THE EFFECTS OF ANTIDEPRESSANT DRUGS ON POLYMORPHONUCLEAR LEUKOCYTE FUNCTIONS AND LEVELS OF FOLIC ACID, VITAMIN B_{12}, ZINC AND COPPER IN MAJOR DEPRESSION

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ABSTRACT

Objective: *Today it is known that frequently used antineoplastic agents, analgesics and antibiotics affect the immune system cells and can modify polymorphonuclear leukocyte (PMN) functions such as phagocytosis, lymphocyte proliferation and production of various cytokines. In this study we investigated the effect of antidepresssant drugs on PMN functions, serum folic acid, vitamin B_{12}, zinc, copper levels and hematological parameters in the patients with major depression (MD).*

Material and Method: *PMNs were isolated by Ficoll-Hypaque gradient centrifugation method. Phagocytosis and intracellular killing activity were assayed by modifying Alexander's method. Serum folic acid, vitamin B_{12}, copper and hematology parameters were detected with the biochemical analyzer. Serum zinc levels were measured by flame atomic absorption spectrometer.*

Results: *White blood cell (WBC), neutrophil counts (NE) significantly increased after 2 months of antidepressant drug treatment ($p<0.05$). The phagocytic activity of PMN of MD patients was found same as healthy volunteers, the intracellular killing activity was found lower than that of healthy volunteers. The PMN's phagocytic activity after one and two months of antidepressant drug treatment insignificantly increased and the intracellular killing activity also insignificantly increased after one month of treatment when compared with those before treatment. The results were not found statistically significant. Serum folic acid, vitamin B_{12}, zinc and copper levels were found in reference value intervals compared to those before treatment.*

Conclusions: *This study showed that WBC, NE counts in patients with MD significantly increased in the reference value intervals after antidepressant drug treatment ($p<0.05$). We conclude that antidepressant drugs have not deleterious effects on PMN functions, serum folic acid, vitamin B_{12}, zinc, copper levels of patients with MD.*

Key Words: *Antidepressant drugs, phagocytosis, folic acid, vitamin B_{12}, zinc, copper* ***Nobel Med 2013; 9(1): 81-88***

MAJÖR DEPRESYONDA ANTİDEPRESAN İLAÇLARIN POLİMORF NÜVELİ LÖKOSİT FONKSİYONLARI, FOLİK ASİT, B_{12} VİTAMİNİ, ÇİNKO VE BAKIR DÜZEYLERİ ÜZERİNE ETKİLERİ

ÖZET

Amaç: Günümüzde yaygın kullanılan antineoplastik, analjezik, antibiyotik gibi bir dizi ilaç polimorf nüveli lökosit (PNL) fonksiyonlarını, bakterilerin makrofajlar tarafından fagositozunu, lenfosit proliferasyonunu ve çeşitli sitokinlerin üretimini modifiye edebilmektedir. Çalışmamızda antidepresan ilaçların, major depresyonlu (MD) hastaların polimorf nüveli lökosit (PNL) fonksiyonları, serum folik asit, vitamin B_{12}, çinko ve bakır seviyeleri ile hemogram parametreleri üzerindeki etkisine bakılmıştır.

Materyal ve Metod: PNL'ler Ficoll-Hypaque gradient santrifugasyon yöntemi ile izole edilmiştir. Fagositoz ve hücre içi öldürme aktivitesi tespiti Alexander metodu modifiye edilerek yapılmıştır. Serum folik asit, bakır, vitamin B_{12} seviyeleri ve hematolojik parametreler biyokimyasal analizör ile çinko atomik absorbsiyon spektrofotometresiyle ölçülmüştür.

Bulgular: İki aylık antidepresan tedavi sonrasında beyaz kan hücreleri ve nötrofil sayısı anlamlı olarak artmıştır ($p<0,05$). MD'li hastaların PNL'lerinin fagositik aktivitesi sağlıklı gönüllülerle aynı bulunmuştur. MD'li hasta PNL'lerinin hücre içi öldürme aktivitesi ise sağlıklı gönüllülerden daha düşük bulunmuştur. MD'li hasta PNL'lerinin fagositik aktivitesi ise 1 ve 2 aylık antidepresan tedavisinden sonra, hücre içi öldürme aktivitesi ise 1 aylık tedaviden sonra tedavi öncesine göre artmıştır. Ancak sonuçlar istatistiksel olarak anlamlı bulunmamıştır. Serum folik asit, B_{12} vitamini, çinko ve bakır düzeyleri tedavi öncesine göre referans değerler içinde bulunmuştur.

