PD-L1 expression occurs in hematopoietic cells such as T cells, B cells, macrophages, dendritic cells and natural killer cells and non-hematopoietic cells. PD-L1 expression is increased especially in Hodgkin Reed-Sternberg (HRS) cells through EBV related mechanisms, gene amplification, and chromosomal translocation in Hodgkin lymphoma. PD-L1 expression is also detected in Non-Hodgkin's lymphoma subtypes such as DLBCL, primary mediastinal B-cell lymphoma and anaplastic large cell lymphoma. 9p23-24 chromosomal amplifications are common in Hodgkin lymphoma patients and associated with good response to PD-1 inhibitor treatment.[7]

Pidilizumab is the first PD-1 inhibitor and particularly has been used for phase 1 and phase 2 studies in DLBCL patients. Although promising in refractory cases when combined with rituximab, desired success could not be achieved because of the low specificity of pidilizumab to PD-1 receptors. In phase 1 studies conducted with nivolumab and pembrolizumab, objective response rate was 36% and 40% in DLBCL and follicular lymphoma patients, respectively.[10] Most common side effects of PD-1 inhibitors are immune system associated pneumonia, colitis, hypophysitis, thyroiditis and hepatitis. The most serious side effects (grade 3 and 4) associated with immune checkpoint inhibitors, are most frequent with the ipilimumab treatment (20%). Immune-modulatory agents such as corticosteroids and infliximab could be used for the side effects of these agents.[8]

Today, frequency of PD-1 inhibitors usage is increasing, especially in relapsed Hodgkin lymphoma cases. Nivolumab has been used more often than pembrolizumab because of it's grade 1 and 2 treatment-related side effects. If the disease persists after autologous stem cell transplantation in relapsed/ refractory Hodgkin lymphoma patients, PD-1 inhibitor treatment seems to be preferred instead of allogeneic stem cell transplantation because of the difficulty of finding suitable donor and long-term strict control requirements.

Brentuximab Vedotin

Despite all the advances in the treatment, long-term remission can not be achieved at approximately 30% of classical Hodgkin lymphoma patients with conventional chemotherapy and radiotherapy.[9] In the present, standard treatment approach of these patients is high dose chemotherapy and autologous stem cell transplantation.[10] However, this treatment provides long-term remission for only 50% of patients and expected median survival time is 27 months for the rest. An antibody drug conjugate brentuximab vedotin is the only drug approved by the FDA in the last 30 years for this group of patients.

It selectively binds to CD30 (+) malignant HRS cells through chimeric monoclonal antibody in it's structure and shows tumoricidal effect by a microtubule inhibitor called as monomethyl auristatin E (MMAE).[11]

When compared with a phase 2 study consisting of 102 patients that conducted by Jones et al., this study also shows similar objective response rates (80% vs. 75%), at least in the early stages of disease.[12] However, PET/CT scan assessment after 6 cycles of treatment with brentuximab vedotin showed that, the objective response rate decreased up to 10% and progression of disease had observed in 7 patients with at least partial response in the early stages. This result demonstrated that brentuximab vedotin is insufficient to obtain long-term remission. In fact, decreasing of high objective response rates in further cycles that reached after the first 3 cycles of brentuximab vedotin treatment, revealed problem of permanent response rate with brentuximab treatment.[12,13]

Blinatumomab

Blinatumomab is a 55 kDa tyrosine protein derivative which is specific for CD3 and CD19 cells. So, it has immune effects on both T and B cells. Half-life of blinatumomab is 2 hours, therefore administration of 3 times a week is recommended in non-Hodgkin's lymphoma and CLL. Blinatumomab administration dose is 0.75-15 microgram/m² intravenously within 2-4 hours. In one study, blinatumomab was administered in doses of 15 microgram/m² in 76 patients with relapsed/refractory Non-Hodgkin's lymphoma (37% follicular lymphoma, 32% mantle cell lymphoma, 18% DLBCL and 12% others) and objective response rate and complete response rate was 69% and 37%, respectively.[14] While best response rate has detected in follicular lymphoma patients with a response rate of 80%, response rate in patients with DLBCL was 55%.[14] Blinatumomab was approved by FDA in December 2014 for refractory Non-Hodgkin's lymphoma treatment.

Small Molecule Inhibitors; Bruton's Tyrosine Kinase Inhibitors (BTKI)

B cell receptor signaling is essential for proliferation of normal and malignant B cells. B cell receptor expression is increased in DLBCL, FL, mantle cell lymphoma and CLL. Bruton kinase is a member of Tec kinase family and has a role in B cell receptor signal cascade with Syk and PI3K.

Ibrutinib

In a phase 3 open label randomized study that consisting of 391 patients with relapsed / refractory CLL or small lymphocytic lymphoma (SLL), 195 patients were randomly assigned to receive oral ibrutinib (420 mg once daily) and 196 patients randomly assigned to receive intravenous ofatumumab.[15] Median age was 67 (30-86) and all patients in both groups previously had a median of three-line treatment. Median follow-up time was 9.4 months and the overall survival rates at 12th month was 90% and 81% in ibrutinib and ofatumumab groups, respectively ($p<0.001$). Objective response rate was 42.6% in ibrutinib group and 4.1% in ofatumumab group ($p<0.001$). Most frequent treatment-related side effects in ibrutinib group was diarrhea, fatigue, pyrexia, and nausea. On the other side, side effects in ofatumumab group was fatigue and cough. Interestingly, development of cataract was seen 3% in ibrutinib group and 1% in ofatumumab group.

In another phase 2 multi-center study, efficacy of ibrutinib treatment (560 mg) was investigated in 111 relapsed or refractory mantle cell lymphoma patients.[16] Patients were enrolled into two groups: those who had previously received at least 2 cycles of bortezomib treatment and those who had previously received less than 2 complete cycles of bortezomib or had received no prior bortezomib treatment. The median age was 68 and patients had undergone a median of three (1-5) previous therapies. Objective response rate was 68% with a complete response rate of 21% and a partial response rate of 47%. Prior treatment with bortezomib had no effect on the response rate, and the median response duration was 17.5 months. Median progression-free survival was 13.9 months and overall survival rate was 58% at 18 months. Grade 3 or 4 side effects were infrequent, neutropenia and thrombocytopenia was detected in 16% and 10% of patients, respectively. As a conclusion ibrutinib shows efficacy in relapsed or refractory mantle cell lymphoma patients who received multiple prior therapies.

