Impact Of Apolipoprotein E4 On Development And Cognitive Function In Giardia-Infected Children

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Citation

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Abstract

Parasitic worms have largely been over looked by medicine, but attitudes are changing with the realization that they can seriously affect child development and cognition while the treatment is easy and cheap. The aim of this study is to elucidate ApoE and neurodevelopment in Giardiasis patients and the role of ApoE isoforms in childhood cognition and infant development.The present study was performed on 75 Giardia-infected patients (1-9 years). All patients suffered from sole Giardia infection with normal blood pictures. They were subjected to anthropometric measurements, lipid profile, cognitive functioning, developmental assessments and determination of apolipoprotein E allelic forms.ApoE genotypes in the studied children were 5, 6, 2, 30, 18 and 14 for E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4, respectively. There were no significant differences in the different genotypes regarding lipid profiles. There were also no significant differences regarding demographic and anthropometric measurement as well as cognitive function between ApoE4 carrier and non carrier, while highly significant differences were observed between ApoE4 carrier and non carrier regarding developmental assay, in children below 4 years.In conclusion, the ApoE4 isoforms have a protective role on cognitive development in children below 4 years suffered from sole Giardia infection. So, children with ApoE4 negative are advised to be treated and protected from any diarrheal disease.

INTRODUCTION

Parasitic worms have largely been over looked by medicine, but attitudes are changing with the realization that they can seriously affect child development and cognition while the treatment is easy and cheap (Warkin and Pollitt 1997). Indeed, a child growing up in an endemic community can expect be infected soon after weaning and constantly reinfected for the rest of his life. It has been estimated that for children of school age in low income countries, intestinal worms account for 12% of the total disease burden (Awasthi et al. 2003). Given the prevalence of high intensity infection in school children, it is particularly worrying that these infections can adversely affect cognition and educational achievement 6 to 9 years later (Lorntz ey al. 2006). Giardia is a well known parasite causing fat malabsorption and fatty diarrhea (Stevens et al., 1997). Diarrhea-induced malnutrition impairs brain development (Morgan et al., 2002) and its effect may be profound when nutritional deprivation occurs between birth and the 3rd year of life (Rice and Barone, 2000). Recently, it was found that, cognitive function in children is affected by environment, genetic determinants and health related factors (Oriá et al., 2005). However, Oriá et al., 2007 reported that genetic marker (ApoE4) appears to be important for cognitive development under the stress of heavy diarrhea.

ApoE encodes apolipoprotein E (glycoprotein, containing 299 amino acids, with a relative molecular mass of 34200 Da.) which plays a basic role in the binding and transport of lipids through the bloodstream and their delivery to the appropriate organs and tissues for processing and use (Wiesgraber 1994a). This function includes the removal of excess cholesterol from the blood (Mahley 1988; Wiesgraber et al., 1994b). There are three major isoforms of ApoE (E2, E3 and E4) that are the products of three allelic forms (e2, e3 and e4) of this single gene, which is located on the long arm of chromosome 19 (Reiss, 2005). The various combinations of these alleles give rise to six different genotypes, of which the most common is ApoE E3/3 (Hallman et al., 1991). Being the richest organ in lipid, the brain lipid balance is dependent on local synthesis of cholesterol and elimination of its metabolite 24(s) hydroxyl cholesterol as well as through the lipoprotein carrier ApoE (Reiss, 2005). Although nearly all brain cholesterol is synthesized in situ (Dietschy and Turley, 2001), the brain is vulnerable to diet-induced changes in serum lipid levels as this may affect cortical apolipoprotein E (Sparks et al., 1995) and myelin gene expressions (Salvati et al., 2002). The blood brain

barrier permeability could be also altered in case of diarrheal-induced oxidative stress (Noseworthy and Bray, 1998). In developing CNS, the need for cholesterol synthesis is much higher than in adult human state as myelination begins during the second trimester and continues through the second year of life (BjÖrkhem and Meany, 2004). Increased ApoE expression induced by dietary cholesterol enhances plasma cholesterol availability for some human brain areas that known to mature late during childhood and adolescence (Rice and Barone, 2000).

The aim of this study is to elucidate ApoE and neurodevelopment in Giardiasis patients and the role of ApoE isoforms in childhood cognition and infant development.

MATERIALS AND METHODS

This study was performed on 75 Giardia-infected patients (43 males and 32 females) attending in- and out-patients clinics of Children Hospital Mansoura University, Egypt. All patients suffered from sole Giardia infection with normal blood pictures. Patients aged from one to 9 years (4.1 ± 2.5). Secondary hypercholesterolemia (children with hypothyroidism, cholestatic hepatitis and familial hypercholesterolemia) nephrotic syndrome, chronic renal failure, and those receiving medications like thiazides, betablockers, oral estrogen and/or clozapine were excluded from the study. Written consent was taken from parents of the studied children patients.