Sonuçlar: Bu çalışma göstermektedir ki majör depresyonlu hastalarda beyaz kan hücreleri, nötrofil sayıları antidepresan ilaç tedavisinden sonra referans değerler içerisinde anlamlı olarak artmıştır ($p<0,05$). Antidepresan ilaçların MD hastalarının PNL fonksiyonları, serum folik asit, B_{12} vitamini, çinko ve bakır düzeyleri üzerinde kötü bir etkisinin olmadığı neticesine varabiliriz.

Anahtar Kelimeler: Antidepresan ilaçlar, fagositoz, folik asit, B12 vitamini, çinko, bakır **Nobel Med 2013; 9(1): 81-88**

INTRODUCTION

Major depression (MD) is a psychiatric disorder that can be triggered by negative life conditions and is influenced by genetic tendency[1]. It has been known for a long time that there was a relationship between depression and immune-inflammatory response. Now a number of studies have been carried out to identify the defect in host defence mechanism in MD.[1-5] That stress and depression affect immune system negatively is a common point of view related to patients with MD.[5] Drugs like interferon-alpha (IFN-α: a cytokine frequently used for treatment of multiple sclerosis) commonly cause depression which can be treated or pre-treated with antidepressants.[1] Investigatoins show that during treatment, cytokine leads to depression and during IFN-α treatment MD has been observed at 50%.[6] It is reported that patients who have MD, had a decreased lymphocyte count and lymphocyte mitogen response. Investigators to date show that patients with MD, levels and receptors of serum serotonin are decreased, many neuroendocrine disorders existed and proinflammatory cytokines (IL-1 and IL-6), acute phase proteins, C-Reactive protein (CRP), haptoglobulin, allergy, infection and cancer frequency are increased. It is also reported that in patients with MD nitric oxide levels increased and serum folic acid, vitamin B_{12}, zinc and selenium levels decreased.[5,7] It is found that depression is associated with immune dysregulation and inflammatory or autoimmune mechanisms are involved in development of depression.[2,4]

Today it is known that some vitamins and essential elements take place in the structure of enzymes and coenzymes that are important in regulation of metabolic functions of our body and they have important roles on cell metabolism and positive effects on immune system.[8-10] Folate and vitamin B_{12} are necessary for normal functions of central nervous system. A deficiency of either folate or vitamin B_{12} causes elevated homocysteine concentrations which may contribute to the pathogenesis of MD.[11]

Zinc is a bio-element that plays role in many biochemical events. It is reported that there was a decrease in many immune system functions during zinc deficiency. Monocytes decrease, cytotoxicity in Natural Killer (NK) cells functions declines. Peripheral T cell count, helper T cell function and cytotoxic T cell activity decreases. Neutrophil functions (chemotaxis) decrease. B cells undergo apoptosis. A decrease is observed in macrophage functions (phagocytosis and intracellular killing activity).[12-14] Briefly; a significant decline occurs in cellular immunity, antibody reactions, antibody affinity, complement system, and phagocytic activity. →

