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Apolipoprotein E Gene Polymorphism and Serum Lipids in Patients with Superficial Fungal Disease

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Superficial mycosis, including dermatophytic infections, tinea versicolor, and cutaneous candidiasis is mostly limited to the outer layers of the skin, nails, and mucous membranes. In this study, Apolipoprotein E (ApoE) polymorphism and lipoprotein cholesterol concentrations were compared between 42 patients with superficial fungal disease and 27 control subjects. Both the patients and controls were found to be normolipemic. The patients with superficial fungal disease had significantly higher concentrations of high-density cholesterol (HDL) compared to the control group (p=0.0462). However, there was no difference in the serum triglyceride, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol concentrations. A significantly higher incidence of heterozygosity E2/3 was found in the patients (p=0.0228), and significantly lower incidence of homozygosity E3/3 in all patients, and those with candidiasis and dermatophytosis (p=0.0139, 0.0194 and 0.0337, respectively) compared to the control group. The E3/4 genotype differences between patients and controls were not statistically significant. There were slight differences in the allele frequencies between the two groups, but these did not reach statistically significant levels. It was concluded that the presence of apoE2/3 genotype, high HDL-cholesterol levels and the absence of apoE3/3 genotype can be regarded as risk factors for superficial fungal disease, especially dermatophytosis.

Key Words: Apolipoprotein E, lipoprotein, dermatophyte, Candida, Malassezia

INTRODUCTION

Superficial mycosis is mostly limited to the outer layers of the skin, nails and mucous membranes, and can be divided into three broad categories: dermatophytic infections, tinea versicolor, and cutaneous candidiasis.[1] The defense mechanisms against superficial mycosis can be categorized as nonimmunological and immunological defense mechanisms. Various factors may be involved in the infection process, such as increased hydration of the skin with maceration, skin surface lipids, trauma, and atopy. The major immunological defense mechanism in superficial fungal diseases is the type IV delayed-hypersensitivity response.[2,3]

Localization of apoE in normal skin has been demonstrated by immunohistochemistry, and the relationship between the apoE allele and some skin diseases has been shown.[4,5] Regarding microbial growth, lipoproteins can modulate the growth of microorganisms, as has been demonstrated for Candida albicans, Klebsiella and Staphylococcus aureus. It was shown that mice deficient in apoE were highly susceptible to microorganisms,[6-8] and it has also been shown that lipoproteins are able to interfere with the interaction between lipopolysaccharide and cytokine-producing cells.[9-11] Bont et al suggest that both the absence of apoE, which is important in the neutralization of lipopolysaccharides, and a defect in granulocytes could explain this increased sensitivity.[8] Viable Candida cells and cell wall constituents are able to induce the synthesis of proinflammatory cytokines in vitro, similarly to gram-negative bacteria and their lipopolysaccharide component.[12,13] Hyperlipoproteinemia has deleterious effects on the course of an acute disseminated Candida albicans infection.[13] Increased outgrowth of Candida albicans

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due to lipoproteins has been demonstrated.[14] However, no epidemiological studies have been performed to show the relationship between hyperlipoproteinemia and increased susceptibility to Candida albicans. Since apoE has both immunomodulatory and anti-infective features, to search apoE polymorphism and lipoprotein compositions, in patients with superficial fungal disease, is plausible. Therefore, it was decided to investigate the apoE polymorphism and lipoprotein compositions in patients with superficial fungal infections.

MATERIALS AND METHODS

The study was designed as a prospective, randomized and controlled study. Forty-two patients with dermatophytosis, 10 patients with cutaneous candidiasis, and 8 with pityriasis versicolor and 27 healthy control subjects were enrolled in the study after giving their informed consents. The diagnosis of superficial fungal disease was based on microscopic direct examinations performed with 20% potassium hydroxide for the presence of fungal elements. All patients and subjects were selected from persons with no histories of cardiovascular disease, primary or secondary hyperlipidemia, medication that may affect lipid metabolism and alcohol abuse. The study was approved by the Ethic Committee of Mersin University.

The plasma lipid and lipoprotein levels were measured following a 12-hour fasting period, with the patients on a standard diet, and taking no drugs. Five ml samples of venous blood were taken from each patient using Vacutainer tubes. Total (CHOD-PAP), HDL (Direct method), LDL (Friedwald formula) cholesterols, and triglyceride (GPO-PAP) were measured by enzymatic methods (Hitachi Modular System, Roche Diagnostics, Mannheim, Germany).

For genotype analysis, 2 ml venous blood samples were taken from each patients into plastic tubes containing 0.1 mol/l trisodium citrate. The whole blood was used for DNA extraction (DNA Isolation Kit for Whole Blood, Roche Molecular Biochemicals, Manheim, Germany). The extracted DNA's were used for the detection of apoE point mutations in codons 112 and 158 of the ApoE gene (Light Cycler-ApoE Mutation Detection Kit, Roche Molecular Biochemicals, Manheim, Germany). This system performs rapid PCR and simultaneously mutation detection employing melting curve analysis by monitoring the fluorescence.