PI3K Inhibitors; Idelalisib

With activation of PI3K pathway, B cell receptor signaling becomes stronger especially in CLL patients. Idelalisib is a first-class selective inhibitor of PI3K pathway. Preclinical studies showed that idelalisib inhibiting PI3K-AKT pathway and inducing apoptosis.[17] Reliable side effect profile and anti-tumor activity of idelalisib shown with phase I and phase II studies in relapsed and refractory indolent NHL and CLL / SLL patients.

In a randomized, double blinded, placebo-controlled, phase 3 study consisting of 220 poor prognosed relapsed/ refractory CLL patients, one group received idelalisib in combination with rituximab and other group received rituximab plus placebo. Objective response rate was 81% in idelalisib group and 13% in placebo group ($p<0.001$). Overall survival at 12 months was 92% in idelalisib group and 80% in placebo group ($p<0.02$). Median progression-free survival was 5.5 months in the placebo group and was not reached in the idelalisib group yet ($p< 0.001$).[18]

Idelalisib received FDA approval in July 2014 for treatment of relapsed CLL and SLL patients who received at least 2 prior therapies.

Immunomodulators; Lenalidomide

Mantle cell lymphoma is 6% of all NHLs and has a heterogeneous clinical presentation. Wait and see policy may be preferred in indolent forms, but in aggressive forms immediate high-dose chemotherapy following induction treatment and allogeneic stem cell transplantation seems to be the ideal approach. New therapeutic agents in resistant and aggressive mantle cell lymphoma patients remain promising. Lenalidomide is an immunomodulator agent and successful response rates was obtained in multiple myeloma (MM) and myelodysplastic syndrome patients. Efficacy of lenalidomide in lymphomas was shown in preclinical studies conducted in guidance of this knowledge. Better response rates (53% vs. 35%) and long term response rates (16.3 vs. 13.7 months) were provided in mantle cell lymphoma patients rather than other subgroups in a phase II study with NHL patients.[19]

In MCL-001 (EMERGE) study,[20] efficacy of lenalidomide (administered 5 mg per oral on days 1 through 21 in every 28 days) examined in 134 mantle cell lymphoma patients who were refractory to prior immunochemotherapy and bortezomib. Median age was 67 (43-83), median of prior therapies was 4 (2-10), objective response rate was 28% (8% partial response, 20% partial response), median duration of response was 16.6 months, median progression free survival was 4 months and median overall survival was 19 months. The most common adverse effects was neutropenia (43%), thrombocytopenia (28%), anemia (11%) and pneumonia (8%).

Lenalidomide received FDA approval in June 2013 for treatment of relapsed mantle cell lymphoma patients after 2 prior therapies.

Proteasome Pathway Inhibitors; Bortezomib, Carfilzomib

MM is 1% of all cancers and 10% of all hematologic malignancies. Not only genetic mutations in plasma cells but also bone marrow microenvironment and most importantly, the loss of strength of the immune system to fight against tumor is responsible for progression in MM patients. Although B cell disorders is seen in MM primarily, T cell functional defects also has been observed. Especially, loss of tumor-specific CD4 and CD8 T cells and natural killer cells in monoclonal gammopathy of undetermined significance (MGUS) patients is seems to be responsible for progression of disease to MM. In the light of these findings use of new immunologic agents for treatment of MM brings the success of treatment with itself.

Ubiquitin-Proteasome pathway is responsible for degradation and elimination of damaged proteins in healthy cells to ensure the healthy cell cycle. Protein synthesis cycle is required for cell life and energy is provided by proteasomes. It has been shown that the usege of proteasome inhibitors in MM, mantle cell lymphoma and some types of leukemia, induce apoptosis and inhibits uncontrolled cellular proliferation.[21] Dexamethasone and bortezomib combination is more effective and well tolerated in MM patients than vincristine, adriamycin, dexamethasone (VAD) combination and seems to be better choice as a first line treatment.[22]

Carfilzomib is a second-generation proteasome inhibitor with selective and irreversible inhibition of pathway and approved by FDA for the usege in treatment of in refractory MM patients.

A summary of Immunotherapeutic agents is given in **Figure 1**.

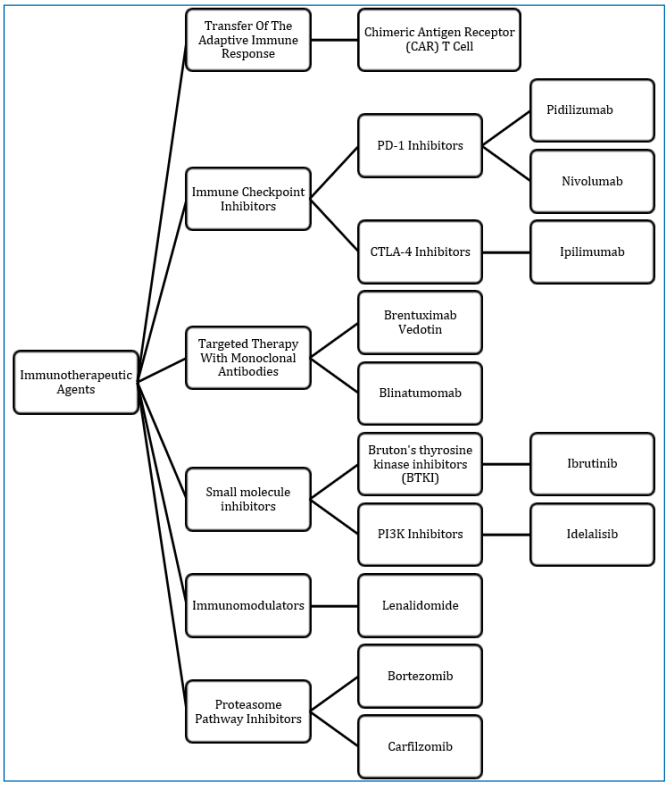

Figure 1. Immunotherapeutic agents

CONCLUSION

Immunotherapy has lead the oncology science into a new era, and hematologic malignancies seems to be the leading group that benefits. Immunotherapeutic approaches had stunning effects at the treatment of some solid tumors, as well as some types of hematologic cancers. There are numerous possible combinations of new drugs to test their efficacy in many tumors and settings. Therefore, the spectrum of immunotherapy is vast and enticing. However, to maximize the therapeutic potential of these strategies, much studies remains to be done in a coordinated fashion.