Recently, it was reported that not only antimicrobial agents that were accepted as biological response altering agents, but also some non-antimicrobial drugs influenced immune system in positive or negative way.[8-10] There were also some studies which were performed to clarify the effects of antidepressants on immune system[3]. As there is evidence associating increased production of proinflammatory cytokines with MD, one might expect that antidepressants suppress the production of those cytokines.[15] It was reported that antidepressants decreased the secretion of proinflammatory cytokines from activated monocytes and macrophages, inhibited chemotaxis and supressed the expression of antiinflammatory cytokines.[5] Antidepressants decreased the synthesis of prostaglandin E2 and nitric oxide by inhibiting enzymes such as cyclooxygenase and induced nitric oxide synthase.[3,16] When human monocytes were incubated with different classes of antidepressants together with lipopolysaccharide which stimulated the release of proinflammatory cytokines, the synthesis and release of IL-1, IL-6 and TNF were inhibited.[3,17-19] IL-1 is a polypeptide derived from mononuclear phagocytes that enhances T cell responses to antigens. It is a costimulator of T-cell activation. IL-6 is also a costimulator of T cells and of thymocytes. It acts as a cofactor with other cytokines for the growth of early bone marrow hematopoietic stem cells.[20] TNF causes vascular endothelial cells to become adhesive for leukocytes, initially for neutrophils and subsequently for monocytes and lymphocytes. TNF also acts on neutrophils to increase their adhesiveness for endothelial cells. These actions contribute to accumulation of leukocytes at local sites of inflammation. TNF activates inflammatory leukocytes to kill microbes. TNF is especially potent at activating neutrophils but also affects eosinophils and mononuclear phagocytes. TNF stimulates mononuclear phagocytes and other cell types to produce cytokines. TNF may function as a costimulator for T cell activation and stimulates antibody production by B cells. However, IL-1 and IL-6 are more potent than TNF at mediating these effects. IL-3 is a product of $CD4^+$ T cells that acts on the most immature marrow progenitors and promotes the expansion of cells.[20] IFN-γ is a potent activator of mononuclear phagocytes. It directly induces synthesis of the enzymes that mediate the respiratory burst, allowing macrophages to kill phagocytosed microbes.[20] It activates neutrophils, NK cells, and vascular endothelial cells. In the presence of IFN-γ, venular endothelial cells become more adhesive for neutrophils and may differentiate to form high endothelial venules, which attract lymphocytes from the circulation.[21] IL-10 inhibits cytokine production by activated T lymphocytes, NK cells and macrophages. It has a direct stimulatory effect on B cells and promotes antibody production.[21] In a study the ratio of IFN-γ to IL-10 in patients with MD treated with one of four antidepressants, imipramine, venlafaxine, 1-5 hydroxytryptophan or fluoxetine was examined compared to healthy controls.[18,19] All four antidepressants were found to increase the production of IL-10 whereas fluoxetine was found to decrease the concentration of IFN-γ. Also it was reported that all four antidepressants significantly decreased the IFN-γ / IL-10 ratio.[15] In another study, it was found that clomipramine, sertraline and trazodone had a significant suppressive effect on IFN-γ and a significant stimulatory effect on IL-10 secretion by whole blood stimulated with polyclonal activators and was suggested that various antidepressants such as SSRIs, tricyclic and heterocyclic antidepressants might affect immune regulation negatively.[22]

Table 1: Comparison of PMN functions of patients with MD and healthy volunteers

	Phagocytic activity (%)	Intracellular killing activity (%)
Patients with MD (n=12)	56.58 ±15.49*	2.08 ± 2.57*
Healthy volunteers (n=10)	59.95 ± 15.11	9.04 ± 3.86

* p> 0.05

In contrast with above findings, increases in IL-1 and IL-3 production have been detected after administration of clomipramine in depressed patients.[23,24] In addition, IL-6 levels have been found to remain unchanged after treatment with antidepressants suggesting that these drugs lack any immunomodulatory effects.[2,23] Thus the issue of interaction between antidepressants and immune system still remain to be elucidated.

The aim of our study is to investigate the effect of antidepressant treatment on immune system cell functions, serum folic acid, vitamin B_{12}, zinc, copper and hematological parameters of patients who are diagnosed as MD and are not hospitalized (n=12, average age 40).

MATERIAL and METHOD

The experiment protocol was approved by Marmara University Ethics Commitee (B.30.2.MAR.0.01.00.02/ AEK-135). Informed consent forms were collected from all patients.

Subjects

Twelve female patients between 20-60 ages that were enrolled to Outpatient Unit of Psychiatry Department in Marmara University were diagnosed as MD for the first time according to the DSM-IV diagnosis scale,

Table 2: Comparison of values of patients with major depression before treatment and after treatment (n=12)

	Before treatment	After treatment (one month)	After treatment (two months)	Reference value
Phagocytic activity %	56.58±15.49	65.25±14.22	65.50±12.69	30*
Intracellular killing activity %	2.08±2.57	4.16±5.40	2.66±2.26	4*
Folic acid level (ng/ml)	5.88±1.80	5.37±1.12	6.20±1.77	2.2-17.5 ng/ml
Vitamin B_{12} level (pg/ml)	532.5±387.46	501.01±320.04	447.73±242.81	197-866 pg/ml
Zinc level (ppm)	2.20±0.76	2.00±0.37	1.876±0.65	0.67-1.99 ppm
Copper level (ppm)	1.37±0.29	1.20±0.18	1.20±0.22	0.58-1.76 ppm

