İnönü Üniversitesi Tıp Fakültesi Dergisi *15 (3) 145-149 (2008)*

Investigation of Serum Fatty Acids' Composition in Patients With Vitiligo

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Objectives: Vitiligo is an acquired skin disorder resulting from the loss of functional melanocytes and is characterized by depigmented macular lesions. The etiopathogenesis of vitiligo has not been explained exactly yet. As a result of the studies that have been made for clarifying this mechanism, three basic theories have been suggested. Autoimmune theory, neural theory and autocytotoxic theory. In this issue, there are a lot of studies and several studies have been continued. Some of the present studies show that inflammatory parameters involves in vitiligo. As known, arachidonic acid is a PUFA and a key molecule for inflammation. In the literature, there is not a comprehensive study that investigates the relation between polyunsaturated fatty acid (PUFA) and vitiligo formation.
Patients and Methods: In this study we aimed to investigate the possible relationship between vitiligo etiopatogenesis and serum fatty acids' composition. In this way we studied serum fatty acids' levels in healthy and patients with generalized active vitiligo. Serum fatty acids' levels were transformed to their methylesters, then analysed by gas cromotography.
Results and Conclusion: We found that serum fatty acid levels except two kinds were similar to those of healty controls. On the other hand, palmitoleic acid levels were found decreased and Docosapenthaenoic acid levels were found increased ($p<0.05$). But, in general, our results clearly show that the serum composition of fatty acids in patients with generalized active vitiligo are not significantly different from those of healthy controls.

Key Words: Vitiligo, PUFA, Gas cromotography

Vitiligolu Hastaların Serum Yağ Asidi Kompozisyonunun İncelenmesi

AMAÇ: Vitiligo fonksiyonel melanositlerin kaybı sonucu oluşan ve depigmente maküler lezyonlarla karakterize bir tür deri hastalığıdır. Oluşum mekanizması henüz aydınlatılabilmiş değildir. Bu mekanizmayı aydınlatabilmek üzere yapılan çalışmalar sonucunda 3 temel teori öne sürülmüştür; otoimmün teori, nöral teori ve otositotoksik teori. Bu doğrultuda araştırmacılar tarafından pek çok çalışma yapılmıştır ve yapılmaya devam etmektedir. Mevcut çalışmaların bir kısmı vitiligoda inflamasyonla ilişkili parametrelerin etkili olabileceği yönündedir. Bilindiği gibi, araşidonik asit çoklu doymamış bir yağ asididir (PUFA) ve inflamasyon prosesinde anahtar moleküldür. Literatürde vitiligo gelişimi ile doymamış yağ sitleri arasındaki ilişkiyi araştıran karşılaştırmalı bir çalışma bulunmamaktadır.
YÖNTEM: Bu çalışmada serum yağ asidi kompozisyonu ile vitiligo oluşum mekanizması arasındaki olası ilişkinin araştırılması amaçlandı. Bu doğrultuda aktif generalize vitiligolu hastalar ile sağlıklı bireylerin serum yağ asidi düzeylerine bakıldı. Serum yağ asitleri önce yağ asidi metil esterlerine dönüştürüldü ve sonra gaz kromotografisi ile analiz edildi.
SONUÇLAR VE YORUM: On yağ asidi düzeyinden ikisi dışındakiler kontrol gurubu ile aynı düzeylerde bulundu. Palmitik asit düzeyi kontrol gurubuna göre azalmış ve dokosapentaenoik asit düzeyleri ise kontrole göre artmış ($p<0,05$) olarak tespit edildi. Fakat, genel olarak aktif vitiligolu hastalar ile sağlıklı kontrol grubu arasında serum yağ asidi düzeyleri bakımından dikkate değer bir farklılık belirlenemedi.