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Review

Obesity: etiology and the problems it causes

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ABSTRACT

Obesity is one of the biggest health problems today and its prevalence is increasing. It develops with excessive fat accumulation in the body secondary to high energy intake in obesity. It is a chronic disease in which the interaction of genetic, endocrine, metabolic, behavioral, psychological and sociocultural factors plays a role. In addition to endocrine (type 2 diabetes mellitus and insulin resistance, metabolic syndrome, etc.), cardiovascular (hypertension, hyperlipidemia, etc.) and cerebrovascular diseases, diseases such as cholecystitis, sleep apnea, osteoarthritis, hyperuricemia and gout, endometrial, breast, gallbladder cancers in women are associated with diseases such as colon, rectum, prostate cancer in men. These diseases both reduce the quality of life and lead to financial losses in individuals and national economies. Today, preventive health policies aim to reduce morbidity, mortality and economic burden by treating obesity without causing chronic diseases.

Keywords: Obesity, morbid obesity, obesity diagnosis, obesity prevention, body mass index

OBESITY

Obesity, one of the biggest health problems of today, is defined by the World Health Organization (WHO) as an abnormal or excessive amount of fat tissue accumulation that poses a health risk.[1] Although the information about the occurrence of obesity is not complete, it is a chronic disease in which the interaction of genetic, endocrine, metabolic, behavioral, psychological and sociocultural factors plays a role.[2] Obesity is associated with endocrinological diseases (type 2 diabetes mellitus and insulin resistance, metabolic syndrome, etc.) and cardiovascular diseases (hypertension, hyperlipidemia, etc.), cerebrovascular diseases, cholecystitis, sleep apnea, osteoarthritis, hyperuricemia and gout, endometrial, breast, gallbladder cancers in women, and colon, rectum, prostate cancer in men. It develops with excessive fat accumulation in the body secondary to high energy intake in obesity.[3-5]

OBESITY EPIDEMIOLOGY

Obesity is a very common health problem all over the world. According to the estimates of the Non-Communicable Diseases Risk Factor Collaboration (NCD-RisC) Group, the prevalence of obesity in the adult population increased from 3.2% in men and 6.4% in women in 1975 to 10.8% in men and 14.9% in women in 2014.[6] According to the Global Burden of Disease (GBD) Obesity Cooperation Group 2015 Report, the obese population in the world has reached 711.4 million (603.7 million adults).[7] According to WHO statements, in 2016, 39% of adults worldwide were overweight and 13% were obese.[8] By 2030, it is estimated that 38% of the world's adult population will be overweight and 20% will be obese. In the US, estimates based on previous lifestyle trends and outcomes indicate that more than 85% of adults will be overweight or obese by 2030.[9]

The prevalence of obesity in Turkey is increasing day by day as it is worldwide. WHO data report that in 2016, there were 16,092,644 obese individuals in Turkey and Turkey was the country with the highest prevalence of obesity in Europe with a prevalence of 29.5%.[4]

OBESITY DEFINITION

Obesity is a complex and multifactorial disease that negatively affects health. Since it is not easy to determine the body fat percentage, obesity is defined as excess weight rather than excess fat. 20-25% of the body weight of adult women and 15-18% of men consists of adipose tissue. Increasing the fat distribution above 30% in women and 25% in men causes obesity.[4]

Considering the fat distribution, there are two types of obesity, android and gynecoid. Android type obesity (central or apple shape obesity) is the type that accumulates hypertrophic fat cells in the abdomen and chest region, is more common in men and has an increased risk of chronic diseases. Gynecoid-type obesity (peripheral or pear shape obesity) occurs with increased fat distribution in the lower parts of the body and hips and is mostly seen in women.[11]

OBESITY DIAGNOSIS

Methods Used in the Evaluation of Obesity

Neutron activation analysis, underwater weight measurement, total body water, total body potassium measurement (Total Body K40), ultrasonography (USG), computed tomography (CT), deuterium oxide (D_2O), magnetic resonance imaging (MRI), bioelectrical impedance

analysis (BIA), Total Body Electrical Conductivity (TOBEC), single photon absorptiometer (SPA), dual photon absorptiometer (DPA), dual X-ray absorptiometer (DEXA) are included in this group.[12]

Bioelectrical impedance analysis (BIA): Basic principle in the use of BIA; weak electrical current (800 μA; 50 KHz) is given to the person's body and the permeability is measured using the lowest resistance value of the person formed, lean tissue mass is calculated·2

Indirect (Anthropometric) Methods in the Evaluation of Body Composition

Among the many methods used in the definition and classification of obesity, the most common and most reliable are body mass index (BMI) and waist circumference measurement.[12]

Body mass index (BMI) (kg/m²): BMI is calculated by dividing the body weight in kilograms by the square of the height in meters. Formula: 'BMI= Weight (kg) /Height (m²)' and if this ratio is ≥30 kg/m², it is considered obesity.[13] This index, which is the most preferred and easily used today, was first described by Quetelet in 1835. There is a correlation between body fat tissue measured directly by densitometer and BMI that varies according to population.14 The World Health Organization uses the classification made according to body mass index in the evaluation and grading of obesity. According to BMI in adults, underweight, normal and overweight and obesity and their degrees are shown in Table 1.[4,15]

