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THE EFFECTS OF THE H1-RECEPTOR BLOCKERS ON ADENOSINE DEAMINASE, XHANTHINE OXIDASE AND TRACE ELEMENTS IN PATIENTS WITH CHRONIC IDIOPATHIC URTICARIA

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ABSTRACT

Background: Chronic idiopathic urticaria (CIU) is widespread symptom of dermatologic diseases. But the etiology is not fully defined. ADA (Adenosine deaminase) ve XO (Xanthine oxidase) are key role in purin metabolism. Cu (Copper), Zn (Zinc), Se (Selenium) are of vital importance for many proteins and enzymes. Our aim in this study is to investigate these parameters after treatment of CIU patients. **Materials and Methods:** Blood samples were obtained from 25 patiens with CIU before and after weeks of treatment with H1 –receptor blokers. Blood samples from 10 healthy volunteers were used as controls. ADA, XO, Nitric oxide (NO) and Malondialdehyde (MDA) were measured as spectrofotometric. Also, the levels of trace elements were measured with atomic absorbsion spectroscopy. **Results:** After treatment Zn, Se levels increased significantly($p<0,05$). After treatment, Zn and Se levels approached the control group. ($p>0,05$) And After treatment MDA, NO, XO, ADA levels significantly decreased ($p<0,05$). **Conclusion**: After treatment,Cu, Zn, Se, MDA, NO, ADA,XO approached normal physiological levels.In CIU diesase,purine metabolism and trace element level deteriorate, lipid peroxidation increases.

Key words ; Adenosine Deaminase, Xhantine Oxidase, Trace elements, MDA.

1. İNTRODUCTİON

The origin of chronic idiopathic urticaria has been to a great degree unknown. Some cases autoimmune aetiology is proposed.[1,2] The role of free oxygen radicals (FOR) in the inflammatory region in the pathogenesis of tissue damage is important. Oxidative stress can be described as impose upon increased oxidant or decreased antioxidant capacity.[3,4] The role of reactive oxygen species (ROS) in the pathogenesis of urticarias has been in adequately studied. But, some recent marks support the presence of modifications suggestive of oxidative stress in CIU.[5,6] Local and systemic changes in enzymatic antioxidant defence system and condensation of lipid peroxidation are associated with different types of immune and inflammatory processes. ROS-mediated membrane lipid peroxidation results in production of malondialdehyde (MDA)—a highly toxic molecule, which is used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress.[7] Nitric oxide (NO) is generated during the conversion of L-arginine to L-citrulline.[6] Trace elements play a vital role in the body. These elements must be present in the body sufficiently and must be present to react with other elements to form critical molecules and participate in various important chemical reactions. Various immunological and inflammatory changes associated with physiological and pathological conditions can effect trace element distribution in the body. For example; plasma or serum Cu/Zn could potentially represent one of the most susceptible clinical markers of these changes.[7,8] As an essential trace element, selenium (Se) has a major metabolic significance for human beings.[9] Selenium deficiency is associated with impaired immune function.[10,11] A number of studies have shown a additive relationship between selenium deficiency and a reduction in CD4 cell counts in HIV-infected patients.[12] İnflammatory cells are activated and produce large amounts of FOR in some allergic diseases.[13] The conflicting information related to oxidative stress status in patients with chronic idiopathic urticaria (CIU) has been reported.[14] Chronic idiopathic urticaria (CIU) is based on the use of H1-receptor antagonists which reason a significant relief of symptoms by inhibiting the action of histamine, the most important agent. H1-receptor antagonists show additional antiallergic and anti inflammatory activities. (Levocetirizine, Loratadine, Desloratadine, Chlorpheniramine, Fexofenadin eg). They are especially used in allergic diseases. such as CIU, Allergic rhinitis, Pruritus.[15,16] The aim of the present study was to search the role of the oxidative stress in the patients with CIU by determining the lipid peroxidation, purine metabolism

of enzymes and trace elements plasma and in erythrocytes of these patients.