Anthropometric measurements. Height and weight were determined when children were lightly dressed and without shoes. Height was measured to the nearest 0.1 cm using a portable sadiometer and weight was recorded to the nearest 0.1 kg using a standardized electronic digital scale.

Lipid profile. Fasting 12 hours, venous blood samples were obtained from every child early in the morning by venipuncture. Plasma total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) levels were determined enzymatically using spinreact kits (Spain). Cut off points for lipid levels, for children, were defined according to the national cholesterol education program guidelines for children (National Cholesterol Education Program,1992), TC 200 mg%, TG 140 mg%, HDL 35 mg% and LDL 130 mg%.

Cognitive functioning. 35 children (over 4 years) were assessed using the Arabic version of Wechsler Intelligence Scale for Children (WISC); (Wechsler, 1951). The WISC include verbal part (subtests, vocabulary, comprehension, arithmetic, similarities and digit span “forward and reverse”) and performance part (picture completion, block design and digit symbol). Test scores were converted into scaled age appropriate scores.

Developmental assessments. Motor and language development were assessed by the parents, for children below 4 years, reporting gross motor and language milestones: a method known to have considerable accuracy and sensitivity for identifying developmental delays (Knobloch et al., 1979; Cowen et al., 1994; Glascoe and Sandler,1995; Ireton and Glascoe, 1995). Mothers were asked if their child could do each of the tasks listed in the rating scales (20 points) for language development scale and (18 points) for motors development scale (Stoltzfus et al. 2001).

DETERMINATION OF APOLIPOPROTEIN E ALLELIC FORMS

DNA extraction: High molecular weight DNA was extracted from frozen EDTA-blood samples using GFX genomic blood DNA purification kit (Amersham Biosciences UK Limited). Extraction yielded an average of 20-40 µg of genomic DNA / ml of whole blood.

ApoE genotyping. DNA was amplified by PCR in a DNA Thermal Cycler (Techne Genius, England) using oligonucleotide primers F4 (5′-GCACGGCTGCCAAGGAGCTGCAGGC-3′) and F6 (5′-GGCGCTCGCGGATGGCGCTGAG-3′), according to a protocol described by (Hixson and Vernier 1990). The amplification reaction volume of 50 µL contained 25 pmol of each primer, 400 ng of genomic DNA and 25 µL of Ready MixTM RedtaqTM PCR Reaction Mix (Sigma, Saint Louis, Missouri, USA). Each reaction mixture was heated at 95°C for 5 min for denaturation, and subjected to 30 cycles of amplification by primer annealing (6O°C for 1 min), extension (7O°C for 2 min), and denaturation (95°C for 1 min).

PCR amplification of the ApoE generated a fragment of 299 bp. eighteen micro liters of PCR product were digested with 8U of HhaI (Promega, USA) at 37°C for at least 3 hours. Digested DNA fragments were analyzed with a 0.5 mm 10% nondenaturing polyacrylamide gel, containing 5% glycerol (Protean IIa vertical slab gel apparatus; Bio-Rad, Richmond,CA). Electrophoresis time was 120 min at 400 V. Separate DNA fragments were visualized by ethidium

bromide staining.

The fragments size from polymorphic HhaI sites after cleavage were as follows: the homozygote E2/E2 sample (91 bp and 83 bp HhaI fragments), the E3/E3 (91 bp, 48 bp and 35 bp fragments) and the E4/E4 (72 bp, 48 bp and 35 bp fragments) and the heterozygote E3/E2 sample (91 bp, 83 bp and 48 bp HhaI fragments), the E4/E2 (91 bp, 83 bp, 72 bp and 48 bp fragments) and the E4/E3 (91 bp, 72 bp and 48 bp fragments). The heterozygote E4/E2 sample, containing many restriction fragments, was used as a control.

Statistical analysis: Data were statistically analyzed using SPSS program standard version 10. Quantitative data were presented as mean ± standard deviation (SD), student's-test and ANOVA were used to compare between means. P≤0.05 was considered to be statistically significant.

RESULTS

The ApoE genotype distribution in the studied children is shown in Table (1). The most prevalent genotype is ApoE3/3 (40%) and the most common allele is e3 (36%). Table (2) shows the mean level of each TC, TG, HDL and LDL in the different genotypes. There were no significant differences regarding lipid profile between each genotype and the other one. However, significant differences appeared only in low birth weight group.

