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REVIEW ARTICLE

Evaluation of serum levels Interferon gamma , Interleukin 10 and the ratio between them in patients with vitiligo

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Abstract:

Background and Objective: Cytokines, which control the immune response and inflammation, are essential in the process of depigmenting vitiligo. In this investigation, serum levels of interferon gamma (IFN-γ) and interleukin 10 (IL-10) and their ratio were compared in healthy people and those with vitiligo in Iraq.

Materials and method: To conduct the study, samples from 60 patients were collected and samples from 60 healthy participants (the control group) were pooled and measured using sandwich ELISA. Data on the patient's gender, family history, disease state, stress exposure, and smoking habits of males were collected as part of the study.

Results: When the vitiligo patients were compared to the controls, they revealed higher amounts of IFN- γ and lower levels of interleukin 10. IFN-γ to IL-10 ratio substantially varied among the patients as well as the controls. Concerning the physical differences, disease stability, and male smokers, there had been substantial distinctions between both the IFN- γ concentrations and the IFN-γ to IL-10 ratio. IFN -γ : IL -10 ratio was substantially correlated with those who had a history of vitiligo in their family, but, not at each molecule's level.

Conclusion: It could be concluded that the patients with vitiligo had an elevated IFN- γ serum level. The levels of serum IL-10 were lower in patients with vitiligo than in healthy controls. A possible solution for potential immunologic indicators for vitiligo was the ratio of serum levels of IFN-γ to IL-10.

Keywords: Vitiligo, Interleukin 10, Interferon gamma, IFN-γ to IL-10 Ratio

Introduction

Vitiligo a gained skin is a complicated condition that is caused by functional melanocyte loss in the epidermis and is characterized by the presence of milk-white spots of diverse forms tending to grow in size on the periphery with time (1). It is an inflaming agent condition that is linked to elevated inflammatory cytokine expression in the blood and skin (2). Multiple aggressions that target the melanocytes result in a notable decrease and loss of vitiligo's pigment cells sufferers (3). Both inherent abnormalities in melanocytes that trigger cellular stress responses and autoimmune processes that target melanocytes using humoral and the causes of vitiligo are linked to cell-mediated immunity (4). Interferons, interleukins, and tumor necrosis factors are examples of cytokines, which are protein molecules that play a crucial role in mediating cytokine and inflammatory responses. The severity and susceptibility of autoimmune diseases can be significantly influenced by their response because of an imbalance or shortage in the cytokine network. Various autoimmune illnesses have been linked to changes in the accumulation of pro-inflammatory and cytokines with anti-inflammatory properties like interleukin-6, interleukin-8, interleukin-10, interleukin-2, and tumor necrosis factor-alpha and interferon–gamma (5). IL-10 is regarded as a potent inhibitor of Th1 cells and macrophage generation of cytokines because it is an effective immune system regulator involved in suppressing inflammation (6). This study aims to estimate the serum levels of IFN-γ, IL-10n, and the ratio between them in vitiligo patients and the control group by using the ELISA technique.

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2.The Material and Methods:

The research groups involved the subjects of a case-control study that had been carried out from December 2022 to the end of March 2023 at Al-Sader- city hospital medical in Al-Najaf, Iraq, and outpatient clinic. In the present investigation, the patients group consisted of 60 participants, there were 24 males and 36 females with ages varied from 15 to 40 years. Under the supervision of a dermatologist specialist physician, the patients were clinically diagnosed with vitiligo. The patients were interviewed directly by using an anonymous questionnaire form that covered age, gender, family history of vitiligo, disease state, exposure to stress, and males smoking. This study also included 22 males and 38 females as a control group 60 presumably healthy individuals. All the participants provided informed consents in accordance with the ethical committee of Al-Sader-medical City Hospital in Al-Najaf. This study was subjected to evaluate IL-10 and INF- γ by ELISA technique, three milliliters of blood were transferred to a sterile Gel tube using disposable syringes, permitted to coagulate at ambient temperature, followed by centrifugation at 2500 revolutions per minute for ten minutes. The serum was then placed in Eppendorf containers and frozen at -20 degrees Celsius for future use.

