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Measurement of the Neutrophils Count and Oxidative Burst in Neutrophils of Patients with Sanjad Sakati Syndrome

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ABSTRACT

Sanjad Sakati Syndrome (SSS) is categorized as a neuroendocrine-related disease due to disorders of the nervous and hormonal systems. Since hormonal changes in these patients may affect the nature and function of the immune system. Thus, in this study, cell count and phagocytotic function of neutrophils were evaluated which may be influenced by changes in the hormonal rate and growth factors.

In this study, the neutrophil count value and the oxidative burst were evaluated in six patients diagnosed with SSS and six healthy individuals.

There was a significant reduction in the neutrophil count observed in SSS patients compared to healthy controls (37.41±7.93 percent vs. 66.5±6.8 percent). However, there was no significant difference in neutrophil oxidative index between patients with SSS and control subjects (172.33±55.08 *vs.* 217.00±77.38).

We concluded that in patients with SSS, the phagocytic activity of neutrophils was not affected by hormonal changes, while the number of neutrophils and neutrophil-to-lymphocyte ratio (NLR) index were decreased.

Keywords: Endocrine system; Neutrophil; Oxidative burst; Sanjad sakati syndrome

INTRODUCTION

Sanjad Sakati Syndrome (SSS) is a rare autosomal recessive congenital disorder found mainly in the Middle East and the Persian Gulf.[1] This syndrome is known as hypothyroidism dysmorphism (HRD) syndrome, in which the genes that cause the disorder are located on chromosome 1 and in the chromosome

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1q42-43 region. This syndrome may be due to a gene mutation in the tubulin-specific chaperone E (TBCE) region that can cause neutropenia as well as cell dysfunction.[2,3] Sanjad Sakati Syndrome is sometimes thought to be the autosomal dominant form of Kenny Caffey syndrome caused by a mutation in the gene encoding FAM111A, which is a protein with a homology similar to peptidases.[4] In two of the twins, a recessive form of the mutation was observed, which is associated with a decrease in T cells and a defect in phagocytosis.[5]

This syndrome is characterized by congenital hypoparathyroidism, recurrent pneumonia, severe growth retardation, dysmorphic features, skeletal disorders, and mild to severe mental retardation.[2,6-8] Other studies have demonstrated that serum immunoglobulin levels in patients with SSS are normal while their cellular immunity is impaired.[9] Generally, patients with SSS are highly susceptible to recurrent infections, which can be associated with splenic shrinkage and PMN dysfunction. In this group of patients, intracellular transfer and cell migration are impaired, which can be the reason for multiple manifestations in these patients.[10] Due to the involvement of the TBCE gene in patients with SSS, one of the cells affected by this gene is neutrophils.

Neutrophils are circulating phagocytic cells that have a high potential for the digestion of particles and microorganisms. These particles are inserted in the form of vesicles called phagosomes and then during the combination of phagosomes with lysosomes containing hydrolytic enzymes, fusion vesicles called phagolysosomes are formed, which ultimately destroy the pathogen through phagocytosis and reactive oxygen species (ROS).[11] Additionally, the neutrophil-to-lymphocyte ratio (NLR) is a marker of subclinical inflammation, with a higher value in many lung exacerbations such as cancer, asthma, bronchiectasis, and cardiac diseases.[12-14] It is noteworthy that the measurement of this marker does not require special techniques and can be checked in a short time through routine laboratory tests.[15]

Given the fact that no documented report to evaluate the immunological cells in patients with SSS, neither in the form of articles nor in reference books, here, we aimed to evaluate the level of neutrophils, NLR, and their potential for phagocytosis. In this research, SSS patients, which until now were mentioned as a rare disease in the category of endocrine disorders, have been examined from the point of view of immunodeficiency, and the results of this study may answer this hypothesis: “can SSS patients be classified in the category Immunocompromised patients or not?”.