Figure 1

Table 1: ApoE genotypes in the studied children

	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4
No of children	5	6	2	30	18	14
%	6.6	8	2.6	40	24	18.6

Figure 2

Table 2: Lipid profile in the different genotypes

Mean ± SD	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4
TC (mg%)	150.3 ± 6.9	152.7 ± 6.3	160.0 ± 5.6	153.4 ± 10.9	166.6 ± 5.9	164.9 ± 21.5
TG (mg%)	131.1 ± 9.9	146.3 ± 3.1	158.8 ± 2.6	135.4 ± 4.6	156.7 ± 14.8	137.3 ± 9.1
HDL (mg%)	39.4 ± 3.1	47.1 ± 1.4	40.5 ± 6.6	40.6 ± 1.4	46.3 ± 2.3	41.0 ± 2.2
LDL (mg%)	93.7 ± 1.8	109.4 ± 2.3	114.3 ± 11.8	100.2 ± 3.6	114.5 ± 8.5	103.5 ± 3.6

Mean values for all demographic and anthropometric variables are shown in Table (3). They were similar between ApoE4-positive children (who harbor genotypes 2/4, 3/4 or 4/4) and ApoE4-negative children (who harbor genotypes 2/3, 2/2 or 3/3). Furthermore, no significant differences were found regarding cognitive function in ApoE4 carrier and non carrier in children above 4 years. However, their scores of performance part of the intelligence test and of the Full scale IQs are at the lower normal level (Table 4). This Table shows also a highly significant increase was found in ApoE4 carrier when compared to ApoE4 non carrier regarding developmental assay in children below 4 years.

Figure 3

Table (3): Demographic and anthropometric measurement of the studied children according to ApoE4 allele

		APOE4 (+) N=34	APOE4 (-) N=41
Age (years) (mean ± sd)		3.9 ± 2.4	4.5 ± 2.1
Sex	Male	19	24
	Female	15	17
Birth weight	Average	21	25
	Below average	13	16
Weight	Average	18	22
	Below average	16	19
Height	Average	15	18
	Below average	19	23

Figure 4

Table (4): Cognitive function and developmental assay in the studied children according to ApoE4 allele

		APOE4 (+) N=34	APOE4 (-) N=41
Cognitive function above 4 years (N=35)	Full scale IQ	92.5±4.3	90.1±4.7
	Verbal IQ	100.3±5.4	101.6±4.9
	Performance IQ	85.3±3.8	87.1±4.1
	Vocabulary	10.2±3.3	9.4±2.9
	Comprehension	10.5±4.0	10.2±3.1
	Similarities	13.4±3.0	12.8±2.3
	Arithmetic	11.1±1.9	10.3±2.8
	Digit span	7.0±2.2	6.2±1.4
	Picture completion	13.1±3.2	11.7±2.9
	Block design	8.1±2.1	7.4±1.3
	Digit symbol	9.0±1.2	8.6±2.8
Developmental assay below 4 years (N=40)	Motor	12.5±2.4	6.3±1.1
	Language	13.4±2.3	6.9±1.7

DISCUSSION

Genetic factors that alter the host response during critical developmental windows that occur during early life, can affect life history of human (Lanting and Boersma, 1996; Roux et al., 1998). Apolipoprotein E is one of almost a dozen protein constituents of plasma proteins that serve various functions (Mahley et al., 1984; Breslow, 1985). In brain, ApoE is synthesized by astrocytes and secreted into the extracellular space where it binds to cholesterol. There, it is taken up by neurons via various Apo E receptors and incorporated into cell membranes structures and myelin

(Yankner, 1996; Teter et al., 1999). The metabolism of cholesterol is believed to play a major role in neurite outgrowth and synaptogenesis (Beffert et al., 1998; Poirier and Sevigny, 1998; and Dierschy and Turley, 2001). Because cholesterol and fatty acids are critical to brain development, genetic factors that regulate their metabolism may influence development independently or may serve as modifiers of the response to maternal diet or maternal exposure to neurotoxin (Wright et al., 2003).

In the present work, the most prevalent phenotype is ApoE3/3 and the most common allele is e3. This in accordance with Utermann et al. (1980, 1982) and Menzel et al. (1983) who considered ApoE3 the parent form of the protein and ApoE4 and E2 are variants. However, ApoE allele frequencies are highly variable among different population. Chinese, Japanese and Mayan Indians have higher e3 and lower e4 allele frequencies than other populations (Hallman et al., 1991; Kamboh et al., 1991). Blacks from Africa, the USA and inhabitants of New Guinea have the lowest e3 and the highest e4 allele frequencies (Kamboh et al., 1989; 1991; Hallman et al., 1991; Hendrie et al., 1995). The e4 allele frequency is also higher in northern Europe than in southern Europe (Gerdes et al., 1992).