Table 1 Rating of obesity in adults according to BMI (kg/m²)	
Underweight	<18.50
Healthy weight	18.5-24.99
Overweight	25.00–29.99
Obesity	≥30.00
Obesity Class 1	30.00–34.99
Obesity Class 2	35.00–39.99
Obesity Class 3 (Extreme)	40.00-49.99
Super Obesity	≥50.00

Women and men are diagnosed as overweight and obese based on the same BMI values. Similarly, in the elderly, the height decreases due to osteoporosis, while the muscle mass decreases, the fat ratio increases, and the body fat distribution changes and more fat accumulates around the waist.[16] The validity of BMI measurement points used to diagnose obesity in all adults is doubtful. The term 'Obesity Paradox' in the elderly actually refers to this incompatibility. Unlike other adults, obesity-related morbidity and mortality begin when BMI>33 kg/m² is over seventy years of age. This situation is interpreted as obesity being protective in the elderly. When using the BMI method, it can sometimes be perceived as a fat mass when evaluating people with excess muscle mass such as athletes and cause misconceptions. While the BMI value of the individuals is the same, the amount of body fat is higher in the yellow race than in whites. BMI is actually related to the amount of fat in the body, not the rate of fat. It is possible to calculate body fat from BMI with the formulas' Body fat % (men) = (1.33 x BMI (kg/m²)) + (0.236 x Age (years)) - 20.2 and Body fat % (women) = (1.21 x BMI (kg/m²)) + (0.262 x Age (years)) - 6.7 '.[17]

It is a cost-effective, easy and noninvasive method that is widely used in the evaluation of obesity. The disadvantage is that the distribution of body composition may be insufficient to distinguish between fat and lean tissue.

Skinfold thickness measurement (SFT): One of the most commonly used supportive anthropometric methods in the evaluation of body fat is SFT. The thickness of the subcutaneous adipose tissue of the areas (suprailiac, subscapular, biceps and triceps) determined as standard in the body is measured(3). However, the statistical relationships between skinfold thickness and total body fat percent body fat are generally not as strong as BMI.[18]

Waist Circumference, hip circumference, waist/hip ratio, waist/height ratio, visceral adiposity index (VAI), neck circumference: The relationship between morbidity and mortality is important according to the localization and distribution of fat tissue instead of the total amount of fat in the body. Another important dimension of obesity is abdominal fat deposition. The amount of intraabdominal (visceral) lubrication is correlated with the waist circumference measurement and the margin of error varies a lot according to the measurement.[19]

According to the data of the obesity-lipid metabolism-hypertension study group of the Turkish Society of Endocrinology and Metabolism (TEMD), ≥100 cm in males and ≥90 cm in females were recommended as abdominal obesity criteria (Table 2).[4]

Table 2. Waist circumference values by societies (cm)		
	Male	Female
Europe	≥ 94	≥ 80
USA	≥ 102	≥ 88
Turkey	≥ 100	≥ 90
South Asia and China	≥ 90	≥ 80
Japan	≥ 85	≥ 90
Africa	If there are no values belonging to the society, European data is appropriate	
Middle and South America	If there are no values belonging to the society, South Asian data is appropriate	

A study of 1898 adult men and 2308 women in 24 centers in all geographical regions of Turkey showed that the waist circumference values that best determine overweight and obese men in adults were 90 cm and 100 cm, respectively, and the values that determine overweight and obese women were 80 cm and 90 cm, respectively.[20]

Visceral adiposity index (VAI) is a new anthropometric method that indirectly indicates the risk of cardiometabolic complications associated with obesity. Data on safety in patients with acromegaly, diabetes, viral hepatitis C, non-alcoholic fatty liver diseases and polycystic ovary syndrome have been presented.[21]

According to the TURDEP-I study conducted in Turkey between 1997 and 1998, the prevalence of obesity over the age of 20 was found to be 22.3% in both genders; 12.9% in males and 29.9% in females. In the TURDEP-II Study, which was repeated in the same centers, the frequency of obesity was found to be 35.9% (female 44.2%; male 27.3%).[22]

According to the Heart Disease and Risk Factors in Turkish Adults (TEKHARF) study, the prevalence of obese people aged 30 and over was 25.3% in men and 44.2% in women, compared to the follow-up of 2001/2002 in Turkey. According to a 1990 survey, the number of obese people had increased by about 90%. Accordingly, it is estimated that 3.2 million men and 5.5 million women have obesity. This shows that the prevalence of obesity has increased by 20% in the last 10 years, regardless of population growth and age.[23]

According to the results of the Turkish Nutrition Health Survey (TNHS 2010), the prevalence of obesity and

overweight in adults was reported as 20.5% and 39.1% in men; 41% and 29.1% in women; 30.3% and 34.6% in both genders, respectively. The overall prevalence of morbid obesity was found to be 2.9% (female 5.3%, male 0.7%) (39). When the data from Turkey Population Health Survey (TDHS) conducted on 15-49 year old women published every five years throughout Turkey are examined, it is seen that obesity in women is increasing. According to the results of TDHS 1998, 2003, 2008 and 2013, the incidence of obesity was reported in **Figure 1** as 18.8%, 22.7%, 23.9% and 26.7%, respectively.[24]

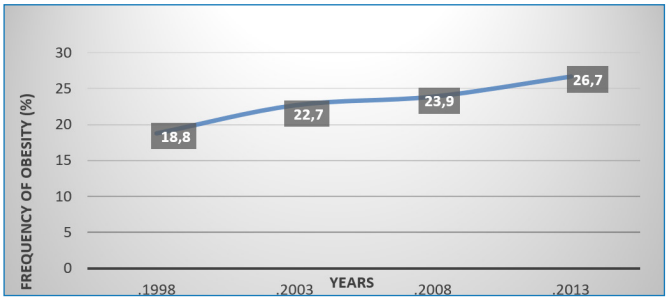

Figure 1. Turkey Population Health Survey (Prevalence of obesity in women aged 15-49 by years (%))

