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Evaluation of Homocysteine, Trace Element, and Vitamin Levels in Male Individuals with Hemorrhoidal Disease

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Abstract

Objectives: Hemorrhoidal disease is a common and uncomfortable condition affecting people worldwide, primarily in the lower rectal region. This study explores the relationship between hemorrhoidal disease and the levels of homocysteine, folic acid, vitamin B12, zinc, and copper in men.

Methods: A prospective study included 38 male patients with internal hemorrhoids (Group I) and 38 healthy male individuals (Group II). Venous blood samples were collected after a 12-hour fast and analyzed for homocysteine, folic acid, vitamin B12, zinc, and copper levels. Statistical analyses, including the Kolmogorov-Smirnov test, Mann-Whitney U or Two-Sample t-test, Receiver Operating Characteristic (ROC) analysis, and Multivariate Binary Logistic regression, were performed.

Results: Group I and Group II had similar age and body mass index (BMI). Homocysteine and copper levels were significantly higher in Group I, while folic acid and vitamin B12 levels were significantly lower. High homocysteine levels (≥11.2 µmol/L) had a sensitivity of 92.11%, while low vitamin B12 (<114) and high copper (≥1004) levels exhibited high specificity (97.37% and 86.8%, respectively). An increase of one unit in vitamin B12 was associated with a 1.04% decrease in hemorrhoid occurrence.

Conclusion: This study suggests that evaluating homocysteine, copper, folate, and vitamin B12 levels may be valuable in patients with or at risk of hemorrhoidal disease. Future research should include larger, more diverse samples to enhance the generalizability of these findings.

Keywords: Hemorrhoidal disease, trace elements, folate, vitamin B12, homocysteine

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INTRODUCTION

Hemorrhoidal disease is a benign anorectal disease with a prevalence ranging from 4% to 17%, which is seen with different frequencies in different populations (1). It shows symptoms such as pain, bleeding, sagging, and itching in the anus region and affects the quality of life by causing important social problems (2). Zinc is an element that plays a role in various metabolic pathways, possessing antioxidant and anti-inflammatory properties. Its deficiency has been linked to increased chronic oxidative stress and inflammation (3). Copper is one of the elements involved in metabolic processes such as the development of connective and bone nerve tissues, electron transport chain, cell proliferation, and vessel formation (4). Since copper deficiency (Menkes disease) and excess (Wilson's disease) cause various diseases and cause changes in antioxidant-oxidant effects, the body copper balance is very important (5).

Homocysteine is a toxic amino acid formed during the conversion of methionine to cysteine (6). It has been shown that the increase in homocysteine in the body causes oxidative stress, DNA damage, neurovascular such as epilepsy dementia, cardiovascular, nephrogenic and vascular diseases such as atheromatous plaque formation, as well as events such as increased inflammatory cytokines (7, 8).

Vitamin B12 (cobalamin) is a water-soluble vitamin that plays a crucial role in neurological development, erythropoiesis, and DNA synthesis. Its deficiency can lead to conditions such as hyperhomocysteinemia, megaloblastic anemia, and neurological diseases (9). Folate, a vitamin primarily responsible for single-carbon transfer, plays a pivotal role in various biochemical processes, including nucleic acid synthesis, neurotransmitter production, phospholipid formation, protein synthesis, and the conversion of homocysteine to methionine (10). Deficiency in folate can result in hyperhomocysteinemia, neural tube defects in pregnant women, megaloblastic anemia, and cardiovascular diseases (11).

This study aims to investigate the alterations in homocysteine, folic acid, vitamin B12, zinc, and copper levels in men with hemorrhoidal disease and assess their potential role in the disease's progression and etiology.

METHODS

This prospective study included 38 male patients (Group I) who presented with complaints of bleeding, pain, itching, or swelling in the anal region and were diagnosed with internal hemorrhoidal disease at the General Surgery outpatient clinic of Adiyaman University Medical Faculty Hospital. These patients met the selection criteria, which required them to be aged 30-40 years, have no prior treatment, be non-smokers, have no chronic illnesses, and possess a comparable body mass index.

The control group (Group II) comprised 38 healthy male individuals with similar demographic characteristics and no underlying diseases. Ethical approval for this study was granted by the Adiyaman University Ethics Committee (Decision number: 2022/2-17, Decision date: 16/02/2023).

Obtaining Serum Samples

Venous blood was drawn from both groups after 12 hours of fasting. Afterwards, blood samples were centrifuged at 4000 rpm for 10 minutes and their serums were separated. Samples were stored at -80 0C degrees until analysis.

