Clinical Research

Relationship between serum metabolic indexes and immune function in patients with insomnia and their mechanism

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Abstract

We conducted a study to examine how certain serum metabolic markers (reactive oxygen species (ROS), homocysteine (HCY), and reduced glutathione (GSH)) impact the levels of IL-4 in patients with insomnia. The study involved 60 insomnia patients, including 20 with primary insomnia and 20 with somatopathy insomnia, aged 23-84 years with a mean age of 61.20 ± 12.59 years and a mean disease duration of 6.97 ± 8.45 years. There were 20 normal controls, 11 males and 9 females, aged 26-63 years, with an average age of 49.95 ± 10.52 years. We measured ROS levels using immunofluorescence, HCY levels using enzyme-linked immunosorbent assay, and GSH levels using ELISA. ELISA also detected the IL-4 level in serum to assess the patient's immune function. Our analysis revealed that changes in ROS, HCY, and GSH levels were associated with changes in IL-4 levels in serum. Therefore, early detection of serum metabolic changes in insomnia patients and proactive intervention can help reduce susceptibility to various infections and tumors.

Keywords: Sleep deprivation, reactive oxygen species (ROS), homocysteine (HCY), reduced Glutathione (GSH), interleukin IL-4, immune function.

1. Introduction

Insomnia patients may have prolonged wakefulness time, poor cellular metabolism Petit, et al. (2015), increased oxidative stress, increased ATP loss. Increased oxidative stress and reactive oxygen species concentrations lead to oxidative damage of organelles and membrane components, which in severe cases can lead to cell death (Hasanuzzaman et. al. 2021; Sachdev et. al 2021).

The study of Trivedi et.al. (2017) has shown that oxidative stress and ATP depletion are associated with sleep deprivation. Before and after sleep deprivation, the levels of glutathione (GSH), ATP, cysteine, and homocysteine (HCY) significantly decreased ($P<0.01$), while the levels of reactive oxygen species (ROS) and free radicals increased.

The change of immune function is one of the serious complications of insomnia. The prevalence rate of insomnia in adults is as high as 38.2% , the imbalance of Th1/Th2 cytokines is also increased, which leads to the increase of the incidence of various infections such as virus, bacteria, fungi, tuberculosis and the incidence of tumor, it is also an important risk factor for anaphylaxis. IL -4 has immunoregulatory effects on B cells, T cells, mast cells, macrophages and hematopoietic cells (Gao et. al., 2022; Xing et. al., 2006).

In our previous work, we also found that long-term sleep deprivation, namely insomnia, can lead to changes in cognitive function (Zhang et. al., 2021; 2022), how does the change of metabolite in blood affect il-4, then lead to the increase of immune function and anaphylaxis.

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2. Information and methods

2.1 General information

2.1.1 Participants

From December 2022 to July 2022, 60 patients with insomnia, including 20 patients with primary insomnia, were 2023 from the Sleep Department of Neurology, Taiyuan Central Hospital. There were 20 cases of emotional insomnia and 20 cases of physical disease insomnia; the age ranged from 23 to 84 years, the average age was 61.20 ± 12.59 years, and the average course of the disease was 6.97 ± 8.45 years. There were 20 normal controls, including 11 males and 9 females, aged 26-63 years, with an average age of 49.95 ± 10.52 years.

2.1.2 Selection criteria

Select different types of insomnia patients; the main performance is difficulty falling asleep, waking up early, waking up again having difficulty falling asleep, excluding patients with serious cardiopulmonary disease and cannot cooperate with the examination. The types of insomnia were divided into the primary insomnia group, emotional insomnia group, and physical disease insomnia group. Primary insomnia is a subjective experience not satisfied with the time and/or quality of sleep and affects social function during the daytime. The main symptom is difficulty falling asleep (sleep latency over 30 minutes), difficulty in sleep maintenance (≥2 awakenings throughout the night), early awakening, decreased sleep quality, and decreased total sleep duration (usually less than 6.5 hours), accompanied by daytime dysfunction, such as fatigue, depression or irritability, physical discomfort, cognitive impairment. Emotional insomnia is accompanied by anxiety and depression after the emergence of insomnia, also known as emotional insomnia. Body disease insomnia refers to the body disease after the occurrence of insomnia.