2. MATERİALS AND METHODS

The study was approved by the local ethical committee of KSU Medical Faculty, Kahramanmaras, Turkey. Blood samples were obtained from 25 patiens with CIU before and after weeks of treatment with H1 –receptor blokers. Blood samples from 10 healthy volunteers were used as controls. They were symptomatic for more than six weeks, with at least three episodes per week. All well-known causes, such as food and drug allergies, infections, any systemic or neoplastic diseases were excluded. Those with physical and cholinergic urticarias, urticarial vasculitis and hereditary angio-oedema were not included in the study. In blood from both patients and controls, Adenosine deaminase (ADA), Xanthine oxidase (XO), Nitric oxide (NO) and Malondialdehyde (MDA) were measured as spectrofotometric. Also, the levels of trace elements (Copper, Zinc and selenium) were measured with atomic absorbsion spectroscopy.

2.1 Biochemical measurements

Analyses were carried out in serum, plasma and erytrocyte. The acvities of ADA, XO in erytrocyte, Nitric oxide and MDA in plasma were measured as spectrophotometric. Activities of ADA, XO in expressed in international units, NO and MDA is expressed as nmol/ml. Also, the levels of trace elements (Se, Zn and Cu) in serum were determined on flame and furnace atomic absorption spectrophotometer using Zeeman background correction.

2.1.1 Measurement of oxidative stress biomarkers

Plasma preparation Fasting venous blood samples (5 ml) from 25 patients (before and after) and 10 healthy control individuals were drawn into vacutainers containing heparin as anticoagulant. Samples were centrifuged at 3000×g for 10 min at 4◦C. Plasma was separated and buffy coat was discarded by aspiration. Erythrocytes were washed four times with cold physiological saline and stored at −80◦C until analysis.

2.1.1.1 Determination of MDA levels

Lipid peroxidation level in the plasma samples was expressed in MDA. Measuremen t was based on the method of Ohkawa etal.[17] There action mixture contained 0.1ml of sample,0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 mLof 20% aceticacid and1.5 mlof 0.8% aqueous solution of thiobarbituricacid (TBA). The mixture pH was adjusted to 3.5 and volume was finally made up to 4.0 ml with distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1, vol/vol) were added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for10 min, the absorbance of the organic layer was measured at 532 nm. MDA levels were expressed as nmol/mL.

2.1.1.2 Determination Xanthine oxidase Activity

Xanthine oxidase activity was determined by measuring the uric acid formation from xanthine at 293 nm.[18]

2.1.1.3 Determination of ADA Activity

Adenosine deaminase activities (ADA; E.C.3.5.4.4) were estimated spectrophotometrically by the method of Giusti based on the direct measurement of the formation of ammonia, produced when AD acts in excess of adenosine.[19]

2.1.1.4 Determination of NO Levels

NO has very short half-life. The oxidation products of NO, nitrite (NO^{2-}) and subsequently nitrate(NO^{-3}), serve as an index of NO production. The method for measuring plasma nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite+nitrate) was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granule [20] A standard curve was established from nitrite standards to analyze unknown sample concentrations. Results were expressed as micromoles/liter. (μmol/L).

2.1.2 The levels of hemoglobin

Hemoglobin levels were measured with cyano methemoglobin method.

2.1.3 Measurement of trace elements

2.1.3.1 *Measurement of serum Se levels*

Se measurement was done in graphite furnace atomic absorption spectrophotometer (Perkin Elmer Analyst 800) using Zeeman background correction. Matrix modifiers were palladium (4 mg in 20-ml sample) and Mg sulfate (3 mg in 20-ml sample). Samples and calibration standards were diluted in1:3 with 0.05% Triton X- 100 to improve the sample viscosity and reproducibility of the results. Se levels in all groups were evaluated according to a standard curve. Se calibration standards were prepared from the commercial Se standard(1000mg/L) by serial dilutions.[21]

2.1.3.2 *Measurement of serum Cu levels*

Serum Cu levels were analyzed in flame photometer of atomic absorption spectrophotometer (Perkin Elmer Analyst 800). Samples and calibration standards for Cu measurement were 1:2 dilutions with 10% glycerol. Commercial Cu calibrators were used as standards.[22]

(1.000mg/L)by serial dilutions and samples were evaluated according to a standard curve.