3. The Results:

3.1. The Serum Levels of IFN-γ

The mean levels of serum IFN-γ were (18.55 ± 1.95 pg./ml) and (9.40 ± 0.764 pg./ml) in the patients with vitiligo and healthy control respectively; the level was highly significantly higher in the patients with vitiligo than in healthy controls (P < 0.001). According to gender, the mean levels of serum IFN-γ were (19.07 ± 2.04 pg./ml) and (18.20 ± 1.83 pg./ml) in males and females respectively, this was greater in males and not females, but the difference was non-significant (P=0.090). Depending on family history, the mean levels of serum IFN-γ were (18.59 ± 1.92 pg./ml) and (18.53 ± 1.98 pg./ml) among the patients with a positive family history and the patients with no family history of the disease respectively; the mean levels was slightly greater among individuals who had a positive family history compared to those who did not; nevertheless, the difference was not statistically significant (P=0.923). In comparing IFN-γ levels according to disease state, the mean levels of serum IFN-γ were (19.16 ± 1.83 pg./ml) and (17.33 ± 1.62 pg./ml) in the patients in a state of activity, and the patients in a state of stability respectively; the mean levels were greater in those individuals whose conditions were active than in those whose conditions were stable, but the difference was significant (P = 0.001). According to exposure to stress, mean levels of serum IFN-γ were (18.48 ± 2.18 pg./ml) and (18.58 ± 1.87 pg./ml) in patients with exposure to stress, and patients without exposure to stress respectively; the mean levels were slightly lower in patients with exposure to stress in comparison with patients without exposure to stress, but the difference was nonsignificant (P=0.856). The comparison of IFN-γ levels according to males smoking. The mean levels of serum IFN-γ were (18.59 ± 1.89 pg./ml) and (18.54 ± 1.98 pg./ml) in the patients with smoking and patients without smoking respectively; the mean levels were slightly greater in the patients who smoke compared to those who do not, but the difference was non-significant (P = 0.948). These results are presented in Table (1).

3.2. The Serum levels of IL-10

The mean levels of serum IL-10 were (10.25 ± 1.16 pg./ml) and (11.52 ± 1.64 pg./ml) in the patients with vitiligo and healthy control respectively; the level was significantly lower in the patients with vitiligo in comparison with the healthy control (P < 0.001). According to gender, the mean levels of serum IL-10 were (10.59 ± 1.17 pg./ml) and (10.03 ± 1.12 pg./ml) in males, and females respectively; the mean levels were greater in the males group in contrast with females group, but the difference was non-significant (P=0.070). According to family history, the mean levels of serum IL-10 were (10.68 ± 1.89 pg./ml) and (10.08 ± 1.22 pg./ml), correspondingly in the individuals with positive family history and people with negative family history, the mean levels were higher in the patients with positive family history in comparison with patients with negative family history, and the difference was non-significant (P = 0.073). Depending on the disease state, the mean levels of serum IL-10 were (10.22 ± 1.23 pg./ml) and (10.31 ± 1.06 pg./ml) in the patients in active states and the patients in stable conditions respectively; the mean levels were slightly lower in the patients with active states compared to those with inactive states, but the difference was non-significant (P = 0.781). According to the exposure to stress, the mean levels of serum IL-10 were (10.07 ± 1.24 pg./ml) and 10.33 ± 1.13 pg./ml) in the patients with exposure to stress, and the patients without exposure to stress respectively; the mean levels were slightly lower in the patients with exposure to stress in comparison with the patients without exposure to stress. However, the difference was not statistically significant (P=0.419). In the comparison of IL-10 levels according to males smoking, the mean levels of serum IL-10 were (10.27 ± 1.11 pg./ml) and (10.25 ± 1.18 pg./ml) in the patients with smoking and the patients without smoking respectively; the mean levels were increased in the patients who smoke compared to those who do not, but the difference was non-significant (P=0.974). These results are presented in Table (1).

3.3. The Ratio of IFN- □: IL-10 in Serum

The ratio of IFN- □ to IL-10 was significantly higher in vitiligo patients than in the controls (1.81 ± 0.295 vs. 0.82 ± 0.171; P<0.001). There was a significant increase in the ratio of IFN- □ to IL-10 among the patients with active and stable vitiligo, both with and without social smoking practices (p<0.05). However, there is no noticeable change between males and females according to interferon gamma to interleukin 10 ratio, (P =0.827). There was no clinically significant distinction between the patients who had a family history and those who did not according to the interferon gamma to interleukin 10 ratios, (p =0.162). The ratio of IFN-□: IL-10 in serum was detected in the patients with Vitiligo present and the results are shown in Table (1).