2.2 Test indicators and methods

A serum ROS detection kit detected the level of in blood to reflect the metabolic function of patients. Detection using the immunofluorescence probe method. The reagent comes from Beibo Biological Reagent Co., Ltd. The detection kit of ROS is a kind of kit that uses a new fluorescence probe, O13, to detect ROS in serum. In the presence of ROS in serum, the O13ROS fluorescence probe was oxidized to produce a red fluorescent substance; the level of ROS in serum can be determined by detecting the fluorescence of O13 products. The level of ROS in serum was measured at an excitation wavelength of 536 nm and an emission wavelength of 610 nm by fluorescence photometer and enzyme-linked immunosorbent assay.

Blood GSH levels were measured using a Glutathione (GSH) Detection Kit, an ELISA method purchased from Bebe Biological Reagents Ltd.

Serum HCY level was measured using the enzyme cycle method. This method uses the circulating reaction of substrate and coenzyme to expand continuously the products of enzyme-catalyzed reaction to facilitate the determination and improve the sensitivity and specificity of detection. It is the development and extension of enzymatic analysis.

Immune Index Detection: the level of IL-4 in the patient's blood was detected by ELISA. The reagent was purchased from Bebe Biological Reagent Co., Ltd.

2.3 Statistical analysis

SPSS17.0 software was used for statistical analysis, measurement data were expressed as mean ± standard deviation, f-test was used for data comparison between groups, and regression analysis was used for data correlation analysis between groups; $P<0.05$ was statistically significant.

3. Results

The basic information collected (see **Table 1**) Primary insomnia emotional insomnia somatic disease insomnia, insomnia patients total normal control group.

Table 1 Basic information for each sub-group

Disease grouping	Example number	Sex		Age	Average Age (years)	Average course of disease (years)
		Male	Female			
Primary insomnia	20	6	14	42-80	63.65±9.84	8.10±8.50
emotional insomnia	20	4	16	23-84	58.75±15.11	7.30±8.29
somatic disease insomnia	20	8	12	36-83	61.20±12.44	5.50±8.76
insomnia patients total	60	18	42	23-84	61.20±12.59	6.97±8.45
normal control group	20	11	9	26-63	48.95±10.52	--

The change of metabolic indexes such as ROS, HCY, GSH and IL-4 in the grouped data (see **Table 2**).

Table 2 Changes of metabolic indexes such as ROS, HCY, GSH and IL-4

Disease grouping	Example number	ROS (Fluorescence intensity)	HCY(mmol/L)	GSH(ng/ml)	Il-4（pg/ml）
Primary insomnia	20	261.54±57.91	15.65±5.77	3.31±0.79	20.59±4.39
emotional insomnia	20	283.15±89.40	13.94±3.37	2.90±0.64	19.77±5.20
somatic disease insomnia	20	296.91±59.94	19.06±12.38	2.99±0.73	19.61±3.10
normal control group	20	277.20±64.16	15.15±7.14	2.89±0.56	19.45±4.25
F value		4746.32	63.55	0.49	17.97
P		0.0005	0.01	-	0.05

Correlation Analysis of ROS and IL-4 changes in each subgroup (see **Figure 1**).

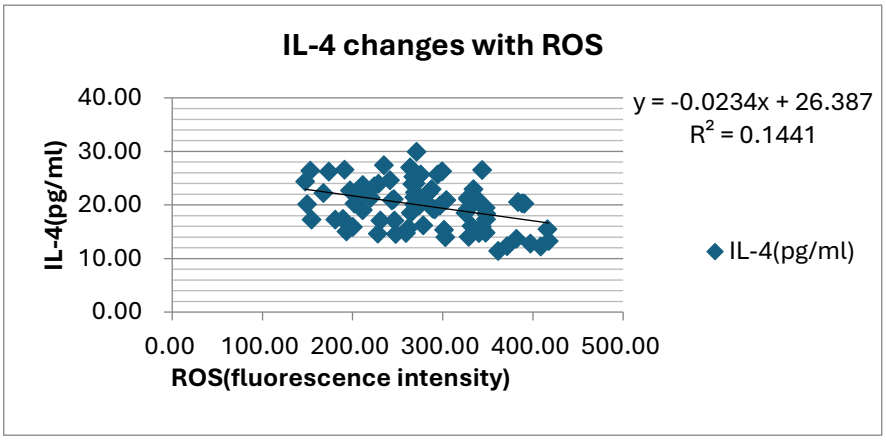

Fig. 1 *Effect of ROS on IL-4. This figure shows the changes in IL-4 as ROS changes in patients with insomnia. As shown from the figure IL-4 in serum decreased with the increase of ROS fluorescence intensity, The changing trend is Y = –0.023x + 26.38, R2 = 0.144. There was a negative correlation between ROS and IL-4. It shows that IL-4 decreases with the change of ROS.*

Correlation Analysis of HCY change and IL-4 change in each subgroup (see **Figure 2**).