The results were expressed as means±SD. Statistical analysis were performed using Spearman Correlation Test. *: Phagocytic activity greater than 30% and intracellular killing activity more than 4% were regarded as normal values.[28] Note: The drugs that were used by the patients and their dosages were Sertraline (50 mg), Citalopram (20 mg), Escitalopram (20 mg), Fluoxetine (60 mg), Mirtazapine (15 mg), Paroxetine (20 mg, 40 mg), Trazodone (100 mg), Reboxetine (4 mg), Venlafaxine (150 mg), Tianeptine (12.5 mg).

had no additional diagnosis, were included in the study.[25] 30 mL of blood were taken from patients, their PMN functions before treatment were detected and the values were compared to PMN function values of healthy individuals that had previously been detected in our department and the values before treatment were compared to the values after treatment (1-2 months).[26] Also serum zinc, copper, vitamin $B_{12,}$ folic acid levels and hematological parameters of patients before treatment and after treatment (one-two months) were detected and compared.

Antidepressant Drugs

The drugs that were used by the patients and their dosages were sertraline; 50 mg, citalopram 20 mg, escitalopram 20 mg, fluoxetine 60 mg, paroxetine 20 mg, mirtazapine 15 mg, paroxetine 20 mg, 40 mg, trazodone 100 mg, reboxetine 4 mg, venlafaxine 150 mg, tianeptine 12.5 mg.

Preparation of PMNs

In our study a modified neutrophil function evaluation method of Alexander et al. was used to detect PMN functions. In the modified method, Ficoll was used in place of dextran and PMNs were counted by microscope instead of standard pour plate technique. The PMNs were isolated from the venous blood by the Ficoll-Hypaque gradient centrifugation method.[27-31]

Phagocytosis and Intracellular Killing Activity

Whole blood in 0.1 g/mL EDTA was centrifuged at 2500 rpm for 30 minutes, the buffy coat layer was removed and transferred to a mixture of Ficoll-Hypaque plus Polymorphoprep Solution and was centrifuged at 3000 rpm for 30 minutes to isolate PMNs. After centrifugation, the PMN layer was removed, washed 3 times in icecold Phosphate Buffered Solution (PBS) and suspended in Hanks's Buffered Salt Solution (HBSS), cell density was adjusted to $1x10^7$ cells/mL. Viability of PMNs was tested by the trypan blue exclusion method by counting the stained (dead) versus unstained (alive) cells on a hemocytometer (0.5%, in 0.9% saline solution).

A clinical strain of *C. albicans* was used in order to detect the activity of phagocytosis and intracellular killing of PMNs. The isolate was grown on Sabouraud agar plate for 24h at 37ºC before the experiments. Under these conditions the *Candida* cells only form blastoconidia without any germ tubes or pseudohyphae. Viability of the yeast cells was determined with methylene blue (0.01% Sigma) staining method. These cells were suspended in HBSS, and their concentration was adjusted to $1x10^7$ cfu/ml with a hemocytometer and the suspension of *C.albicans* into which a pool of human serum was added at a portion of 4:1, was incubated at 37ºC for 30 min in order to opsonize.

The PMNs that were obtained from patients with MD were incubated for 30 minutes at 37ºC on a shaker incubator. After incubation the opsonized yeast cells were added to the PMN and the final cell density of the mixture contained $5x10^6$ PMNs/ml and $5x10^6$ yeast cells/ml.

The intracellular killing of the blastoconidia was tested by the methylene blue staining (0.01%, Sigma). Wet mounts were prepared, and the phagocytic activity of PMNs was determined by counting PMNs that ingested alive and dead yeast cells and the intracellular killing activity was determined by counting PMNs that included killed yeast cells on a slide under a microscope and the result was expressed as a percentage.[27-33]

All samples were analyzed in triplicate.

Measurement of Serum Folic acid and Vitamin B_{12}

The levels of serum folic acid and vitamin B_{12} were detected with the device Roche Elecsys 2010 (Boehringer Mannheim, Germany).