OBESITY ETIOLOGY

It is a multifactorial chronic disease with many genetic, epigenetic, physiological, behavioral, psychological, neurogenic, endocrinological (hormonal), sociocultural and environmental factors in its pathogenesis.[25]

Genetic Factors

Interactions between the biological environment (genetic/epigenetic factors) and environmental factors are responsible for the regulation of energy balance and the formation of fat stores.[26] Familial inheritance of obesity is the most important supportive condition. In addition, the incidence of obesity in monozygotic twins is higher than in sesquizygotic twins. Individuals in a subgroup in the healthy weight class can maintain their weight with continuous diet and exercise efforts and easily switch to the overweight or obese class by gaining weight when they are not paying attention. In these individuals, depending on the genetic background, metabolic mechanisms work similarly to those in obese individuals, and the term "metabolic obese" has been used for this group in recent years. Researchers have also identified another subgroup, called "healthy obese," which falls into the overweight or even obese class 1 category but is completely normal from a metabolic point of view.[4]

Obesity can be a component of many genetic diseases (such as Prader-Willi syndrome, Laurence Moon Biedl syndrome, Down syndrome, Turner syndrome, Albright's hereditary osteodystrophy, Fröhlich syndrome (adiposogenital dystrophy), hyperostosis frontalis interna). There is rarely a single gene mutation in the genetics of obesity, and obesity is more common as a result of the interaction of many genes with behavior and environmental factors.[27,28]

Neurogenic and Endocrinological (Hormonal) Factors

It has an inhibitory effect on the satiety center in the ventromedial hypothalamus and the appetite center in the ventrolateral hypothalamus in the brain. Affecting these centers for reasons such as inflammation, trauma or tumor leads to a change in dietary habits and eventually 'hypothalamic obesity'. In addition, many people with hypothalamus lesions or pituitary adenoma extending towards the hypothalamus may develop a tendency to obesity.[29]

Table 3. Peptides and hormones that affect food intake

• Appetite Boosters	• Appetite Suppressants
• Ghrelin	• Leptin
• Galanin	• Glucagon-Like Peptide- 1 (GLP- 1)
• Neuropeptide Y (NPY)	• Insulin
• Nitric oxide*	• Corticotropin-releasing factor (CRF)
• Orexin A-B	• Seratonin
• Cannabioids*	• α-melanocyte-stimulating hormone (α-MSH)
	• Calcitonin
	• Cocaine-Amphetamine-Regulated Transcript (CART)
	• Bombesin
	• Nesfatin-1

* not in peptide structure

Obesity develops in people who do not release leptin from adipose tissue due to genetic defect. Hormonal causes of obesity include Cushing's syndrome, insulin resistance, growth hormone (GH) deficiency, insulinoma, hypothyroidism, polycystic ovary syndrome (PCOS), diabetes mellitus (DM), pseudohypoparathyroidism, male hypogonadism, craniopharyngioma and hypopituitarism. Hypothyroidism occurs very often and leads to a decrease in the rate of metabolism and a decrease in energy expenditure.[30,31]

Demographic (Age, Gender, Ethnic Origin) Factors

Demographic factors such as age and gender are the unchangeable causes of obesity. As you get older, the muscle tissue and brown adipose tissue in the body decrease and the white adipose tissue increases. As a result of the decrease in physical activity, decrease in basal metabolic rate, change in food intake habits, oxidative stress, hormonal changes and disruption of regulatory mechanisms in the elderly, energy balance is negatively affected and "sarcopenic obesity" occurs. Although it is higher in women, the rate of obesity increases with age in both genders.[4,31]

Physical Activity

With the developing technology, industrialization and urbanization and the facilitation of lifestyle have caused a decrease in physical activity and daily energy expenditure. As a result of the rapid change in dietary habits, the increase in energy intake has made obesity inevitable. Excessive calorie consumption and sedentary lifestyle are known risk factors for obesity and DM. In the "Let's Eat Healthily, Protect Our Hearts" study conducted with 15,468 individuals over the age of 30 in 7 pilot provinces selected from all geographical regions, it was determined that only 3.5% of people in the society regularly (at least 3 days a week and 30 minutes of moderate-intensity) did physical activity.[32,33]

Dietary Habits

The second main reason for the increase in the prevalence of obesity is the increase in energy intake as a result of the rapid change in dietary habits. Reduction in physical activity and unhealthy diet are thought to have a share of at least 10% in the global burden of disease.[34,35]

Psychological Factors

There is a relationship between psychological factors and obesity. Obese individuals have problems with their eating attitudes and have poor quality of life and especially in physical space (self-confidence). It is known that behavioral changes are effective in weight loss. Due to the effects of situations such as stress, depression, sadness and anxiety on hypothalamic centers, changes in appetite, as well as poorly compatible coping response and overeating attitude as a way to get rid of tension are seen. Sleep disorders are also associated with obesity.[36,37]

Environmental Factors (Sociocultural and Socioeconomic Factors, Drug Use)

It has been revealed that factors such as the environment (place and region of residence), social status, income and education level, number of marriages and births, drug use, and lifestyle contribute to the development of obesity.[34-37]

OBESITY-RELATED PROBLEMS

Metabolic Syndrome and Prediabetes

Obesity has a very close relationship with prediabetes and type 2 DM. Obesity has important implications for morbidity, disability, and quality of life, and like many other health problems, the risk of developing type 2 DM is higher in obese people. According to World Health Organization data, overweight and obesity are responsible for 80% of type 2 DM cases in adults in Europe. The weight distribution of patients with type 2 diabetes mellitus is shown in Figure 2.[38-40]