Anahtar Kelimeler: Vitiligo, PUFA, Gaz kromotografi

Vitiligo is a pigmentary anomaly of the skin expressed by depigmented white patches of different size and shape; it affects 1–4% of the world population.[1] The family history is positive in approximately 30-40% of cases and there is no gender or racial bias. The onset is mostly early in life and it has an estimated worldwide incidence of 0.5-4%.[2] The etiology and pathogenic mechanism of vitiligo is still unclear. Several hypotheses have been proposed for the loss of functioning melanocytes in the skin of these patients. These include the presence of autoantibodies against various tissues; cytotoxic T-cells; autodestruction of melanocytes by intermediates of the melanogenesis pathway; the presence of intrinsic/extrinsic metabolic defects in the melanocytes themselves or in the epidermal melanin unit, leading to oxidative stress; and the neural hypothesis. Recently, a `convergence theory' has been suggested, combining all hypotheses of this disease.[3]

Fatty acids are common components of biological systems that are known to play important roles in considerable celullar mechanisms such as mediators in the intracellular signaling network, precursors for ligands that bind to nuclear receptors, regulating intracellular protein turnover et. However, ω-3 polyunsaturated fatty acids (PUFA) have several health benefits on cardiovascular disease, immune disorders and inflammation, oxidative damage, renal disorders, allergies, diabetes, cancer and skin disease.[4]

Recently, regulatory properties of PUFA on some intracellular proteins such as tyrosinase (monophenol, L-dopa:oxygen oxidoreductase; EC 1.14.18.1) are drawing attention. Tyrosinase is a type I membrane glycoprotein that is the critical rate-limiting enzyme involved in melanin biosynthesis.[5] Previous studies have shown that tyrosinase is synthesized in the endoplasmic reticulum and processed rapidly through the Golgi apparatus, in/after which active degradation of tyrosinase occurs spontaneously.[6,7] It has also been reported that tyrosinase can be degraded endogenously by proteasomes.[8,9] There was an ideal balance between tyrosinase synthesis and degradation is necessary for regulating pigmentation in mammalian skin, hair, and eyes. It is possible that the untimely or excessive degradation of tyrosinase may be result in hypopigmentation and low melanine levels can cause degeneration of melenocytes.

In this study we aimed to investigate the possible relationship between vitiligo etiopatogenesis and serum fatty acids' composition. In this way we studied serum fatty acids' levels in healthy and patients with generalized active vitiligo.

MATERIAL AND METHODS

Study Groups

Sixteen (10 male, 6 female) patients with generalized stable vitiligo and sixteen (10 male, 6 female) healthy controls were included in this study. Patients' ages were ranged from 17 to 33 years old. The control group consisted healthy volunteers, ages ranged from 16 to 42 They were not under a therapeutic regimen for the previous 2 months and had not received drugs containing iron and/or vitamins. All individuals with any history of smoking and alcohol habits were excluded.

Samples

All blood samples were drawn at the same time. Ten ml blood was drawn from cubital median vein of the patients and control group into tubes. The blood samples were centrifuged at 1000 x *g* for 10 minutes at +4 ^{0}C, and upper serum phase was drawn with pipette and transferred to into polypropilen tubes and used immediately.

Fatty Acid Analysis

The total lipids in serum samples (1 ml) were extracted three times with 5 ml of choloroform:methanol (2:1, v/v) containing 1 mg butylated hydroxytoluene (BHT) by the method of Folch et al.[10] The combined $CHCl_3$:MeOH phases were washed with 0.5 M KCl thus nonlipid contaminants were removed. Fatty acids in lipid extracts were converted to methyl esters by using 2% sulfuric acid prepared as volume in methanol.[11] Fatty acid methyl ester (FAME) forms were extracted four times with n-hexane. FAMEs were quantified by using gas chromathography equipped with a flame-ionizion detector. Choromotography was performed with capillary column (lenght 30m, diameter 0.32 mm) using helium as carrier gas (flow rate 2.5 ml/min). The temperatures of the column, detector and injection port were 200, 260 and 250 ^{o}C, respectively. Retention times and peak areas were automatically computed by a HP Agilent 6890 recorder. Identification of the individual methyl esters was performed by frequent comparison with Supelco 4-7015-U PUFA standart mixtures analyzed under the same conditions.