MATERIALS AND METHODS

Study Design and the Patients

This research was approved by the ethical board of Ahvaz Jundishapur University of Medical Sciences with a code of ethics: *IR.AJUMS.REC.1399.215).* Six patients with the diagnosis of SSS (4.66±1.36 years old, mean±SD), referred to Abuzar Hospital (Ahvaz, Iran) with complaints of recurrent pneumonia and hormonal disorder, were enrolled in the study. Additionally, 6 age-matched healthy individuals (4.83±1.17 years old) without a family history of immunological disorders, were considered as a healthy control group. Informed consent was signed by all participants before joining the project. Diagnosis of SSS was established with a combination of relevant history and findings in hormone-physical examinations that were confirmed by the endocrinologist. Patients were known for active infection, hypoparathyroidism (Ca^{+2}=6.93±1.18 [normal range: 9–12 mg/dL] and PTH=7.12 ±2.6 [normal range: 15-65 pg/mL]), recurrent pneumonia, severe growth retardation, skeletal growth disorders, and other chronic inflammatory diseases. Control subjects consisted of healthy children who visited our institution for a routine check-up. General characteristics and laboratory data of all participants were obtained. The inclusion criteria included obvious skeletal growth disorders, a history of seizures at a young age, retardation, recurrent infections, and hypoparathyroidism.

In this research, the neutrophil cells in SSS patients have been focused on. For this purpose, the sample containing EDTA taken from the patients studied and the healthy control group was assessed in terms of neutrophil count. NLR was also calculated simply by dividing the neutrophil count (Neu) value by the lymphocyte count (Lym) value.

Measurement of Oxidative Burst in Whole Blood Using DHR (dihydrorhodamine) Method

The oxidative burst was evaluated in human whole blood using a DHR kit (Sigma-Aldrich, America)

according to the manufacturer's instructions. Heparinized whole blood (100 µL) was placed in three sterile 1.5 mL eppendorf centrifuge tubes, and DHR (3 µL) was added into tubes 2 and 3 and mixed with blood. All the tubes were incubated in a water bath for 15 min at 37°C. Then, phorbol myristate acetate (PMA, Sigma-Aldrich, America) was added into tube 3, and incubated for 15 min at 37°C. In the next step, 1 ml RBC lysis buffer was added to each tube and kept tubes on ice for 4 min to lyse erythrocytes. After washing twice, samples were measured with a flow cytometer as soon as possible. The forward and side scatter detectors were adjusted to clearly identify the PMN population and then adjusted fluorescence gain using unstimulated and stimulated control samples.

RESULTS

In this research, it is focused on neutrophil cells in SSS patients. For this purpose, the samples containing EDTA taken from the patients under study and the healthy control group were checked for neutrophil counts and NLR and DHR were calculated.

Comparison of Neutrophil Count and NLR Index between SSS Patients and Healthy Controls

A statistically significant difference was found between SSS patients (37.41±7.93) percent and healthy controls (66.5 ±6.8) percent in terms of neutrophil count (p=0.002) [normal range of neutrophil percentage: 40% to 60%] (Figure 1A). Additionally, the neutrophil-to-lymphocyte ratio (NLR) was calculated to achieve a better understanding of the status of neutrophils compared to lymphocytes. We found that there is a significant statistical difference in NLR value in patients with SSS (0.70±0.28) when compared with healthy subjects (2.07±0.56) ($p=0.002$) (Figure 1B).

Comparison of the DHR Test Results between SSS Patients and Healthy Controls

Results are expressed as neutrophil oxidative index (NOI) which is the ratio of PMA stimulated over unstimulated. A neutrophil oxidative index greater than or equal to one hundred is considered normal. There was no significant difference in neutrophil oxidative index between patients with SSS (172.33±55.08) and control subjects (217.00±77.38) (p=0.31) (Figure 2A and B).