Measurement of Serum Zinc and Copper Levels and hematological parameters

In order to determine the level of serum zinc and copper, the venous blood that has been taken in sterile silicon coated tubes from patients with MD was centrifuged at 3500 rpm for 5 minutes and the serum on top of the tube was transferred to another sterile tube and kept at -20ºC. To measure the zinc and copper levels, the serum samples were taken from -20ºC to the room temperature, then were diluted →

with trichloroacetic acid (TCA) in the ratio of 1:4 and incubated at room temperature for 10 minutes. The proteins that exist in the serum were precipitated by centrifuging the tubes at 3000 rpm for 5 minutes. The clear supernatant was analysed.[29,34,35]

Coulter LH 750 Analyzer was used to determine the level of hematological parameters.

Statistical Analysis

The results were expressed as means±SD. Statistical analyses were performed using Spearman Correlation test in SPSS. *p* values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

As shown in Table 1, there was no significant difference between phagocytic activity of PMNs of patients with MD before treatment and healthy volunteers, but intracellular killing activity was insignificantly low when compared with that of healthy volunteers.

The differences between the effects of antidepressant drugs on the PMN functions and the blood parameters in patient with MD were statistically insignificant. (p>0.05) All of them were found in reference value intervals. Therefore the data shown Table 1, 2 and 3 are by means±SD of 12 individual experiments.

PMN functions, serum folic acid, vitamin B_{12}, zinc and copper levels of patients with MD before and after treatment (one and two months) are shown at Table 2 and as it is seen from Table 2, phagocytic activity (65.25±14.22) and intracellular killing activity (4.16±5.40) of patients with MD after one month of antidepressant treatment increased compared to those before treatment, after two months of antidepressant treatment, the phagocytic activity (65.50±12.69) was found at the level after one month of treatment and intracellular killing activity (2.66±2.26) decreased to the level of before treatment. The results were not found statistically significant.

Serum folic acid levels of patients before and after treatments were found in the reference (2.2-17.5 ng/ml) value intervals. After two months of treatment, the folic acid values (6.20±1.77) insignificantly increased compared to those before treatment (5.88±1.80) (p>0.05). Vitamin B_{12} levels of patients with MD were found in reference value intervals (197-866 pg/ml). After one and two months of drug treatment, vitamin B_{12} levels insignificantly decreased (501.01±320.04-447.73±242.81) in reference value intervals compared to those before treatment (532.5±387.46).

Table 3: Hematological parameters of patients with MD (n=12)

	Reference Values	Before Treatment	After Treatment (One month)	After Treatment (2 months)
WBC (10^3/µL)	4-10	7.04 ± 2.10	7.99 ± 2.41	8.49 ± 1.70*
NE (10^3/µL)	1.4-6.2	4.14 ± 1.51	4.97 ± 1.69	5.45 ± 1.46*
LY (10^3/µL)	1.2-3.1	2.13 ± 0.67	2.30 ± 0.70	2.25 ± 0.65
MO (10^3/µL)	0-0.7	0.56 ± 0.13	0.55 ± 0.16	0.58 ± 0.14
EO (10^3/µL)	0-0.7	0.12 ± 0.08	0.14 ± 0.13	0.14 ± 0.11
BA (10^3/µL)	0-0.2	0.02 ± 0.06	0.01 ± 0.03	0.01 ± 0.02
RBC (10^6/µL)	3.5-5.7	4.52 ± 0.37	4.42 ± 0.34	4.35 ± 0.34
HGB (g/dL)	12-17	13.51 ± 1.31	13.19 ± 1.42	12.99 ± 1.37
HCT %	36-50	38.97 ± 3.72	38.28 ± 3.57	37.72 ± 3.26
MCV (fL)	82-97	86.08 ± 3.47	86.48 ± 3.45	86.64 ± 3.90
MCH (pg)	27-34	29.80 ± 1.26	29.70 ± 1.50	29.78 ± 1.37
MCHC (g/dL)	32-36	34.62 ± 0.59	34.35 ± 0.78	34.40 ± 0.95
RDW %	11.6-16.5	13.97 ± 0.63	13.76 ± 0.84	13.82 ± 0.86
PLT (10^3/µL)	150-440	257.33 ± 65.76	270.25 ± 55.39	267.25 ± 69.93
MPV (fL)	7.4-11	8.36 ± 0.93	8.27 ± 0.92	8.45 ± 0.90
PCT %		0.21 ± 0.04	0.219 ± 0.02	0.22 ± 0.04
PDW ratio		16.82 ± 0.77	16.85 ± 0.56	16.86 ± 0.66

*: p<0.05 (values compared with those before treatment) WBC: White Blood Cell, NE: Neutrophil Count, LY: Lymphocyte Count, MO: Monocyte Count, EO: Eosinophil Count, BA: Basophil Count, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PLT: Platelet, MPV: Mean Platelet Volume, PCT: Procalcitonin, PDW: Platelet Distribution Width

Serum zinc levels of patients before treatment were found higher than (2.20±0.76 ppm) reference values (0.67-1.99 ppm). After antidepressant treatment in the second month it reached the normal serum zinc level (1.876±0.65).