2.13.3Measurement of serum Zn levels

Serum Zn levels were analyzed in flame photometer of atomic absorption spectrophotometer (Perkin Elmer Analyst 800). Samples and calibration standards for Zn measurement were prepared in 1:4 dilutions with 5% glycerol. Commercial Zn standards (1.000 mg/L) were used by serial dilutions and samples were evaluated according to standard curve.[23]

2.2 Statistical analysis

Statistical analysis was carried out using SPSS 17.0 for Windows statistical software. The conformability of the quantitative data to the normal distribution was examined using Kolmogorov–Smirnov test. Cu,Zn,Se were distributed normally, the descriptive statistics were presented as mean ± standard deviation. The student's t-test was used to compare mean values between groups. XO and ADA enzyme activities and MDA, NO levels were not in concordance with normal distribution and Mann–Whitney U test was used in terms of groups. The statistical difference was taken as p value < 0.05.

3. RESULTS

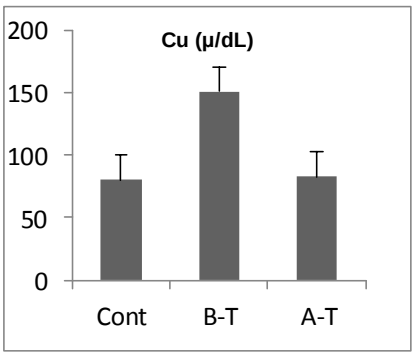
The CIU patients group (N=25) includes. control subjects (N=10) was includes. The Cu level decreased significantly after treatment. (83,25±9,52, p<0,05). The treatment group approached the control group (80,23±11,05). However, unlike Cu levels, Zn (97,00±17,84 p<0,05) and Se (72,00±7,46- p<0,05) levels increased after treatment. MDA (32,55±8,73, p<0,05) and NO (32,55±8,73 ,p<0,05) levels of CIU patients decreased after treatment. XO (18,75±3,35 p <0,05) and ADA (1,06±0,23 p <0,05) activities of CIU patients decreased after treatment. After treatment the groups approached the control group.

Table. 1. Comparison with Cu, Zn, Se, NO, MDA, XO, ADA levels and control group before and after treatment of CIU patients. The results are given as the mean ± standard deviation of the arithmetic mean.

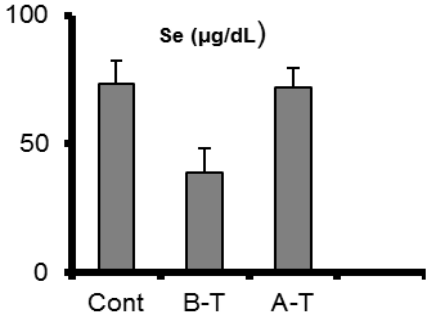
	Before treatment	After treatment	Control group	p
Cu (µg/dL)	151,25±30,24*	83,25±9,52**	80,23±11,05	< 0.05
Zn (µg/dL)	56,89±13,35*	97,00±17,84**	98,85±11,29	< 0.05
Se (µg/dL)	39,00±9,45*	72,00±7,46**	73,55±8,67	< 0.05
NO (µmol/L)	89,65±11,58*	32,55±8,73**	34,20±7,12	< 0.05
MDA (nmol/mL)	3,80±0,33*	2,15±0,36**	2,02±0,25	< 0.05
XO (U/L)	41,20±9,40*	18,75±3,35**	18,85±4,34	< 0.05
ADA (U/g Hb)	1,95±0,72*	1,06±0,23**	1,09±0,16	< 0.05

** Compared with control group (p <0.05),

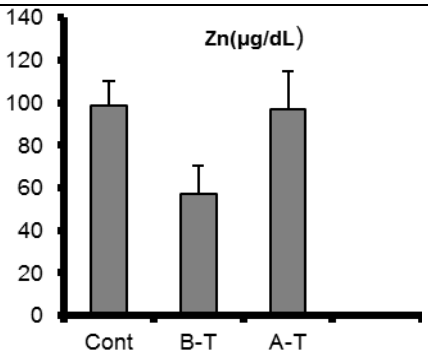
* The before- and after-treatment values of the CIU patients were compared (p <0.05).



