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ELEVATED INFLAMMATİON AND NEUTROPHİL-LYMPHOCYTE RATİO İN MALE WİTH IDİOPATHİC HYPOGONADOTROPIC HYPOGONADISM

Medical Science

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ABSTRACT

Objective: The goal of this study is to determine the NLR in the young male with idiopathic hypogonadotropic hypogonadism (IHH).
Methods: A total of 33 isolated and untreated IHH male patients and 56 healthy control males were included in the study. In addition to the blood sampling, anthropometric measures, and physical examination were undertaken to all participants.
Results: As inflammatory markers, the NLR and C-reactive protein (CRP) levels were significantly higher in the IHH group than the controls. Also, the uric acid level was higher in the patients group, but it was not statistical significant. This high NLR in the patients with IHH were independent of the fasting glucose, age, and body mass index. Using the cut-off value of NLR>1.46, there was sensitivity at 72.6% and specificity at 73%.
Conclusion: In addition to several systemic inflammatory markers, the NLR was higher in males with IHH compared to the healthy ones.

KEYWORDS

inflammation, hypogonadism, neutrophil-lymphocyte ratio

INTRODUCTION

Male idiopathic hypogonadotropic hypogonadism (IHH) may be congenital or acquired factors. These IHH patients have chronic low-grade systemic inflammation and evidence of inflammatory metabolic disorders such as dyslipidemia, obesity, hypertension, and type 2 diabetes mellitus (T2DM), and consequently increased mortality. [1,2] This inflammation is characterized by increased levels of proinflammatory cytokines and chemokines in serum from the sustained activation of monocytes in the periphery that differentiate macrophages, absorption of oxidized lipoproteins, migrate through the endothelial barrier to the intima-media layer of arteries and form foam cells, the primary component of atherosclerotic lesions. [2] It is known that cardiovascular disease (CVD) due to the atherosclerotic lesion is the most common cause of death worldwide. Considering that IHH patients are young, systemic chronic inflammation which leads to atherosclerotic lesion and CVD is gaining more importance.

For years, hemogram parameters such as neutrophils and lymphocytes have been used for the assessment of diseases. These neutrophils and lymphocytes are members of the immune system elements that the system plays a fundamental role in the control of inflammation. Recently, the neutrophil-lymphocyte ratio (NLR) which can be determined from peripheral blood neutrophil/lymphocytes values was shown to be an indicator of systemic inflammation that is inexpensive and routinely available. [3]

The NLR has been used to determine the severity of inflammation, disease activity, and predict the disease progression and the mortality in CVD, T2DM, malignancies, and autoinflammatory diseases. [4,5,6,7,8,9,10] In this study, we evaluated the NLR value as an indicator of chronic systemic inflammation in young males diagnosed with IHH.

Materıals And Methods

Thirty-three isolated and untreated IHH male patients were recruited in Erzurum Region Education and Research Hospital Outpatient Clinic of Endocrinology, and 56 healthy control individuals were recruited into the study at the same hospital Outpatient Clinic of Internal Medicine. All patients didn't previously receive any medical treatment for IHH. The disease was identified as total testosterone <229 ng/dl and free testosterone <5.1 pg/ml due to the absent or inadequate of pituitary gonadotropins. Patients were not taking any medicine affecting platelet function at least 2 weeks (e.g. acetylsalicylate, antiepileptics, heparin and so on) before the initiation of the study. Exclusion criteria were chronic illness, panhypopituitarism, hypo and hyperthyroidsm, nephrotic syndrome, steroid use or use of any drug causing hypogonadism. None of the study subjects was smoking or drinking alcohol. All participants gave their written informed consent to participate in the study. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local ethics institute of Health Sciences University Training and Research Hospital, Erzurum, Turkey (2018/20-208). Body mass index (BMI) was calculated by the ratio between weight and height squared in kg/m^2.