This study revealed no significant differences in lipid profile in different phenotypes of ApoE. Significant differences appeared only in low birth weight group. This agreed with Garcés et al. (2002) who reviewed that boys and girls with e2e3 genotypes had significant lower TC, LDL than those found in e3e3. Also, children with e3e4 genotypes have higher values of these parameters than children with e3e3 genotypes, and concluded that, ApoE allelic frequencies did not differ in boys and girls with different birth weights. A different allele seems to impact TC, LDL-C depending on birth weight. Henry et al. (1997) also recorded that the LDL lowering effect of e2 allele and the raising effect of e4 allele was greater in a group of adult with low infant weight as compared with a group with high infant weight, and the authors suggested that changes in the ApoE gene expression had been programmed by in utero nutritional events. This could be attributed to that ApoE2 isoforms have a lower affinity to ApoE receptor than E3 and E4 isoforms (Weisgraber et al., 1982). The differences in cholesterol absorption and postprandial remnant clearance between phenotypes due to the different isoforms may lead to up-regulation of hepatic LDL-receptors in subjects with E2 isoforms and a lowering of serum cholesterol levels. Conversely, efficient uptake of ApoE4 containing triglyceride-rich particles causes hepatic lipid accumulation with down-regulation of LDL receptors and increase in serum cholesterol levels.

There are no significant differences in ApoE4 carrier regarding age, sex, birth weight, the present weight and height. There were no significant differences in cognitive testing by Wechsler Intelligence Scale for Children (WISC) for older children in ApoE4 carrier and non carrier. This agreed with Turic et al. (2001) who found no difference in case control study of 101 subjects with high general cognitive ability when tested between 6 and 15 years of age. This may be owed to the nearly complete brain development and the effect of ApoE4 may be minimal. The risk factors that interfere with cognitive function are especially important during infancy, because the first two years of life is the essential period of rapid growth and development. However, in this study, it was found that the scores of performance part of the intelligence test and of the Full scale IQs in children above 4 years are at the lower normal level. These results are in agreement with Lorntz et al. 2006 who concluded that childhood diarrhea hinder school performance by impairing cognitive function as measured by performance on TONI-3 non verbal intelligence test; and with Niehaus et al. 2002 who found a significant lower scores, in 17 from 46 studied children, at Wechsler Intelligence Scale.

On the other hand, developmental assay regarding language and motor scales showed highly significant difference in ApoE4 carrier than non carrier. This agreed with Rask-Nissila et al. (2002) and Wright et al. (2003) who reported higher scores among E4 carriers. Malnutrition by intestinal parasites (including Giardia) impairs brain development by decreasing the number of cell replication cycle (Morgan et al., 2002), reducing total brain DNA (Zagon and McLaughlin, 1982), restricting dendritic arborization (Andrade et al., 1991) thus reducing connections between neurons.

Synaptic connectivity is particularly affected if nutritional deprivation occurs between birth and 3rd year of life (Rice and Barone, 2000) which are essential period of rapid growth and development (Oriá et al., 2005). In impoverished setting, children experiencing repeated diarrheal illness and malabsorption in their first years of life may have significant cognitive impairment (Mendez and Adair, 1999; Ivanovic et al., 2000). Giardia, like most eukaryotic cells (Luján et al., 1996), membrane biogenesis requires cholesterol (Jarroll et al., 1981; Kaneda and Goutsu, 1985). Because Giardia is

unable to synthesize cholesterol denovo (Jarroll et al., 1981), it must obtain this compound from the milieu of the upper small intestine, which is particularly rich in biliary and dietary cholesterol (Field et al., 1990; Thompson et al., 1993). In fact, Giardia consume host bile salts which deplete the bile salt pool and thus contributing to fat malabsorption by impairing micellar solubilization of ingested fat (Halliday et al., 1988), interacting with lipolysis hydrolytic enzymes independent of bile salt concentration (Smith et al., 1981; Katelaris et al., 1991) as evidenced by reduction of pancreatic lipase activity in infected children (Gupta and Mehta, 1973). The presence of ApoE4 deprives the parasite from cholesterol and shifting it away from availability to the enteric pathogens including Giardia to the developing brain (Oriá et al., 2005). ApoE4 also interferes with LDL endocytosis or cholesterol translocation by the parasite as Giardia use host cholesterol via receptor mediated endocytosis involving LDL-receptor pathway thus reducing viability (Stevens et al., 1997; Das et al., 2002) and enhance encystations specific gene expression (Luján et al., 1996).

In summary, we found that the ApoE4 isoforms have a protective role on cognitive development in children below 4 years suffered from sole Giardia infection. So, children with ApoE4 negative are advised to be treated and protected from any diarrheal disease.

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