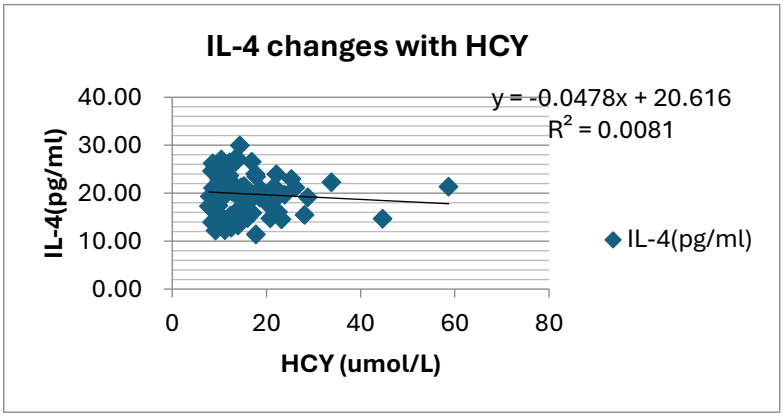

Fig. 2 *Effect of HCY on IL-4. This figure shows the changes in IL-4 as HCY changes in patients with insomnia. As shown from the figure Serum IL-4 decreased correspondingly with increasing HCY, Y = –0.047x + 20.61, R2 = 0.008. The change of HCY was negatively correlated with the change of IL-4. It shows that IL-4 decreases with the change of HCY.*

The correlation between GSH and IL-4 was analyzed (**Figure 3**).

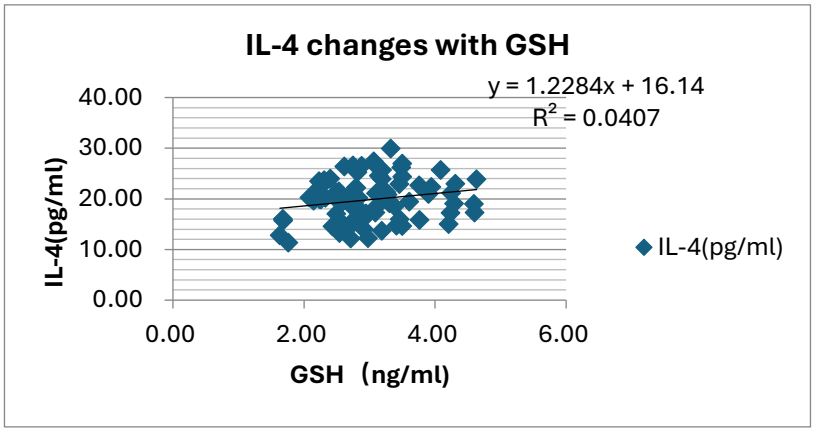

Fig. 3 *Effect of GSH on IL-4. This figure shows the changes in IL-4 as GSH changes in patients with insomnia. As can be seen from the figure, the serum IL-4 level increased with the increase of GSH (y = 1.228 x + 16.14, R2 = 0.040). GSH was positively correlated with IL-4. It shows that IL-4 increases with the change of GSH.*

4. Discussion

Prolonged insomnia can lead to impairment of mitochondrial function to varying degrees, including the production of reactive oxygen species (ROS) and consequent oxidative stress, dysregulation of intracellular calcium, and apoptosis. These associations with mitochondrial dysfunction stem from the need for adequate production of Adenosine triphosphate (ATP) and biosynthetic intermediates to maintain antioxidant capacity, calcium buffering, and various repair processes, and direct links between mitochondrial failure and cellular stress responses such as autophagy and apoptosis (Nunnari & Suomalainen, 2012). Thus, the patient's immune function changed, the incidence of various infections increased, and the incidence of tumors increased.