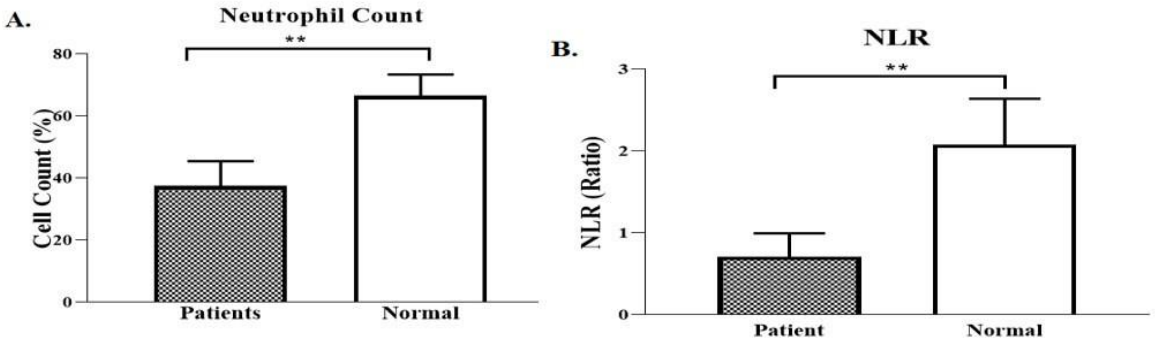

Figure 1. The comparison of the neutrophil count and NLR index in SSS patients and control. A) There was a significant statistical difference in both neutrophil counts (neutrophil counts in percent) in patients with SSS when compared with healthy subjects (p =0.002) . B) There was a significant difference in terms of NLR value between patients with SSS and healthy controls (p =0.002). **p<0.01. [SSS : Sanjad Sakati Syndrome; NLR : Neutrophil-Lymphocyte Ratio]

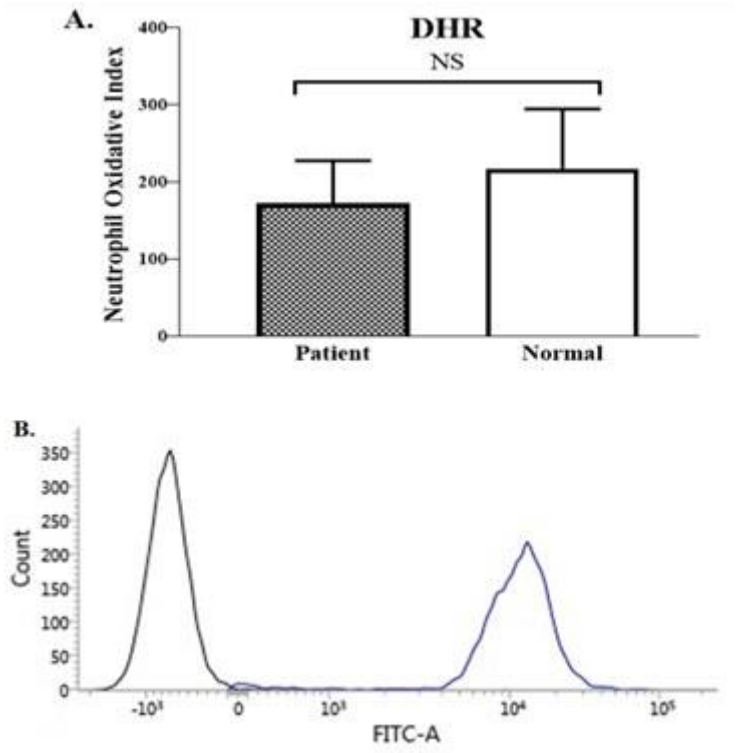

Figure 2. The comparison of the neutrophil oxidative index in SSS patients and the control.A)There was no significant statistical difference in the neutrophil oxidative index in patients with SSS, when compared to healthy subjects *(p=0.31)*. B) Flow cytometric charts that show the results of the DHR test are not significantly different between the SSS and healthy groups [Results are expressed as a NOI which is the ratio of PMA stimulated over unstimulated].
[SSS : Sanjad Sakati Syndrome; DHR : Dihydrorhodamine; NOI : neutrophil oxidative index]

DISCUSSION

Sanjad Sakati Syndrome is a rare disease that is classified as a multifactorial disease (a combination of immunodeficiency and neuroendocrine disorders). This disease affects the hormonal system related to both growth and thyroid and consequently, problems due to abnormal secretion of these hormones are observed in the individuals. To our knowledge, the current study is the first to demonstrate the phagocytic activity of neutrophils in patients with SSS; due to the rarity of the disease, previous reports have been case studies and to date, only hypothesizes about the function of the immune system in these patients have been presented, which is justified by the possible effects of the endocrine system on immune function.