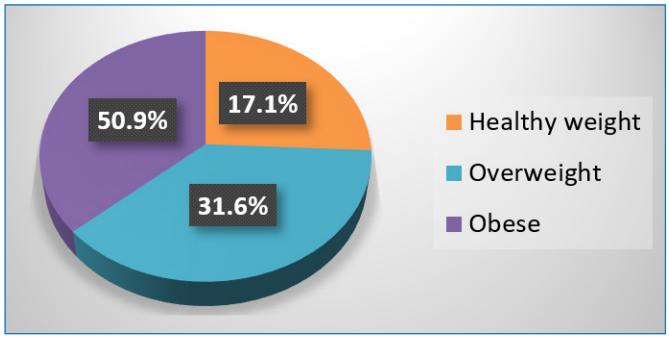

Figure 2. Weight distribution in patients with type 2 diabetes mellitus (%)

Dyslipidemia

One of the common complications of obesity is dyslipidemia, a disorder in lipid metabolism. The increase in the frequency of hypertriglyceridemia in obesity is due to an increase in hepatic TG production due to insulin resistance and hepatic VLDL production due to high carbohydrate consumption and a defect in the lipolysis of triglyceride-rich lipoproteins. With the increase of lipolytic activity in visceral fat tissue, free fatty acids in the portal system increase and cause a decrease in insulin intake to the liver and an increase in the amount of fat. Plasma lipoprotein profile in obese patients is generally high in TG, total cholesterol and LDL cholesterol and low in HDL cholesterol. Dyslipidemia is one of the common metabolic disorders with obesity and its frequency increases in direct proportion to BMI.[40]

Cardiovascular Diseases (CVD) and Hypertension

Studies have shown that obesity is effective in the development of atherosclerosis, symptomatic coronary artery disease, heart failure and atrial fibrillation and is an independent risk factor in the development of CVD. Obesity and atherosclerosis are considered congenital and acquired inflammatory conditions. Dyslipidemia accompanies both atherosclerosis and obesity, and LDL cholesterol and free fatty acids trigger inflammation. Inflammation is a condition associated with obesity, insulin resistance, and type 2 DM, which initiates and accelerates atherosclerosis. Adipocytokines and inflammatory markers released from adipose tissue contribute to atherosclerosis by causing insulin resistance, endothelial dysfunction, hypercoagulability and systemic inflammation. The increase in biomarkers such as Hs-CRP, IL-6 and IL-18 reflects the relationship between obesity-related metabolic disorders and also facilitates the follow-up of treatment.[41]

The Framingham Heart Study found that 26% of hypertensive men and 28% of women were overweight or obese. In the "National Health and Nutrition Examination Survey" (NHANES-3) study, it was shown that an excess of 5-9.9 kg increased the risk of developing hypertension in women with a BMI above 25 kg/m^2, and an excess of 25 kg and above increased the risk by 5.2 times. It has been reported that a weight increase of ten kg causes an increase of 3 mm Hg in systolic blood pressure and 2.3 mm Hg in diastolic blood pressure, which increases the risk of coronary artery disease (CAD) by 12% and the risk of stroke by 24%. In the NHANES-3 study, the frequency of hypertension was found to be around 15% in men and women with a BMI of <25 kg/m^2, while this frequency increased to 38% in women with a BMI of >30 kg/m^2 and up to 42% in men.[42-44]

Respiratory System Diseases (Obstructive Sleep Apnea Syndrome (OSA), Asthma, Reactive Airway Diseases)

Obstructive sleep apnea syndrome is a condition characterized by recurrent partial or complete upper respiratory tract obstructions resulting in hypoxia/reoxygenation during sleep and is the most important and modifiable risk factor in respiratory system problems associated with obesity. OSA is associated with an increased risk of developing cardiovascular diseases, metabolic diseases, insulin resistance and diabetes. Male gender and obesity are the main risk factors for sleep apnea. In obese patients, lung function abnormalities such as increased lung residual volume, ventilation-perfusion disorders, increased chest wall impedance and bronchospasm, decreased lung compliance and respiratory muscle endurance can be seen as a result of increased diaphragm pressure. In addition, OSA causes a decrease in leptin levels and an increase in ghrelin, thus causing a feeling of hunger and weight gain. Obesity causes OSA and OSA causes obesity, linked in a dangerous vicious circle. A BMI >29 kg/m^2 increases the risk of OSA by 10 times. The prevalence of OSA is even higher in patients with obesity and diabetes. For OSA, risky people need to be identified and followed up on in terms of complications that may occur. A 10% increase in weight causes a 30% increase in the apnea-hypopnea index (AHI), and a 10-15% decrease in weight reduces AHI by 50%. Therefore, weight loss programs should be added to treatment in all sleep apnea patients who are obese.[45]

All overweight or obese patients should be evaluated for asthma and reactive airway disease.

Gastrointestinal System and Liver Diseases

The most common are non-alcoholic fatty liver disease (NAFLD), gastroesophageal reflux disease, cholelithiasis, pancreatitis.[46-48]

Reproductive System Diseases

With obesity, the frequency of polycystic ovary syndrome (PCOS), male infertility (hypogonadotropic hypogonadism), female infertility increases significantly. With the fact that obesity changes hormonal balances, problems such as menstrual irregularities, PCOS, infertility, hirsutism, decreased libido and potency are seen. There is an increase in the secretion of free estrogen and testosterone stored in the adipose tissue, which is increased in obesity. In women with abdominal obesity, androgen production from the ovaries increases directly due to hyperinsulinemia. Lifestyle changes and nutritional treatment are the most important treatments in patients with overweight or obese PCOS. The use of orlistat, metformin or GLP-1R agonists in women with overweight or obese PCOS is effective in promoting weight loss and correcting PCOS findings and providing ovulation. In selected cases, improvement in symptoms and ovulation can be achieved with laparoscopic gastric bypass surgery.[49-51]

Musculoskeletal System Diseases (Osteoarthritis, Carpal Tunnel Syndrome, Osteoporosis)