Statistically significance differences between the serum lipoprotein levels of patients and controls were analyzed by independent t-test. The t-test was used to compare the differences between the genotype frequencies of two groups. To compare the allelic prevalence between the patients and controls, the Pearson Chi-square test was used. A level of $p<0.05$ was considered statistically significant.

RESULTS

All patients and controls were normolipidemic. The distribution of apoE alleles and genotypes, and their p values, compared to the control group are shown in Tables 1 and 2. Table 3 shows the concentrations of the serum lipids in patients with superficial fungal disease, and their p values, compared to the control subjects. The patients with superficial fungal disease had significantly (p=0.0462) higher concentrations of HDL-cholesterol than the control subjects. The total cholesterol, triglyceride, and LDL cholesterol concentrations of the patients tended to be higher than those of the

Table 1. ApoE Allele Frequencies among Patients and Controls

	ε 2	ε 3	ε 4	Total	p values
Candidiasis	3 (15.0%)	13 (65.0%)	4 (20.0%)	20	p=0.099
Dermatophytosis	16 (19.0%)	61 (72.6%)	7 (8.3%)	84	p=0.072
Pityriasis versicolor	2 (12.5%)	12 (75.0%)	2 (12.5%)	16	p=0.492
All patients	21 (17.1%)	86 (71.7%)	13 (10.6%)	120	p=0.066
Controls	3 (5.6%)	47 (87.0%)	4 (7.4%)	54	

Table 2. ApoE Genotype Frequencies among Patients and Controls

	ε 2/2	ε 2/3	ε 2/4	ε 3/3	ε 3/4	ε 4/4	Total
Dermatophytosis	---	16 (38.1%)* p=0.0165	---	20 (47.6%)* p=0.0337	5 (11.9%) p=0.7108	1 (2.4%)	42
Candidiasis	---	3 (30%) p=0.1717	---	3 (30%)* p=0.0194	4 (40%) p=0.1106	---	10
Pityriasis versicolor	---	2 (25%) p=0.3263	---	4 (50%) p=0.2082	2 (25%) p=0.5157	---	8
All patients	---	21 (35%)* p=0.0228	---	27 (45%) p=0.0139*	11 (18.3%) p=0.7073	1 (1.7%)	60
Controls	---	3 (11%)	---	20 (74%)	4 (15%)	---	27

*$p<0.05$.

Table 3. Serum Lipid Concentrations (mg/dl) in the Patients and Controls

	Dermatophytosis (n=42)	Candidiasis (n=10)	Pityriasis versicolor (n=8)	All patients (n=60)	Controls (n=27)
Total cholesterol	190.1±35.5 p=0.3505	209.4 ± 53.3 p=0.0718	194.9 ± 36.7 p=0.3609	193.9 ± 39.0 p=0.1750	182 ± 34
HDL-cholesterol	42.1 ± 9.9 p=0.0756	43.2 ± 12.8 p=0.1469	44.9 ± 13.9 p=0.0822	42.7 ± 10.8* p=0.0462	38 ± 8
LDL-cholesterol	120.0 ± 32.6 p=0.4497	131.1 ± 54.3 p=0.2367	120.5 ± 24.2 p=0.5902	121.9 ± 35.7 p=0.3235	114 ± 31
Triglyceride	146.2 ± 80.6 p=0.8241	176.6 ± 97.9 p=0.2357	148.0 ± 77.9 p=0.8350	151.5 ± 82.6 p=0.6038	142 ± 69

*$p<0.05$.

controls, although these differences did not reached statistically significant levels. A significantly higher incidence of heterozygosity E2/3 was found in all the patients, and those with dermatophytosis (p=0.0228 and 0.0165, respectively). A significantly lower incidence of homozygosity E3/3 was found in all the patients, and those with candidiasis and dermatophytosis (p= 0.0139, 0.0194 and 0.0337, respectively) compared with the control group. The E3/4 genotype differences between the patients and controls were not statistical significant. The Apo E2 allele was detected in 17.1 and 5.6% of the patients and control subjects, respectively, although this difference was not statistically significant. Among the patients with superficial fungal disease, the prevalence of the apo E3 allele was lower (71.7%) than that in the control subjects (87.0%), while that of the E4 allele was higher (10.6%) than in the control subjects (7.4%), but the difference was not statistically significant.