Serum copper levels of all patients before and after treatment were found in reference value intervals (0.58-1.76 ppm).

As it is seen from Table 3, the hematological parameters of patients with MD were in reference value intervals. WBC and NE counts significantly increased after two months (p<0.05). The other hematological parameters remained in reference values intervals.

DISCUSSION

It was previously known that activation of proinflammatory cytokines had causative roles on MD. And because antidepressants are used in treatment, they must suppress the production and function of proinflammatory cytokines.[1] Although this idea has not totally been proved, researches have still been continued. In a study it was reported that nefazodone treatment in stressed mice had indicated immunoprotective effects against the adverse effects of stress on immune system[36]. There is no much knowledge about the in vivo effects of antidepressant drugs on PMN functions. Our data demonstrated →

the increasing tendency of phagocytic activity after antidepressant drug treatment.

In our research phagocytic activity values of patients with MD was in reference values, whereas intracellular killing activity was lower than that of healthy volunteers.[28] After antidepressant drug treatment (one-two months) the phagocytic activity of PMNs of patients insignificantly increased compared to those before treatment and intracellular killing activity increased insignificantly after one month of treatment but after two months it decreased to the level of before treatment.

According to the researches in patients with depression, the levels of folic acid and vitamin B_{12} are low compared to those in general population; it is indicated that there was a relation between low folate level and resistance to the treatment.[7] In our study; serum folic acid levels in patients with MD before treatment was found to be reference value intervals. Folic acid levels after two months of treatment indicated an insignificant increase in reference value intervals compared to those before treatment and to the values that were gained after one month of treatment (Table 2). It was reported that the addition of folic acid (2 mg) in treatment of patients with MD, could increase the therapeutic effect of antidepressants and could decrease the period of treatment.[38,39] It was shown that in female depressed patients the co-administration of folic acid substantially and significantly improve the response to fluoxetine.[40] That is why we think that folic acid supplementation could increase PMN functions by stimulating and could strengthen the immune system. Our results demonstrated that serum folic acid level and phagocytic activity in patients with MD increased insignificantly after two months of antidepressant treatment. In another study a significant relationship between serum folate level and the time of clinical improvement during treatment with fluoxetine was shown and either folate or metabolic end products of folate such as S-adenosyl methionine (SAMe) supplementation was suggested.[41]

It was also reported that unlike folic acid, vitamin B_{12} level did not influence antidepressant treatment in positive or negative way.[11] In a study that cognitive function and depression degree during three months were investigated, positive effect of vitamin B_{12} could not be detected[42]. In our study, vitamin B_{12} levels after two months of treatment also insignificantly decreased in reference values compared to those before treatment. Literature knowledge supports our findings. In a study performed to determine the effects of pharmachotherapy on serum folic acid and vitamin B_{12} levels in patients with schizophrenia, bipolar disorder and major depressive disorder, serum folic acid and vitamin B_{12} levels were not found significantly different among the patient groups and the controls in pretreatment and after treatment. Also when the vitamin levels were compared after treatment, a significant difference among patient groups could not be detected.[43] In our study also a positive effect of vitamin B_{12} could not be detected and our results were not significant to report a negative effect. In another study, no significant relationship was found between vitamin B_{12} or homocysteine level status and the time to onset of clinical improvement.[41]