There are studies showing that obesity reduces the risk of developing osteoporosis as it has positive effects on bone mineral density, bone mass and strength. Increased body weight; degeneration of the cartilage structure secondary to trauma caused by increased load on weight-bearing joints such as knees (most often), hips, spine causes osteoarthritis. Osteoarthritis is also more common in joints such as the hand joint that does not carry a load. The effect of obesity on osteoarthritis is thought to be due to the complex interaction of genetic, metabolic and inflammatory factors, in addition to biomechanical stress. Obesity is effective in developing or progressing low back pain and knee osteoarthritis (OA) in women. The frequency of carpal tunnel syndrome (median nerve neuropathy) has also increased in obese patients.[52,53]

Psychological Disorders

Mental disorders related to depression, bulimia, sleep disorders, incorrect diet side effects, increased tendency to bad habits, night eating syndrome and perception of appearance are common in obesity. By paying particular attention to the effects of chronic psychosocial stress, exposure to chronic stress can play a role in the development of obesity. Overweight and obese patients should be evaluated for depression and all patients with depression should be evaluated for obesity and lifestyle changes should be recommended. It is not known how much weight loss can improve depression in obesity accompanied by depression.[54,55]

Cancer

Obesity increases the risk of gallbladder, colon, rectum and prostate cancer in men and gallbladder, ovary, cervical, endometrial and breast cancer in women. Obese patients should be monitored for cancer risk and supported in terms of weight loss.[56]

Anamnesis and Evaluation

When evaluating an obesity patient, the habits of the person, the social environment in which he/she lives, the history of obesity, previous weight loss attempts, the factors involved in etiopathogenesis and accompanying comorbid diseases, his/her expectation and compliance with treatment, etc. should be considered. Appropriate environment and conditions should be provided for the ideal evaluation and physical examination of obese patients. Evaluation of patients should be performed in clinics with sleep apnea monitors, psychologically evaluable, hormonal and molecular genetics laboratories. A comprehensive history, physical examination and laboratory evaluation of the patient's obesity should be performed. It is recommended to study obesity-related blood tests (fasting blood glucose, HbA1c, fasting lipid profile, liver enzymes, renal function tests (creatinine, blood urea nitrogen), uric acid, TSH) and general laboratory tests (hemogram, complete urine analysis) in each obese patient. If necessary, additional examinations and evaluations such as glucose tolerance test, abdominal ultrasonography, dexamethasone suppression test or 24-hour urinary cortisol or 23:00 at night salivary cortisol, prolactin, estradiol, FSH, LH, pregnancy tests, androgens, apolipoprotein B and/or lipoprotein particle count, cranial CT or MRI, resting electrocardiogram (ECG), cardiac stress tests, echocardiogram can be performed. However, the measurement of insulin resistance in patients with excess weight and obesity has no clinical meaning and benefit in terms of diagnosis, treatment plan and follow-up. Since patient motivation and compliance with treatment are the most important elements of weight control programs, they should be questioned using a visual scale or some guided questions.[1,2,4,38]

CONCLUSION

Today, obesity, which is the second most important cause of preventable deaths after smoking, is a complex and multifactorial disease that negatively affects our health and causes disorders and diseases in many systems in the body. First of all, approaches to prevent the development of obesity should be emphasized as a society. Early diagnosis, early intervention and reduction in the prevalence of obesity will not only provide people with a healthy life, but also prevent many conditions that may develop, preventing obesity will prevent great economic losses as well as mental and physical benefits.

ETHICAL DECLARATIONS

Referee Evaluation Process: Externally peer-reviewed.

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Case Report

Late diagnosed prolactinoma in men: a case report

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ABSTRACT

Endocrinologic examinations encounter hyperprolactinemia, which is the release of prolactin hormone in excess of normal amounts, more frequently than any other pathological condition. In this case, we present a case of ıt is explained that a 42-year-old male patient can be diagnosed with prolactinoma by deepening the anamnesis without specifics symptoms. It is aimed to raise awareness on this issue.

Keywords: Hyperprolactinemia, macroadenoma, male sex

INTRODUCTION

Endocrinologic examinations encounter hyperprolactinemia, which is the release of prolactin hormone in excess of normal amounts, more frequently than any other pathological condition.[1] Prolactinomas are the most prevalent type of pituitary adenoma associated with the hypersecretion of hormones. Women should have prolactin levels of less than 25 ng/mL, while men should have levels of less than 20 ng/mL.[2] Prolactinomas are typically categorized as macroprolactinomas (less than 1 cm) or macroprolactinoma (greater than 1 cm) (greater than 1 cm).[3] Although prolactinomas are almost always benign, they frequently necessitate treatment due to gonadal dysfunction caused by prolactin hypersecretion or tissue compression caused by mass effect.[4] In this case report, we present an unusually early diagnosis of prolactinoma in men.

CASE REPORT

A 42-year-old man with no comorbidities and no history of substance abuse was referred to the ophthalmology outpatient clinic with complaints of blurred vision in the right eye for approximately one year, which resolved spontaneously 1-2 times per month, and headache for the past three months. The patient was referred to the neurology outpatient clinic following an ophthalmologic examination that revealed no pathology. In his anamnesis, he described localized pain in the anterior portion of the head for three months, which did not resolve with pain medication and rest, and he had complained of impotence for the past year. Non-contrast cranial magnetic resonance imaging (mrg) and testosterone hormone level for the complaint of impotence were studied. He was referred to the endocrinology outpatient clinic due to the presence of a pituitary mass in non-contrast cranial mrg and a testosterone value of 0.061 ng/mL. On physical examination, fever: 36.8°C, pulse: 80/min, blood pressure: 110/70 mmHg, saturation: 96%, respiratory rate: 13/min; decrease in the frequency of hair in the pubic and chest area and thinning of the existing hair (**Figure 1**). Other system examinations were normal. Anterior pituitary hormone levels were studied, and contrast-enhanced pituitary mrg was taken. Prolactin: >4700 µg/L (4.04-15.2), Testosterone: 0.061 ng/mL (2.49-9.36), TSH: 1.08 mU/L (0.27-4.2), Free T4: 0.59 ng/dl (0.93-1.70), Free T3: 1.91 pg/ml (2.0-4.4), FSH: 1.47 U/L (0.95-11.95), LH: 1.13 U/L (0.57-12.07), ACTH: 41.7 pg/mL (7.2-63.3), Cortisol: 2.29 µg/dL (6.2 - 19.4). The patient's pituitary hormone response was not at the expected level, and contrast-enhanced pituitary mrg: "At the level of coronal images in the sellar region, a mass lesion measuring ML 33 mm, CC 22 mm, AP 27 mm with a smooth lobule contour with hemorrhagic areas, significantly enlarged the sellar region, and slightly compressed and arched the adjacent vascular structures was observed. The patient was diagnosed as a prolactin hypersecreting macroadenoma based on the postcontrast series report "(**Figures 2** and **3**). Treatment was started with methylprednisolone 40 mg (milligram), levothyroxine 50 mcg (microgram), and cabergoline 0.5 mg 2 times a week. The patient was referred to neurosurgery for a surgical procedure because of hemorrhage foci in the macroadenoma.