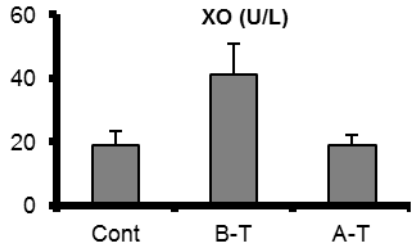
Grafic.1: Groups of Cu levels



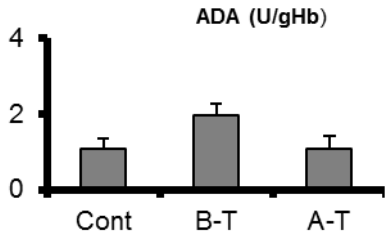
Grafic.2: Groups of Se levels



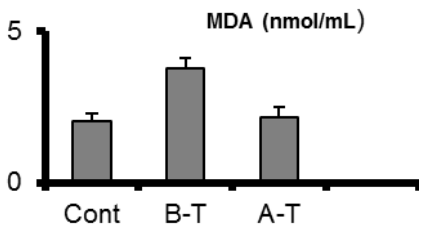
Grafic.3: Groups of Zn levels



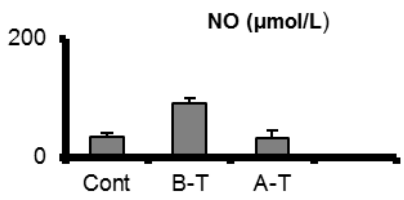
Grafic.4: Groups of XO activities



Grafic.5: Groups of ADA activities



Grafic.6: Groups of MDA levels



Grafic.7 : Groups of NO levels

Cont : Control
B-T : Before treatment
A-T: After Treatment

4. DİSCUSSİON

There is limited information regarding the use of alternative agents in chronic idiopathic urticaria (CIU), also recently termed chronic spontaneous urticaria, that is refractory to traditional, widely accepted therapies such as antihistamines, leukotriene modifiers and corticosteroids.[24] Oxidative stress plays an important role in allergic disorders. And increased levels of oxidants are considered as markers of the inflammatory course. Malondialdehyde (MDA) is a major product of lipid peroxidation. MDA is the oxidative stress biomarker.[4] MDA increase in the plasma of patients with chronic idiopathic urticaria is in line with our results in previous studies.[25,26] We think that inflammation increases the MDA levels. ADA and XO enzyme activities, as an indicator of DNA oxidation. ADA is considered one of the key enzymes of purine metabolism and metabolism. Xanthine oxidase (XO) is the last enzyme in the purine metabolism, which converts hypoxanthine and xanthine to uric acid with production of hydrogen peroxide. XO produces large amounts of ROS, especially superoxide during the above-mentioned reaction. Therefore, the increased XO activity may cause further blood damage because of free radical-generating effect.[27,28] The other histamine H1- antagonist—diphenhydramine, has been also demonstrated to possess antioxidant activity. Some antihistamine preparations inhibit free radical reactions.[29] As can be seen, the XO activity decreased after treatment. And XO activity approached the control group. ($p<0.05$-As shown in Table 1.) ADA activity increased after treatment. And after the treatment; ADA activity approached control group. We think that; H1 receptor blockers suppressed H_2O_2 from XO. However, Andrushkevich, V. V., et al They have observed that ADA activity has increased and They say that in bronchial asthma, the enzymatic reactions of purine metabolism are impaired.[30] Nitric oxide may also regulate mast cell function. including histamine release from mast cells. it is directly inhibits the IgE-mediated secretory function of mast cells.[31] In our study; NO levels were increased after treatment. The level of NO before treatment is high. This may be reporter of histamine. Zn, Se plays a fundamental role in human health by providing protection against ROS.[32,33] Generally, these metals show an increase or a deficiency of antioxidant defense. For example ; Zn has been serving as a metal that prevents lipid peroxidation and DNA damage.[32,34] It may be associated with the active purine metabolism Increased XO activity produces ROS. The trace elements in the environment participate in the redox reaction and their levels (Zn, Se) can be reduced. ($p<0.05$-As shown in Table 1.). After treatment, purine metabolism returns to normal physiological conditions. And trace element levels approach control.

CONLUSİON

As a result, it is very clear from this study that there are abnormalities in patients with CIU. In particular, trace element levels of these patients (serum, plasma, erythrocyte fluids) were lower than control grups. And MDA their levels were very high. Significant findings were found after treatment. The parameters approximated normal physiological conditions. More work needs to be done to understand this.

Conflict of Interests

The authors declare that there are no commercial or associative interests that represent a conflict of interests in connection with the work submitted.

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