After an overnight fasting of 12 h, venous blood was collected from the antecubital vein. The blood glucose and lipid panel tests were measured by standard laboratory methods on a biochemistry autoanalyser (Beckman Coul-ter AU 2700 Plus AQ4 clinical chemistry autoanalyzer) with the company's original kits. The FSH, LH, DHEAS, prolactin, total testosterone (Siemens Advia Centaur XP Immunassay) and free testosterone (Algen diametra micro ELISA) levels were measured by chemiluminescent immunometric methods. The complete blood count which is used to evaluate the red and white blood cells, and determine the number of each type of subgroup cell present were determined with a Sysmex XE-2100 Hematology Analyzer (Sysmex Corp, Kobe, Japan). The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

Statistical Analysis

After analysis of the variance, we used the Mann–Whitney U Test or the Independent Sample t Test. Further, the Pearson correlation test or Spearman correlation test was used for the correlation analysis, and the multiple logistic regression analysis was uesd to exclude possible confounding effects of other variables in the results of each analysis. The receiver operating characteristic (ROC) curve analysis assessed the cut-off of NLR with the best diagnostic accuracy for detecting IHH. Unless otherwise stated, the results were expressed as mean ± SD, and considered a result of $p<0.05$ as statistically significant. All statistical analyses in this study were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA) for Windows.

RESULTS

Sociodemographic characteristics, complete blood count, and biochemical parameters for both groups are summarized in Table 1 and 2. Mean age of the 33 patients with IHH was 22.5±7.6 years while the mean age of the 56 control subjects was 23.1±6.5 years, and there was no significant difference between the baseline demographic characteristics of the groups (Table 1). The liver, kidney, thyroid function tests and cholesterol levels didn't show any significant difference between the patients and the controls while IHH patients' hemoglobin levels were lower than the control cases' (14.3±1.6 and 15.7±1.0, p<0.001) (Table 2).

Table 1: The demographic characteristics of the patients with Idiopathic Hypogonadotropic Hypogonadism (IHH) group and the control group.

Characteristics	IHH Patient Group (n=33) Mean±SD	Control Group (n=56) Mean±SD	P
Age (years)	22.5±7.6	23.1±6.5	0.68
Height (cm)	167±9.9	168±8.0	0.42
Weight (kg)	61.2±17.7	58.6±11.4	0.48
BMI (kg/m2)	21.7±5.0	19.5±3.2	0.063

Table 2: The clinical and biochemical features of Idiopathic Hypogonadotropic Hypogonadism (IHH) group and the control group.

Characteristics	IHH Patients (n=33) Mean±SD	The Controls (n=56) Mean±SD	P
Hemoglobin (gr/dl)	14.3±1.6	15.7±1.0	<0.001
White blood cell (x10.e3/uL)	8.0±3.2	6.7±1.2	0.05
Neutrophil (x10.e3/uL)	4.7±2.7	3.7±0.8	0.04
Lymphocytes (x10.e3/uL)	2.4±0.7	2.8±0.7	0.05
Neutrophil-lymphocyte ratio	1.9±1.3	1.5±0.4	0.03
C-reactive protein (mg/dl)	2.1±1.4	0.9±1.0	0.01
Uric acid (mg/dl)	4.15±1.0	4.9±1.5	0.07
Urea (mg/dl)	29.7±9.6	31.5±8.1	0.40
Creatinine (mg/dl)	0.7±0.1	0.8±0.1	0.51
Fasting blood glucose (mg/dl)	90.7±6.2	87.6±7.7	0.07
Alanine amino transferase (U/L)	20.2±10.3	19±8.4	0.58
Aspartate amino transferase (U/L)	23.8±6.7	22.8±7.0	0.59
Gamma-glutamyl transferase (U/L)	14.5±8.2	17.0±6.7	0.23
Alkaline phosphatase (U/L)	496.1±354.1	307.1±189.8	0.23
Total cholesterol (mg/dl)	157.6±28.3	152.7±21.1	0.44
LDL-cholesterol (mg/dl)	90.9±25.5	95.4±20.3	0.43
HDL-cholesterol (mg/dl)	49.5±16.5	46.8±8.5	0.42
Triglycerides (mg/dl)	111.1±107.2	95.1±44.2	0.42
Total thyroid stimulating hormone (mIU/L)	2.4±1.9	1.8±0.8	0.09
Free triiodothyronine (pg/ml)	3.6±0.7	3.8±0.5	0.96
Free thyroxine (ng/dl)	1.2±0.6	1.2±0.2	0.31
Total testosterone (ng/dl)	44.6±44.5	549.6±277.5	<0.001
Free testosterone (ng/dl)	8.9±13.7	19.7±10.5	<0.001
Estradiol (pg/ ml)	32.5±14.7	45.5±32.7	0.25
Estrogen (pg/ ml)	32.8±9.9	46.8±32.1	0.22
Progesteron (ng/ml)	0.3±0.2	0.4±0.2	0.09
Dehydroepiandrosterone (µg/dl)	165.3±129.8	218.4±82.5	0.29
Luteinizing hormone (mIU/ml)	0.8±0.9	3.4±1.0	<0.001
Follicle-stimulating hormone (mIU/ml)	1.7±2.3	3.8±2.1	0.004
Adrenocorticotropic hormone (pg/ml)	26.4±17.4	21.7±11.1	0.26
Cortisol (µg/dl)	13.6±5.6	15.4±4.5	0.19
Prolactin (ng/dl)	9.2±17.5	8.4±5.1	0.86
Growth hormone (ng/ ml)	1.7±2.7	1.1±1.2	0.41
Insulin-like growth factor (ng/ ml)	263.9±123.3	310.1±88.4	0.23