In the current research, the patients were not normal in terms of bone and overall growth and their growth chart was consistently less than normal during their lifetime. Neuroendocrine hormones, including prolactin (PRL), growth hormone (GH), and histamine are released caused by stress and contribute to the regulation of phagocytic cell activity.[16] The GH receptor is present in neutrophils, monocytes, lymphocytes, and fibroblasts.[17] GH might stimulate neutrophil production and/or release via granulocyte colony-stimulating factor (G-CSF). Therefore, both G-CSF secretion and the neutrophil count might be influenced by GH administration.[18] On the other hand, Sohmiya M et al, showed that recombinant human GH (rhGH) administration elevated neutrophil counts and plasma G-CSF levels.[18] Hence, GH can enhance superoxide anion secretion from activated human neutrophils.[19] In addition, Cheng et al. have reported that respiratory bursts in neutrophils can be stimulated by GH.[20]

According to previous studies, we expected that the neutrophil count and the phagocytic potential of these cells would decrease due to the physical growth conditions of the patients studied, while our results demonstrated that there is no significant difference in phagocytic activity of these cells in SSS patients compared to healthy individuals. However, there was a significant reduction in the patient group in terms of both the neutrophil count and NLR index.

On the other hand, the patients studied also experienced hypothyroidism, and as a result, their immune systems may have been affected by the level of secretions from this gland. There is increasing evidence that innate immune cells have a profound effect on TH target cells.[21]. The research found that phagocytosing neutrophils can generate both T3 and rT3 from T4 in the granule fraction of the cells. Circulating TH levels affect reactive oxygen species (ROS) generation by stimulated neutrophils. Hyperthyroidism results in increased ROS production by activated neutrophils *ex vivo* compared to cells from euthyroid controls, whereas hypothyroidism limits neutrophil ROS generation.[22,23]

In both hypothyroidism and hyperthyroidism, the changes in neutrophil ROS generation are partially reversed by restoring TH levels to within the normal range.[22] Moreover, thyroid hormones promote the production of reactive oxygen species through neutrophil NADPH oxidase (NOX) activity. This phenomenon may be mediated via the binding of TH to a G-protein-coupled receptor (GPCR), which stimulates NADPH oxidase activity.[24] This suggests that TH metabolism has a profound effect on infiltrating neutrophils during infection. In contrast with previous studies, our results showed that there was no significant difference in the phagocytic activity of these cells in SSS patients who experienced hypothyroidism compared with healthy individuals. Whilst, the number of neutrophils and NLR index were decreased in the patients when compared to control subjects.

However, in vitro studies demonstrated that TH stimulation increases neutrophil ROS generation,[24] whereas other research has reported a decrease in ROS generation after TH incubation[25] or no effect at all.[26] These conflicting results suggest that the effects of TH on neutrophil ROS production cannot be exactly elucidated by the direct effects of TH on these cells. One of the proteins involved in the neutrophil-killing mechanism is myeloperoxidase (MPO). Hyperthyroidism did not affect superoxide dismutase activity and glutathione content indicating that the increase in ROS generation found was not due to changes in antioxidant defenses.[23]

Another previous study has reported that respiratory burst activities of both unstimulated and stimulated neutrophils were relatively reduced in subclinical hypothyroidism. Thus, a raised TSH level is associated with altered functional characteristics of neutrophils. According to one study, there is no obvious reduction of phagocytic activity of neutrophils in hypothyroid individuals when compared to euthyroid controls.[27] This conflicting activity could be because of different methods and different cell populations studied. Our data demonstrated that the NLR index is decreased in patients diagnosed with SSS compared to the healthy subjects. In contrast, another study reported that NLR is higher in patients diagnosed with Hashimoto's thyroiditis compared to the control group. These conflict results can be related to the clinical course or the severity of the disease.[28,29]

Based on the clinical characteristics and hormonal examinations of SSS patients, we expected a decrease in the number of neutrophils, as well as a decrease in the phagocytic activity of these cells in SSS patients, which could be a justification for recurrent infections in patients studied. However, we concluded that in patients with SSS, despite multiple hormonal disorders, part of the innate immune system, including the phagocytic activity of neutrophils, was not affected by hormonal changes, while the number of neutrophils and NLR index were decreased. Therefore, it is suggested that in order to better justify the cause of recurrent infections in SSS patients, further studies be done and other dimensions of the immune system, including cellular immunity, hemoral immunity, as well the other cells or mediators involved in the immune responses, be examined.

STATEMENT OF ETHICS

The research was approved by the ethical board of Ahvaz Jundishapur University of Medical Sciences with a code of ethics: IR.AJUMS.REC.1399.215).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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