Determination of Total Lipid Levels

This was determined according to the method of Frings et al.[12] Twenty microliters of each solutions from extracted lipids was treated with 200 µl of concentrated H_2SO_4 and heated in a boiling water bath for 10 min. After cooling, 10 ml of phosphovanilin reagent was added. The mixture was incubated at 37°C for 15 min, the samples were read at 540 nm. A good U.S. grade of olive oil (Sigma, St. Louis, MO) was used as a standard. The total lipid was calculated according to standard curve.

Statistical Analysis

The collected findings from groups were reported as mean ± SE. Statistical analysis was performed using SPSS 10.0 Software. Independent t-test were used for comparison between groups.The results were considered significant if the P value was 0.05 or less.

RESULTS

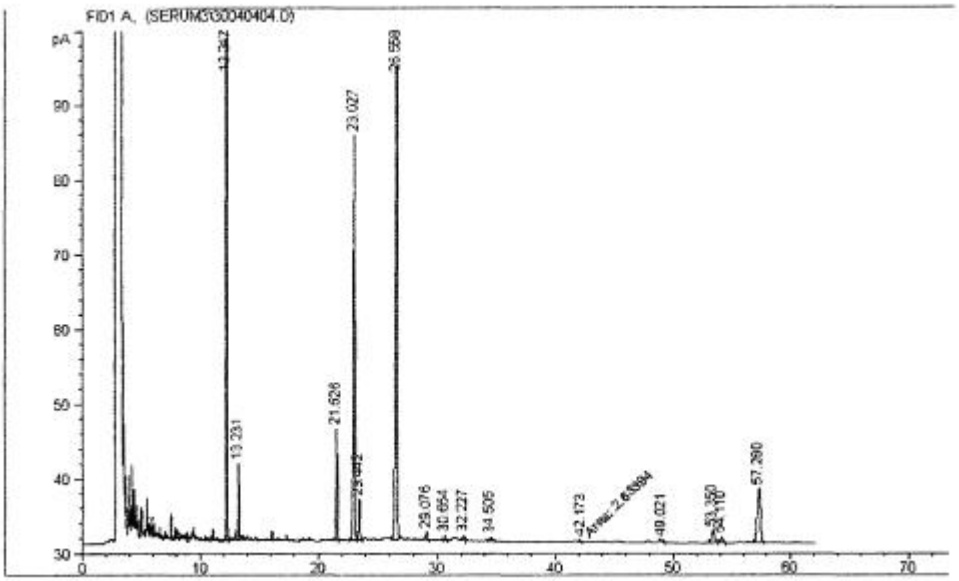
All results were summarized at Table I.

Table I: FA percentage and total lipid levels of serum samples, expressed as mean and standard deviation (SD)

	CONTROLS(% FA) n=16	VITILIGO(%FA) n=16
16:0	21.816±2.962	22.122±2.685
16:1, w-7	2.050±1.056	1.442±0.701*
18:0	7.081±0.760	7.347±0.777
18:1, w-9	19.958±3.561	18.312±3.199
18:2, w-6	34.838±4.926	36.670±4.495
20:1, w-9	0.370±0.139	0.429±0.052
20:4, w-6	1.906±0.452	1.609±0.408
20:5, w-3	0.397±0.182	0.284±0.123
22:4, w-6	0.761±0.232	1.108±0.365*
22:6, w-3	7.685±1.968	6.984±1.796
Σ Sature Fatty acid	28.897±3.722	29.379±3.462
ΣUnsature fattys acid	67.965±12.516	66.838±11.619
Others	3.138	3.783
Total lipid (g/ml)	0.035±0.0016	0.037±0.0027

*: p<0,05 when compared with control group.

Figure I: FAME's analyses in serum samples of a vitiligo group.