When we look at the inflammatory markers; the neutrophils, the NLRs, and the C-reactive protein (CRP) levels were higher in the IHH group than in the control group (p=0.04, p=0.03, and p=0.01, respectively), but the white blood cell (WBC) and the uric acid levels (UA) were not statistically significant higher in the IHH groups than in the control group (p=0.05 and p=0.07, respectively) (Table 2). This higher values of the NLR in the patient group was independent of the fasting blood glucose, age, and BMI (Table 3). The NLR had also some positive correlations between the other inflammatory markers: CRP (r=0.38, p=0.33), UA (r=0.17, p=0.29), WBC (r=0.56 p<0.001), neutrophils (r=0.85, p<0.001), and lymphocytes (r=0.39, p=0.001).

Table 3: The multiple logistic regression analysis of clinical factors possibly affecting the neutrophil-lymphocyte ratio (NLR) in male with Idiopathic Hypogonadotropic Hypogonadism (IHH) individuals adjusted for age.

Characteristics	β	p
The NLR	1.43	0.03
Age (year)	-0.060	0.68
BMI (kg/m^2)	0.199	0.06
Fasting blood glucose (mg/dl)	0.084	0.07

β: simple regression coefficient, BMI: body mass index

The levels of the total testosterone, free testosterone, FSH, and LH were significantly lower than the controls as expected (p<0.001, p<0.001, p=0.004, and p<0.001, respectively). The serum progesterone level was also slightly lower in IHH group (p=0.09). The serum prolactin, dehydroepiandrosterone, estradiol, estrogen, growth hormone, insulin-like growth factor, ACTH, and cortisol weren't significantly different between the patients and controls (p=0.86, p=0.29, p=0.25, p=0.22, p=0.41, p=0.23, p=0.26, and p=0.19, respectively).

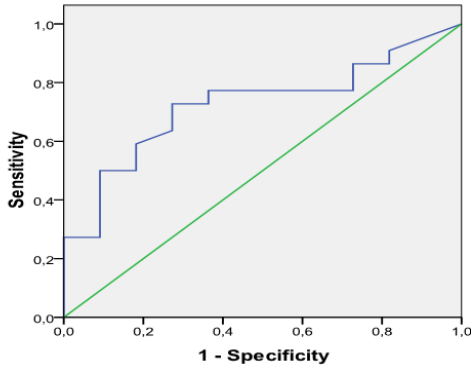

Figure: The ROC curve for the neutrophil-lymphocyte ratio (NLR) >1.46. *The Diagonal segments are produced by ties.*

ROC analysis, diagnostic, and screening test was used to determine a cut-off value for the NLR. Using the cut-off value of NLR>1.46, there was sensitivity at 72.6%, specificity at 73% (AUC: 0.725 [0.549–0.901]; p=0.037) (Figure).

DISCUSSION

This is one of the early studies to determine significant higher NLR levels in patients with IHH comparing with a healthy control group. Our study is important to evaluate mutual links among the NLR, systemic inflammation status, and biochemical parameters in young and non-treatment naive hypogonadotropic patients. Early detection of systemic inflammation status in the IHH is substantial because we already know that systemic inflammation status is one of the major factors in the development of atherosclerosis, endothelial dysfunction, CVD, insulin resistance, and metabolic syndrome. [1] Therefore, increased mortality is connected to them. [1] In this study, we detected that the NLR was higher in patients with IHH than the healthy controls, and this higher values of the NLR was independent of the fasting blood glucose, age, and BMI. The NLR cut-off point in our study was >1.46 (sensitivity:72% and specificity:73%). Considering that the age of the IHH people is young, we can understand why the early evaluation of systemic inflammation status is very important in this patient group. Improving potential noninvasive/invasive markers are important for