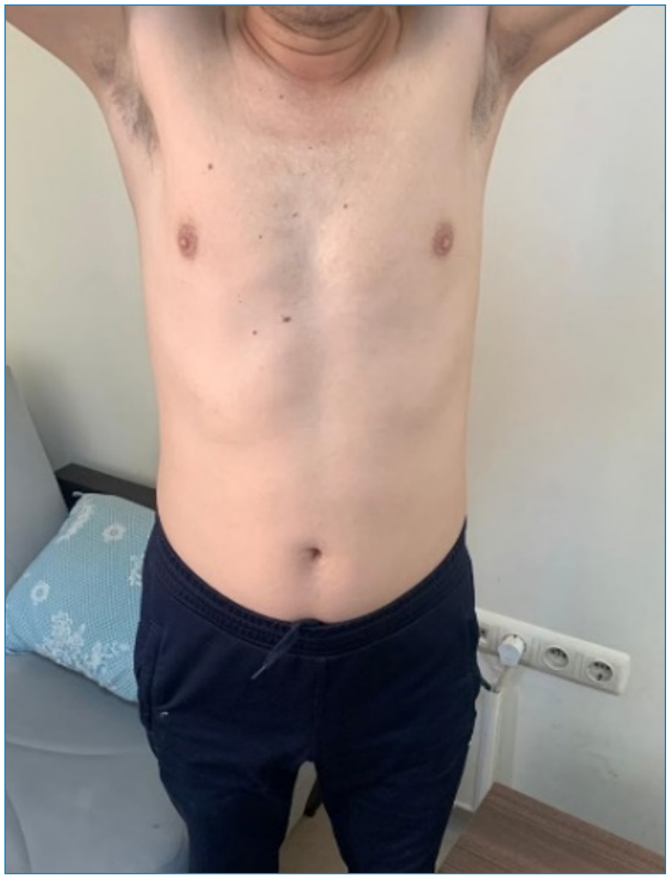

Figure 1. Reduction in the patient's body hair

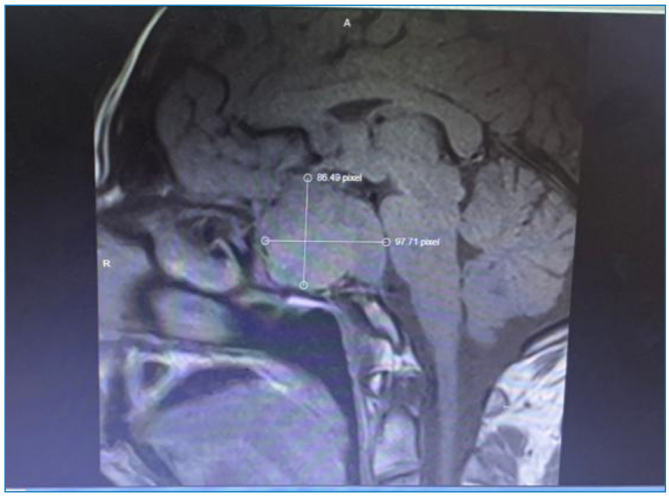

Figure 2. MRI image of the patient's pituitary adenoma

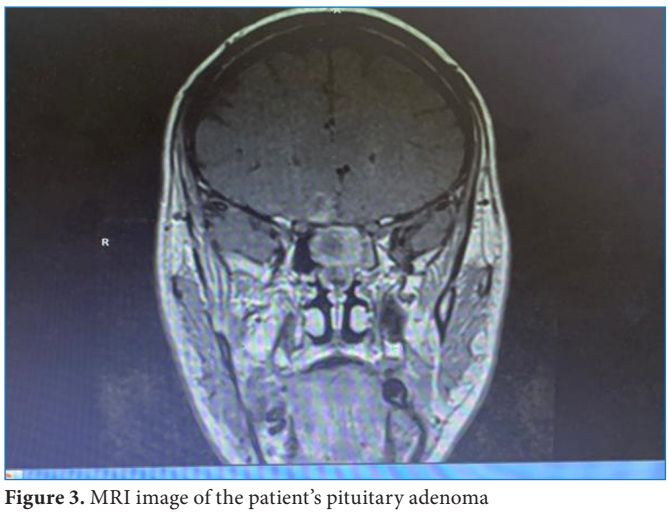

Figure 3. MRI image of the patient's pituitary adenoma

DISCUSSION

Although prolactinomas are the most common type of hormone-secreting pituitary adenoma, the onset of clinical symptoms in men may be delayed, or the diagnosis may be missed due to the absence of specific symptoms, as was the case in our patient.[5] The purpose of treatment is to normalize prolactin levels, ensure fertilization, correct gonad functions, reduce tumor size, treat visual field loss and cranial nerve palsies, if present, and, if possible, reverse pituitary dysfunctions.[6] Since prolactinomas do not present with specific symptoms, particularly in male patients as in our case, we wanted to raise awareness of this issue by emphasizing that the diagnosis can be made by obtaining a more in-depth medical history.

ETHICAL DECLARATIONS

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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