Analysis of folic acid, vitamin B12 and homocysteine levels

Electrochemiluminescence immunoassay method and Beckman Coulter autoanalyzer (Beckman Coulter DxI 800, Kraemer Blvd. Brea, CA 92821 USA) were used for vitamin B12 and folate levels.

For homocysteine levels; electrochemiluminescence immunoassay based Siemens Immulite autoanalyzer (Diagnostic Products Corporation, Los Angeles, CA, USA) was used.

Analysis of selected trace element levels

In the analysis of serum zinc and copper levels microwave digestion was performed in Berghof brand MSW-4 model device and then reading was performed in Perkin Elmer brand NexION 350X model ICP-MS device. The working principle of this device was the same as our previous study (12).

Statistical Analysis

The SPSS version 25.0 (IBM Statistics for Windows version 25, IBM Corp., Armonk, NY, USA) and Medcalc software, version 20.006. programme was used to analyze the study data. The Kolmogorov Smirnov test was used to determine the normal distribution of the data, and then the Mann-Whitney U or Two Samples t-test was used for intergroup comparisons. The Receiver Operating Characteristic (ROC) analysis test was used for the diagnostic features of the parameters used in the disease, and the Multivariate Binary Logistic regression analysis test was used to determine the disease risk. $p<0.05$ was considered statistically significant for all the analyses.

RESULTS

The study was conducted in Group I, comprising male patients with internal hemorrhoids, and Group II, consisting of healthy male individuals. The average age for patients was 36.0 ± 3.99, while the control group had an average age of 35.87 ± 3.91. Body mass indexes were 24.79 ± 2.97 for patients and 23.64 ± 2.12 for the control group. There was no statistically significant difference in terms of age and BMI between both groups. In the study, copper, zinc, vitamin B12, folate and homocysteine levels were examined in both groups. Copper and homocysteine levels were statistically significantly higher in group I, and

folic acid and vitamin B12 levels were significantly lower. There was no significant difference between the two groups in terms of zinc. Table 1 summarizes the results for the parameters.

The results of the Diagnostic criteria of biochemical parameters measured in hemorrhoid patients are given in Table 2. Especially when the cut of sensitivity value for Homocysteine was determined as 11.2, sensitivity of 92.11% (95% CI = 78.6-98.3%) was found at a high rate. On the other hand, the specificity of 97.37% (95% CI = 86.2-99.9%) and 86.8% (95% CI = 71.9-95.6%) was found to be high when vitamin B12 and copper and parameters were considered as 114 and below, 1004 and above, respectively (Table 2).

Taking into account the analysis, an increment of one unit in vitamin B12 was associated with a decrease in the occurrence of hemorrhoids by 1.04% (Table 2).

Table 1. Comparisons of the serum levels of laboratory parameters

Parameters	**Group I: Hemorrhoids N=38**	**Group II: Control N=38**	**P value**
Age[1] (year)	36.0± 3.99	35.87±3.91	0.885
BMI (kg/m²)	24,79 ± 2,97	23,64 ± 2,12	0,902
Homocysteine[1] (µmol/L)	17.87±7.53	14.06±5.14	0.012
Folat[2] (pg/mL)	6.53 (3.14-14.57)	7.15 (4.04-15.72)	0.012
B12[2] (pg/mL)	131 (56-312)	222 (102-662)	<0.001
Zinc[1] (ppb)	607±188.33	582.85±112.98	0.498
Copper[1] (ppb)	972.39±218.14	833.72±130.85	<0.001

[1]: Independent two sample t test was used. Mean±SD.
[2]: Mann Whitney U test was used. Median (min-max).

Table 2. Cut of values for certain parameters.

Variables	**AUC ROC [95 % CI]**	**P value**	**Cut off [95 % CI]**	**Sensitivity [95 % CI]**	**Specificity [95 % CI]**	**+LR [95 % CI]**	**-LR [95 % CI]**
Homocysteine	0.651 [0.533 to 0.757]	0.017	>11.2 [>8.9 to >17]	92.11 [78.6 - 98.3]	39.47 [24.0 - 56.6]	0.52 [1.2 - 2.0]	0.20 [0.06 - 0.60]
Folat	0.667 [0.549 to 0.771]	0.008	≤6.7 ≤4.7 to ≤11.22	60.53 [43.4 - 76.0]	71.05 [54.1 - 84.6]	2.09 [1.2 - 3.7]	0.56 [0.4 - 0.9]
B12	0.765 [0.654 to 0.855]	<0.001	≤114 [≤96 to ≤207]	44.74 [28.6 - 61.7]	97.37 [86.2 - 99.9]	17.00 [2.4-121.4]	0.57 [0.4 - 0.8]
Copper	0.688 [0.571 to 0.789]	0.002	>1004.97 [>770.2 - >1100.75]	47.37 [31.0-62.0]	86.8 [71.9 - 95.6]	3.60 [1.5 - 8.7]	3.60 [1.5 - 8.7]