determining early disease activity, treatment, and following of chronic inflammatory disease and systemic inflammation status. The recent studies have shown that there is an association between simple inflammatory markers (blood neutrophil, lymphocyte, and platelet counts) and adverse effects in certain types of chronic inflammatory diseases and systemic inflammation status. NLR is also a novel noninvasive marker to detect the severity of diseases like ulcerative colitis, different kinds of cancer, CVD, and rheumatologic diseases such as Behcet Disease and Rheumatoid Arthritis. Total WBC count is less useful than NLR because WBC is affected by various physiological conditions such as dehydration and exercise, even though these conditions may affect an absolute number of individual cell types. [4,5,6,7,8,9,10] Hence, we detected that the NLRs and the C-reactive protein (CRP) levels were higher in the IHH group than in the control group while the white blood cell (WBC) was not statistically significant higher in the IHH groups than in the control group. Recently, the elevated mean platelet volume (MPV) which is the accurate measure of platelet size and a marker of platelet function has been reported in patients with vascular risk factors such as diabetes, hypertension, hypercholesterolemia, smoking, subclinical hypothyroidism [11,12], and polycystic ovary syndrome [13]. Carlioglu A. et al., 2015, also showed that the MPV level has been increased in young men (22.5±7.6 years) with IHH. [14] In our study, the NLR and the CRP levels as inflammatory markers were found to be significantly higher in young patients with IHH, but the UA levels as another inflammatory marker was not found significant different between the patients and control cases.

In the last decades, adipose tissue has been considered as an endocrine organ secreting various biochemical factors such as adipokines, so these biochemicals affect the metabolism of lipid, glucose homeostasis, and may influence cardiovascular risk factors such as hypertension as well as thrombotic and inflammatory processes. [15] Also, we know that some of the inflammatory markers are increased by age and some drugs. Fort that reason, we adjusted patients and the control subjects for their BMIs and ages. Parameters of lipid that they are known component of the metabolic syndrome such as total cholesterol, TG, HDL, and LDL levels didn't show any significant difference between the patients and control groups while the inflammatory markers were increased in the IHH group.

It was thought that men have an increased risk of CVD relative to premenopausal women because testosterone contributed to this elevated risk. However, this idea has started to change due to recent studies. One of them, Philips G. et al. showed that testosterone deficiency might contribute to increased CVD. [16] Then, so many studies supported this new idea although some of the studies do not support to this idea. For example, Hak A.E. et al. revealed an inverse association between levels of endogenous testosterone and progression of aortic atherosclerosis in elderly men (age range: 55–89 years). [17] On the other hand, based on a meta-analysis of 19 studies, made by Ruige et al, revealed a weak association between endogenous testosterone and risk for CVD in elderly men but not middle-aged men, and raised the question whether low androgens are a cause of CVD or, rather, a marker of poor general health. [18] Also, the other meta-analysis was made by Corona et al, showed that both low testosterone and high estradiol levels are independently associated with overall CVD in cross-sectional surveys, however, diabetes, obesity, and hypertension, are associated with increased testosterone differences between cases and controls, confirming numerous clinical observations. [19] In addition, Bobjer et al. showed that lowering of serum testosterone concentrations were significantly associated with elevated levels of the pro-inflammatory cytokine in relatively young men (mean age: 37±4.3 years). [20] This chronic low-grade systemic inflammation due to the elevated pro-inflammatory cytokine in IHH patients lead to some metabolic disorders such as dyslipidemia, obesity, hypertension, and T2DM as well as the early development of atherosclerotic plaque and CVD. [1,2] Awareness of this inflammation in this young people is very important to reduce atherosclerotic lesion and CVD which is the most common death reason in developed and developing countries. As an inflammatory markers, the NLR had a positive correlation between the CRP, UA, WBC, and neutrophils, and the height of this NLR was independent of the fasting blood glucose, age, and BMI in our study.

It is known that serum total testosterone levels are positively associated with Hb and hematocrit. [21,22] As expected, the study group Hb level was significantly lower than the control group's ($p<0.001$) because the study group participants who had IHH had lower serum testosterone levels than the control group's. The liver, kidney, thyroid function tests and cholesterol levels were similir levels in the patient and the control groups.

In the present study, it was shown that the NLR as an inflammatory marker was statistically higher in young patients with IHH which could be important in the assessment of systemic inflammation. Furthermore, it is inexpensive and easily available. Our study had the cross-sectional design and a relatively small sample size. We need more study about the proinflammatory cytokines and chemokines to explain the pathways of higher NLR in chronic systemic inflammation. More study should be done to show how the NLR affects the prognosis of this disease.

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