4.The Discussion:

The present results showed that the mean level of serum IFN -γ concentration in the patients with vitiligo was (18.55 ± 1.95 pg./mL) significantly greater than those in the control group (9.40 ± 0.764 pg./mL), this was in accordance with Ala et al (1), who discovered a significant increase in vitiligo cases compared to controls (12.4 ± 3.2 pg./mL versus 9.9 ± 4.4 pg./mL ; P <0.05). IFN-γ is a key proinflammatory cytokine implicated in a number of biological processes (7). Interferon gamma is a cytokine that is primarily involved in the inhibition of cell proliferation, apoptosis, and immunomodulation.

Both IFN-γ and TNF-a are overexpressed in vitiligo. IFN-γ is strictly involved in the development of the disease (8). IFN-γ associated between expression and disease activity where the present results demonstrated that the patients with active vitiligo had higher levels of IF-γ than those with stable illness (p<0.05) and the outcomes were comparable to Shi and Erf (9), who observed increased INF-γ expression during the early and active stages of autoimmune vitiligo. IFN-γ suppresses melanogenesis, raises reactive oxygen species production, and triggers senescence and death in melanocytes via CD8+ cells. Biological reaction to IFN-γ is regulated by a signal transduction pathway including Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) (10). Both JAK 1 and JAK 3 are up-regulated in perilesional and lesional vitiligo skin relative to control levels, and STAT1 inhibition can attenuate the effects of IFN- γ on melanocytes, such as their senescence. STAT1 signaling can be implicated in IFN- γ mediated inhibition of Treg (11). Concerning gender; statistically, there were no differences between men and women (19.07 ± 2.04 pg./mL versus (18.20 ± 1.83 pg./mL); p=0.090), and these results were similar to Ala et al (1), who showed that there were no statistically significant differences between males and females (14.7±5.6 pg./mL versus 14.5 ± 6.1 pg./mL; p=0.091), and this did not correspond with the results by Abdel Mawla et al (12), which showed that IFN-γ was significantly higher in females than in males in vitiligo patients females (452.60 ± 313.19 versus males 230.38 ± 119.04 pg./mL; p=0.012). The present results showed that the mean levels of serum IFN-γ were (19.16 ± 1.83, and 17.33 ± 1.62 pg./mL), in the patients with active states, and the patients with stable states respectively; the mean levels were higher in the patients with active states in comparison with patients with stable states, and the difference was significant (P = 0.001). In consistency with the findings of Ala et al (1), the patients with a family history of vitiligo had lower IFN- γ levels than those with no family history of vitiligo, and the patients with stable vitiligo had higher IFN- γ levels.

Interleukin-10 is a potent anti-inflammatory immune reaction modulator; thought to inhibit the generation of cytokines by T helper 1 (Th1) cells and macrophages (13). The skin has been shown to exhibit a high level of receptors associated with the interleukin-10 family, indicating that it can be a target of these cytokines (14). The present results showed a significant difference in mean serum concentrations of the anti-inflammatory cytokine IL-10 between the patients and the controls (10.25 ± 1.16 pg./mL versus 11.52 ± 1.64 pg./mL; □ < 0.05) and this corresponded with the results by Ala et al (1), who found that the mean serum concentrations of the anti-inflammatory cytokine IL-10 between the patients and the controls were (9.3 ± 1.7 pg./mL versus 11.5 ± 5 pg./mL; □ < 0.05). The patients had reduced serum concentrations of the anti-inflammatory cytokine IL-10 compared to healthy controls, indicating the importance of a reduced Th2 cytokine expression in the development of vitiligo. Active vitiligo lesions were shown to have decreased amounts of IL-10, a cytokine that naturally induces the activity and proliferation of T regulatory cells (9). The patients with vitiligo who were re-pigmented after being treated with tacrolimus and narrow band ultraviolet B showed higher levels of this immunosuppressive cytokine (15). There could be an increase in the anti-inflammatory cytokine IL-10 as a counterbalance to the pro-inflammatory effect, but this increase can not be enough to control the pro-inflammatory cascade of events responsible for the destruction of melanocytes (9). Nonetheless, there were no statistically significant differences in IL-10 levels were observed among the patients based on gender, disease state, family history, smoking status, or stress exposure. These results corresponded with the results by Ala et al (1), who found that there was no correlation between clinical variants, disease stability, social behaviours, or family history and IL-10 levels in the patient group (□ > 0.05).