DISCUSSION

Our results clearly show that the serum composition of fatty acids in patients with generalized active vitiligo are not significantly different from those of healthy controls. The skin is the largest tissue of the human body with an approximate size of 1.8 m^2 where numerous fine-tuned mechanisms act in a concerted action to keep the homeostasis in place. Recently, it has been shown *in vivo* and *in vitro* that patients with the pigmentation disorder vitiligo accumulate mM levels of hydrogen peroxide (H_2O_2) and high concentration of reactive oxygen species (ROS) in their epidermis.[13] It is well established that as high as millimolar levels of hydrogen peroxide lead to the inactivation of antioxidant enzymes such as catalase and glutathione peroxidase despite normal mRNA expression.[14,15] Possible sources of endogenous H_2O_2 production are increased the activities of epidermal monoamine oxidase A, NADPH-oxidases, inducible nitric oxide synthase, increased levels of TNFα and photo-oxidation of epidermal 6-biopterin and sepiapterin.[16] Increased generation or decreased removal of hydrogen peroxide has been shown to lead to lipid peroxidation, protein oxidation and DNA damage in skin and blood.

The oxidative destruction of polyunsaturated fatty acid (PUFA) of phospholipids known as lipid peroxidation, can be in fact considered as a hallmark of the oxidative stress. PUFA are important for the normal function of most mammalian cells both to provide fluidity to cellular membrane lipid bilayer and to function as precursors fort he synthesis of regulatory eicosanoids.[17] Malondialdehyde (MDA), an end-product of lipid peroxidation induced by ROS, is well correlated with degree of lipid peroxidation.Koca et al.[18] reported increased MDA levels and decreased SOD enzyme activity in patients with generalized vitiligo. In the previous studies, high levels of oxidation products of lipids and proteins and low activities of several enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase(GPx), and tyrosinase have been shown.[14,15,19,20]

It is certain that ROS and oxidative damage play an important role on ethiopathogenesis of vitiligo. In such a way that spoilt oxidant/antioxidant balance affects several parameters in not only skin but also other components of organism such as blood. We found increased MDA levels and decreased Se and GPx activity levels of plasma in patients with vitiligo at one of our study which has finished recently in our research laboratory and has not been published yet. Recently, the relationship between fatty acids levels of blood and/or tissue and several pathology is published in the literature.[21,22] Particularly, their regulatory role on intracellular synthesis and destruction reactions of many important biomolecules such as melanin is very attractive. Ando et al [23] reported that fatty acids play a critical regulatory role on turnover of tyrosinase protein which is a key enzyme of melanogenesis. These researchers emphasized that fatty acids regulate the degradation of tyrosinase activity via post transcriptional events. At the similar studies in the literature, the inhibitory effect of palmitic acid and the stimulatory effect of linoleic acid on proteolysis and degradation of tyrosinase were discussed.[13,24,25]

We could found only one study related with fatty acid levels of blood and vitiligo in the literature. Picardo et al [26] reported that the blood levels of seven fatty acids in patients with active vitiligo were not significantly different from those of healthy controls. Our results support these findings. In our study, there is no significant difference between serum fatty acids' compositions of generalized active vitiligo and healthy control groups. Among ten fatty acids levels, two of them were found significantly different. When we compared the fatty acids levels of healthy and patient groups we found decreased palmitoleic acid (16:1 n-7) and increased docosatetraenoic acid (22:4 n-6) levels in vitiligo group. This findings were statistically significant.

It is known that; in active vitiligo, inflammation, though it is not so severe, associated the illness as well. However, we did not recognize any difference regarding arachidonic acid levels in our study. Docosatetraenoic acid level was found to be high is a by-product (elongation) of arachidonic acid. That high level can be explained by either the result of elongation of arachidonic acid which was released from the degenerated membranes of melanocytes or more likely by dieting.

As a result, serum composition of fatty acids of patients with generalized active vitiligo is compared to that of the healthy controls, and no statistically significant difference was datected. However it is a known fact that, many molecular changes which are dominant in the skin may partly reflect to the parameters in the blood in vitiligo. Thus we can say that serum composition of fatty acids does not notably change. From this viewpoint, tissue and cell

studies may be designed to investigate the possible relationship between the vitiligo and PUFA.

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