DISCUSSION

Apolipoprotein E (ApoE) is a secretory glycoprotein involved in the transport and redistribution of lipids between tissues. Its has a molecular weight of 34.2 kDa (299 amino acid residues) and is a component of several lipoproteins, including VLDL, LDL, HDL, and chylomicrons. ApoE interacts with the remnant receptor (apoE receptor) and the LDL receptors (apoE/B receptor), of the liver and other organs, to modulate the catabolism of triglyceride-rich lipoprotein particles.[15] ApoE exhibits a genetically determined polymorphism. Genetic and biochemical studies have shown that three different apoE alleles, E2, E3, and E4, at a single gene locus on chromosome 19, produce three major apoE isoproteins, E2, E3,

and E4, resulting in six different genotypes (called ε22, ε32, ε42, ε33, ε43, and ε44), and thus, six corresponding phenotypes in plasma. The well-documented pattern of increasing cholesterol levels, from ε2 to ε3 to ε4, seems invariant across different populations. Relative to ε33 individuals, ε32 women may be protected, while ε43 and ε44 men may be particularly susceptible to ischemic hearth disease.[16]

Although the mechanism by which apoE modifies immune responses remains to be clarified, there is growing evidence suggesting an interplay between lipid metabolism and immunity. ApoE effects innate and acquired immune responses *in vitro*, as shown by its ability to suppress lymphocyte proliferation, generation of cytolytic T cells, and stimulation of cultured neutrophils.[17] Laskowitz et al, showed that ApoE deficient mice had impaired delayed-type hypersensitivity responses, and generated higher levels of antigen-specific IgM relative to control mice.[17] The phagocytic capacity of granulocytes seems to be decreased in apoE deficient mice.[8] At present, it is unknown whether there is a differential immunomodulatory effect between the alleles and genotypes *in vivo*.

Dermatophytes are ubiquitous keratinophilic fungi, which cause dermatophytosis, usually limited to the skin, hair, and nails, but rarely cause an invasive disease resulting from the impaired immunity and natural defense mechanisms of the host. Resistance to dermatophyte infections may involve nonimmunologic as well as immunologic mechanisms. The fungistatic fatty acids and sphingosines produced by keratinocytes appear to limit the growth of dermatophytes.[2,18] The unsaturated fatty acid in sebum, palmitoleic acid, provides an effective barrier against Candida by blocking the adherence of Candida albicans to the stratum corneum.[19] However, the intravenous administration to volunteers of an emulsion of medium-chain lipids, but not of an emulsion of pure long-chain lipids or a placebo, increased the growth of Candida albicans in the serum and modulated the production of Candida induced cytokine by mononuclear cells. Wanten et al, in their study, observed that infusion of pure long-chain triglycerides (LCT) and mixed long- and medium-chain triglycerides (LCT-MCT) equally increased triglyceride concentrations. However, the *ex vivo* cytokine production by mononuclear cells was distinctly influenced by lipid treatment. The Candida-induced productions of TNF-, IL-1 ß, and IL-10 were increased after LCT-MCT exposure. However, the Candida-induced IFN-production tended to decrease, but this value did not reach statistical significance. The infusion of LCT or placebo did not influence the production of any cytokine. With LCT-MCT, significantly increased rates of growth of Candida were observed after 8 and 24 h compared with those of the placebo and LCT.[20] The elimination of Candida albicans by neutrophils is decreased after exposure to medium-chain fatty acid containing emulsions.[21] Candida albicans growth was 10- to 100-fold higher in the plasma of volunteers infused with 80 or 100 mg/kg high-density lipoproteins than in the plasma collected before infusion.[14] Malassezia species are lipophilic yeasts, which are emerging as nosocomial pathogens, particularly in low-birth-weight neonates receiving lipid emulsions.[22] The lipids presumably provide growth factors required for replication of the organisms. Cultures of Malassezia furfur are best achieved with a solid medium supplemented with a lipid source.[23]

In the light of clues on apoE metabolisms in fungal diseases, some differences were found in the apoE genotype frequencies in patients with superficial fungal disease compared to those of the controls. The apoE was observed to potentially contribute to the pathogenesis of superficial fungal disease, due to the immunomodulatory and anti-microbial effects. Our findings indicated an association between superficial fungal disease and high HDL-cholesterol levels, a high incidence of the apoE2/3 genotype and the low incidence of the apoE3/3 genotype, although no correlation could be established between the serum total cholesterol, LDL and triglyceride concentrations and the E3/4 genotype. It seems that high HDL-cholesterol levels are affected by other factors, such as the environment, genetics, and exercise, rather than apoE genotypes. This is the first report of an association between the apoE gene polymorphism and lipoprotein compositions and superficial fungal disease in a Caucasian population. The results of this study suggest that the

presence of the apoE2/3 genotype, high HDL-cholesterol levels and the absence of the apoE3/3 genotype can be regarded as risk factors for superficial fungal disease, especially dermatophytosis. Further studies, on larger patient series, are needed to determine the frequencies of lipoprotein compositions and the apoE polymorphism in superficial fungal disease and if they contribute to the development of the disease state.

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