+LR: Positive likelihood ratio, -LR: Negative likelihood ratio

DISCUSSION

It is known that hyperhomocysteinemia causes adverse effects in many systems such as oxidative stress and free radical increase, suppression of antioxidant system, disruption of endothelial integrity, especially cardiogenic, nephrogenic and neurogenic tissues (13). Plasma homocysteine levels have been evaluated in various studies. For instance, research has demonstrated that elevated homocysteine can lead to cell proliferation and subsequently contribute to colorectal cancer through oxidative effects (14). In another example,

a statistically significant increase in homocysteine levels was observed in the group with osteoarthritis (15). Furthermore, homocysteine levels in female patients with gallstones were significantly higher than those in the control group (12). In our study, we found that homocysteine was significantly higher and folate and vitamin B12 were significantly lower in the patient group. In the ROC analysis, the sensitivity was found to be 92.11% (95% CI = 78.6-98.3%) when the cut-off homocysteine value was accepted as 11.2 (µmol/L). We think that levels above this value can be used as a diagnostic criterion for hemorrhoidal disease. Considering that the hemorrhoids in the anal region are composed of smooth muscle, connective tissue and vascular structures, we think that the endothelial integrity is disrupted by the oxidative effect in hyperhomocysteinemia, the release of nitric oxide, an important vasodilator molecule, and the collagen in the connective tissue are damaged, thus facilitating the development of hemorrhoids. Decreased levels of vitamin B12 and folat prevent the excretion of homocysteine from the body and support hyperhomocysteinemia. In a study, homocysteine, folic acid, and vitamin B12 levels were evaluated in pregnant individuals with habitual abortion and it was reported that folic acid and vitamin B12 levels were low, while homocysteine levels were high. (16). Both vitamin deficiency and hyperhomocysteinemia are particularly prevalent in Asia (14).

An element that is important in many metabolic processes in the body is copper. Some of those; redox reactions, cellular respiration, neuropeptide transmission, connective and bone tissue development, angiogenesis, antioxidant defense (17). Superoxide dismutase (SOD), cytochrome c oxidase, lysyl oxidase, and tyrosinase are some of the enzymes that need copper to function. In copper deficiency, it has been shown that the SOD enzyme activity in the antioxidant system decreases, so the oxidant system efficiency increases (4). In excess of copper, hydroxyl radical is formed by Fenton reaction, which causes oxidative damage (18). It has been shown that copper levels increase in breast, ovarian, lung, stomach, and colon cancers (19). In addition, it has been shown that especially iron-containing proteins are lipoylated in the tricarboxylic acid cycle with an increase in copper, so that these proteins cannot function and cause cell death by accumulating (20).

In this study, copper levels were found to be significantly higher in the patients. We think that high copper levels damage the hemorrhoidal tissue by forming hydroxyl ions with the Fenton reaction, and may contribute to the development of the disease by causing angiogenesis and inflammation in the hemorrhoidal tissue. Among the parameters in our study, Vitamin B12 was found to be a significant factor in determining the disease risk in the Multivariate Logistic Regression analysis. It has been determined that the risk of hemorrhoidal disease decreases by 1.04% with each unit increase in vitamin B12.

CONCLUSION

As a result, we think that homocysteine, copper, folate, vitamin B12 levels should be determined in patients with or showing symptoms of hemorrhoidal disease. One significant limitation of this study is the relatively small number of participants included in our research,

which may limit the generalizability of our findings. Furthermore, it is essential to acknowledge that our study exclusively comprised male participants, thus potentially restricting the applicability of our results to a broader population. Future research should aim to include a more diverse and representative sample to address these limitations and enhance the external validity of our findings.

Ethical Approval: Ethics committee approval for this study Adıyaman University Received from the Clinical Research Ethics Committee of the University Clinical Research Ethics Committee (Approval number: 2022/2-17, Decision date: 16/02/2023).

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