Lower serum zinc in MD is a sensitive marker of treatment resistance and of the immune/inflammatory response in that illness.[44] In the studies, it was reported that high zinc level had negative effects on immune system.[12,29] Our study confirmed the literature knowledge. It was seen that serum zinc levels which had been over the reference values before treatment, decreased after two months of treatment and reached reference values and as a result of this, the phagocytic activity increased but had no statistically significant effect. The intracellular killing activity was found identical to the values before treatment. According to the studies, zinc deficiency has become a worldwide nutritional problem and several studies were performed in vivo in animals and humans to find out zinc status and immune function. Although there is extensive evidence on the effects of zinc deficiency on leukocyte functions and little is known about its role in bone marrow functions. In many supplementation studies, high doses of zinc were used but adverse effects such as low density lipoprotein cholesterol increase, high density lipoprotein cholesterol decrease and immune alterations resulting from neutrophil-impaired function were observed on immune functions and lipid indexes were seen. The addition of zinc to the diet of elderly people could be an effective and simple strategy to improve their immune functions, but it is important to determine the adequate dose of zinc to obtain successful results.[28,45]

Copper and zinc are essential trace elements. They both are important parts of the enzymes superoxide dismutase, lysyl oxidase and ceruloplasmin, which protect cells from oxidative damage.[46] In our study it was detected that serum copper levels of patients with MD were in reference values.

Stress and depression caused an increase in leukocyte and neutrophile counts and a decrease in lymphocyte count.[5] In our study although hematological parameters of patients with MD were in reference value intervals, WBC and NE counts after two months of antidepressant treatment significantly increased when compared to →

the values after one month of treatment ($p<0.05$). Song and Leonard reported that long term sertralin treatment increased neutrophil percentage and proliferation activity of T lymphocytes.[47] Maes et al. reported that in depression related leukocytosis phagocytic cells such as monocyte and neutrophile increased.[48]

As a conclusion in our study, it was detected that after one and two months of treatment, antidepressant drugs insignificantly increased the phagocytic activity compared to those before treatment, after one month of treatment they increased the intracellular killing activity, in the second month of treatment it was in the value of before treatment. We expect that intracellular killing activity could decrease more if the treatment period gets longer. We think that in long term treatment intracellular killing activity of PMNs in patients and the levels of enzymes that are responsible from this activity should be controlled and if required supplementation treatment that stimulate the intracellular killing activity could be given. Intracellular killing occurs as summarized below;

A foreign particle is recognized and engulfed by the neutrophils. These particles are initially contained within membrane-bounded vacuoles called phagosomes. Seconds after engulfment, degranulation process occurs in which storage granules in the neutrophil cytoplasm begin to fuse with each phagosome, emptying their contents into its lumen. The neutrophil granules contain an extensive array of enzymes such as myeloperoxidase, elastase, collagenase, lysozyme, NADPH oxidases, lipases and other substances that can kill and degrade bacteria or dissolve other phagocytized materials. NADPH-dependent oxidases act to convert molecular oxygen into highly reactive singlet oxygen, which spontaneously dismutates to form hydrogen peroxide. In the presence of the abundant granular enzyme myeloperoxidase, this hydrogen peroxide combines with chloride ions to form hypochlorous acid, a potent oxidizing agent that is the active ingredient of household bleach. The hypochlorous acid is consumed almost instantaneously as it oxidizes amines, thiols, nucleic acids, proteins, and other biomolecules in the target particle, but a substantial portion reacts to form organic chloramines, a less powerful but much longerlived class of oxidizing agents. Together, these oxidative pathways provide some of the most important antimicrobial effects of the neutrophil.[21]

In a study, in neutrophils of patients with depression, a decrease in glutathione peroxidase and catalase enzymes, after all an increase in amount of superoxide dismutase was indicated.[49] PMN elastase is an important enzyme for PMN function, and the level of this enzyme in major depressed patients decreased after 3 months of antidepressant drug treatment and it was also reported that SSRI treatment reduced antioxidant enzyme level.[37,50] As it was mentioned above, myeloperoxidase is a leukocyte-derived enzyme and a component of azurophilic granules of leukocytes, neutrophiles, monocytes and some type of tissue macrophages. It is effective in catalyzing formation of reactive oxidant species.[51] More research should be done in patients with major depression to determine the effects of antidepressant drugs on myeloperoxidase and all other enzyme levels that have functions in intracellular killing.

Limitation: Some immunological, hematological and biochemical effects of different drugs were determined on 12 patients. It would be better if one kind of a drug was investigated on more patients but because some of the patients did not complete the treatment, in order to reach the required n value to perform statistical analysis, the combined effect of the drugs were determined. Also clinical results could be included in this study to determine the effects of antidepressant drugs on immune system and at the same time on major depression.

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