Interferon gamma to interleukin 10 ratios were found to be substantially greater in vitiligo patients versus healthy people (1.81 ± 0.295 and 0.82 ± 0 .171), respectively; (□<0 .05). However, there was no noticeable change between males and females according to interferon gamma to interleukin 10 ratios, (P =0.827). Additionally, there was no clinically significant distinction between the patients who had a family history and those who did not according to the interferon gamma to interleukin 10 ratios, (p =0.162). These results corresponded with those by Ala et al (1), wherein the patients with vitiligo, they discovered that interferon gamma to interleukin 10 ratios were found to be considerably greater than that was seen in the control group (1.3 ± 0.3 and 0.9 ± 0.7) respectively ; p<0 .05). The present study patients, in contrast to the controls, showed a greater interferon gamma to interleukin 10 ratios, supporting the findings of cell-based investigations of Dwivedi et al (16), who found that vitiligo patients had a lower proportion of CD4+/CD8+ T cells than controls, indicating the role of T cells in causing disease in the condition. Immune tolerance-promoting Tregs can be diminished or lost if the cytokine network is not functioning properly. This demonstrates that the ratio of pro inflammatory to anti inflammatory cytokines is crucial in the pathophysiology of Vitiligo (1). The present results showed that IFN-□ and IL -10 were proportionally higher in those with active status compared to patients with stable status, (P=0.011). The current findings are corroborated by cell-based research showing that the patients with active vitiligo had CD8+ T cells outnumber CD4+ T cells in their blood than those with stable vitiligo. (16), the finding of a lower CD4+/CD8+ count corresponded with the current own findings of interferon gamma to interleukin 10 ratio when comparing the former to the latter group. Interferon gamma to interleukin 10 ratios were significantly higher in males smoking, which can lead to an inflammatory response. Smoking is found to influence the ratio of oxidative agents to anti-oxidants (17).

Table (1): The ratio of IFN -□ / IL -10 in the serum of Vitiligo patients and controls :

Characteristic	IFN -□	IL -10	IFN-□: IL-10
Study groups			
Vitiligo patients	18.55 ± 1.95	10.25 ± 1.16	1.81 ± 0.295
Controls	9.40 ± 0.764	11.52 ± 1.64	0.82 ± 0.171
P value	P<0.001	P<0.001	P<0.001
Gender			
Male	19.07 ± 2.04	10.59 ± 1.17	1.81 ± 0.269
Female	18.20 ± 1.83	10.03 ± 1.12	1.82 ± 0.301
P value	0.090	0.070	0.827
Disease status			
Active	19.16 ± 1.83	10.22 ± 1.23	1.88 ± 0.302
Stable	17.33 ± 1.62	10.31 ± 1.06	1.68 ± 0.238
P value	0.001	0.781	0.011
Family history			

Positive	18.59 ± 1.92	10.68 ± 1.89	1.74 ± 0.204
Negative	18.53 ± 1.98	10.08 ± 1.22	1.83 ± 0.320
P value	0.923	0.073	0.162
Exposure to stress			
Positive	18.48 ± 2.18	10.07 ± 1.24	1.86 ± 0.229
Negative	18.58 ± 1.87	10.33 ± 1.13	1.80 ± 0.199
P value	0.856	0.419	0.277
Smoking			
Positive	18.59 ± 1.89	10.27 ± 1.11	1.81 ± 0.332
Negative	15.78 – 22.0	10.25 ± 1.18	1.53 ± 0.131
P value	0.948	0.974	0.008

n:number of cases;SD:standard deviation;:independent samples t-test;¥:Chi-square test;NS:not significant at P> 0.05

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