$CD4^+$T cells play a crucial role in the host's defense against harmful microorganisms and may also play a pathogenic role as drivers of autoimmune diseases and allergies (including asthma) (Guo et al., 2020). Based on the secreted cytokines and the biological functional characteristics of effector cells, they can be divided into helper T cell 1 (Th1), helper T cell 2 (Th2), regulatory T cells (Treg), and Th17. Th1 cells secrete IL-2 and INF - γ, mainly involved in cellular immunity; Th2 cells secrete IL-4, IL-l0, and IL-13 are mainly involved in humoral immunity. Th1 cytokines and Th2 cytokines mutually inhibit the differentiation and function of each other's phenotypes. Cellular and humoral immunity in the normal human body maintains a dynamic balance. The dominance of Th1 cells will promote cellular immune response and facilitate the clearance of viruses and carcinogens. In contrast, the dominance of Th2 cells will promote humoral immune response and inhibit cellular immune response. The trend of Th1 Th2 imbalance and transition to Th1 or Th2 state is called Th1 Th2 drift. Traditionally, the state in which Th1 cells and their cytokines dominate is called the Th1 state, while the state in which Th2 cells and their cytokines dominate is called the Th2 state (Hirahara & Nakayama, 2016).

Under normal conditions, the helper cells Th2/Th1 in the body are in a dynamic equilibrium state. However, in allergic patients, this balance is disrupted, and the Th2 lymphocyte system is overactivated, while the Th1 lymphocyte system is in a relatively inhibited state.

In the early stages of type I hypersensitivity reactions, specific allergens are first introduced into the body and

presented to B cells by Th2 lymphocytes, which are antigen-presenting cells. Under the action of IL-4, this interaction stimulates B cells to begin synthesizing and secreting a large number of specific types of antibodies, namely IgE. The secreted IgE antibodies bind to the specific Th2 receptor on the membrane of local mast cells or eosinophils in the bloodstream (a type I Fc segment ε receptor, Fc ε RI), causing the body to be in a sensitized state. When the same allergen enters the body again, the allergen can specifically bind to IgE antibody molecules on the surface of mast cells or eosinophils, causing cross-linking between Fc ε RI and activating sensitized cells. Activated mast cells and eosinophils undergo degranulation reactions, releasing histamine and other inflammatory chemical mediators (cytokines, interleukins, leukotrienes, and prostaglandins), which enter the surrounding environment. Organization can cause systemic effects such as vasodilation, mucus secretion, nerve stimulation, and smooth muscle contraction, leading to symptoms such as runny nose, itching, and difficulty breathing (Zaijun et al., 2004).

IL-4 is a multifunctional inflammatory mediator that mediates the humoral immune response and plays an important role in resisting extracellular pathogens (Luo & Tang, 2021; Shilovskiy et. al., 2022; Nur et. al., 2022). IL-4 can directly activate inflammatory cells, continuously enhance the killing cell lysis function, promote and amplify the inflammatory response, and has important reference value for evaluating the degree of inflammation in the early stages of infection (Hu et. al., 2005). High mobility group box-1 protein B1 (HMGB1) can widely participate in the inflammatory response of lung tissue and cause damage to lung tissue. On the one hand, HMGB1 promotes the release of pro-inflammatory cytokines such as IL-4; on the other hand, pro-inflammatory cytokines such as IL-4 can also stimulate the release of HMGB1. The two interact to form a positive feedback loop, amplifying the body's inflammatory response (Niu et al.,2021; Tian et al., 2021).

Long-term insomnia patients will produce ROS and metabolites, and the focus of this study is on how these metabolites affect the cytokine IL-4, which plays a crucial role in allergic reactions. Through this study, we found that there is a certain correlation between changes in serum reactive oxygen species and metabolites in patients with insomnia and changes in IL-4: (1) As the fluorescence intensity of ROS increases, serum IL-4 correspondingly decreases, with $y = -0.023x + 26.38$ and $R^2 = 0.144$. ROS is negatively correlated with changes in IL-4; (2) As HCY increases, serum IL-4 correspondingly decreases, $y = -0.047x + 20.61$, $R^2 = 0.008$. There is a negative correlation between changes in HCY and changes in IL-4; (3) As GSH increases, serum IL-4 correspondingly increases, with $y = 1.228x + 16.14$ and $R^2 = 0.040$. GSH is positively correlated with changes in IL-4. Further experimental verification is needed to determine the specific impact mechanism.

Early identification of changes in serum ROS and metabolites in patients with insomnia and active intervention can prevent and treat the imbalance of Th1/Th2 cytokines and reduce infections of various viruses, bacteria, fungi, tuberculosis, and allergic reactions.

This study concluded that IL-4 correlates with allergic reactions such as asthma. With the increase of ROS, IL-4 decreased, which may indicate that an appropriate amount of ROS has anti-allergic effects. This may also be related to the selection of specimens, the selected patients' short course of insomnia, or other factors that need to be further studied.

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