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Full Length Research Paper

Hypofibronectinaemia in leprosy

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The aim of this study is to determine the levels of plasma fibronectin in leprosy patients of Nigerian origin. Seventy leprosy patients, 42 men and 28 women, aged 20 to 70 years, of Mile 4 Hospital Abakaliki in Ebonyi State of Nigeria and seventy age and sex-matched apparently normal subjects were used. Some (44) of the patients were on multiple drug therapy with dapsone, rifampicin, and clofazimine as the mainline drugs. The remaining 26 patients had stable treated leprosy but were living in the colony, (on-colony). Study protocol was approved by the Ethic Committee of the University of Nigeria Teaching Hospital, Enugu and informed consent was obtained from all subjects before collection of data and samples was made. Plasma fibronectin was assayed by an ELISA method. The leprosy patients had mean plasma fibronectin levels of 70.51 ± 17.83 µg/ml, men 85.39 ± 18.0 µg/ml, women 63.19 ± 3.63 µg/ml compared with the mean control value of 286.2 ± 130 µg/ml, men 395.5 ± 114.5 µg/ml, women 208.9 ± 71.69 µg/ml ($p < 0.001$). Peak control values were observed in the fifth decade of life, 40 to 49 years, and declined steadily thereafter. The peak values for the patients were observed in third decade of life, 20 to 29 years, and declined steadily with advancing age. Patients on drugs had lower fibronectin values, 71.32 ± 13.83 µg/ml, than those living on colony, 85.30 ± 20.50 µg/ml ($p < 0.05$). There is marked hypofibronectinaemia in leprosy patients that may be due to several possible causes. More work is needed to elucidate the exact cause(s) of hypofibronectinaemia in leprosy.

Key words: Leprosy, hypofibronectinaemia, plasma fibronectin, adhesion protein, Nigerian lepers.

INTRODUCTION

Fibronectin (FN), is an acute phase connective tissue glycoprotein which has numerous functions that ensure the normal functioning of vertebrate organism. Its interaction with cells affects the morphology, motility, gene expression and survival of the adherent cells (Hynes, 1992). FN regulates cell cycle progression and modulates cell signaling, (Hocking and Kowalski, 2002). Many cells require attachment to fibronectin in order to evade apoptosis, (Ruosalahti and Pierschbacher, 1987). It plays crucial role in blood coagulation and wound healing (Qui et al., 2007; Valenicks et al., 2005), and it is important in cell adhesion to extracellular matrix, (ECM) (Sottile and Hocking, 2002) and enhances nonopsonic phagocytosis of Pseudomonas aeruginosa by macrphages (Kluftinger et al., 1989). Fibronectin has been shown to form complex with antigen 85 (Ag85), a major secretory product of mycobacteria (Stuart et al., 1999). It is speculated that mycobacteria could bind to fibronectin to ascape immune detection or as a bridge to interact with molecules and cells of the host (Espitia et al., 1992). FN plays important role in embyogenesis and tumourigenesis (George et al., 2000). It functions as biological glue attaching the foetal sac to the uterine lining (Yamada, 1990).

It exists in the body in two main forms: insoluble tissue bound form and soluble dimmer found in plasma. The former is synthesized by connective tissue cells while the later is produced by hepatocytes (Vincent, 2000). Venous and arterial endothelia of human umbilical cord synthesize FN up to the 23rd week of gestation. Thereafter it ceases and disappears from the vagina secretion so the detection of foetal FN, (fFN), in cervico-

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vaginal secretion between the 24th and 34th weeks of gestation is indicative of possible preterm delivery (ACCOG, 2001; Andersen, 2000).

In humans, FN decreases early after operative injury, burns or trauma followed by a rapid restoration with a secondary decline typically observed if such patients become septic (Thompson et al., 1989). Excess FN deposition has been implicated in open angle glaucoma, (Babizhyev and Brodskaya, 1989; An-Fei et al., 2004). The plasma level is raised in morbidly obese individuals as a result of increased synthesis by lipocytes, (Diegaard et al., 1984). Raised plasma glucose has been found to increase fibronectin synthesis in trabecular meshwork cells, (Sato and Roy, 2002). Depletion of FN has been reported in sickle cell disease, (Bolarin and Adenuga, 1986; Emeribe et al., 2000), and in conditions associated with increased plasma metalloproteinase activity, (Hope et al., 2001). FN and other matrix protein deposition on wall may result in a re-modeled arterial structure with a smaller lumen and increased media/lumen ratio and so contribute to the development and complications of hypertension, (Hope et al., 2001). Reference plasma levels are between 350 to 450 µg/ml (Thompson et al., 1989).

Leprosy is a chronic disease condition caused by infection with acid-fast bacilli known as “Mycobacterium leprae”. It is characterizes by initial patchy lesions on the body, numbness and later by the presence of wounds and disfiguring deformities of the limbs and the face. Raised plasma fibronectin levels have been reported in untreated leprosy patients (Viashnavi et al., 1991) but there are no reports of the level in patients receiving treatment and those with stable treated leprosy, hence this work.

MATERIALS AND METHODS

Seventy (70) leprosy patients (42 men) of Mile 4 Hospital Abakaliki, Ebonyi State of Nigeria were used for the study. The Hospital is a major referral center for tuberculosis and leprosy in the eastern states of Nigeria and has a large population of these patients. They were aged between 20 to 70 years, and were made up of two groups; 44 patients on multiple drug therapy with dapsone, rifampicin and clofazimine as the mainline drugs and 26 patients who were no longer receiving drug treatment but were still living within the hospital, (on-colony). Clinically, the patients were classified as borderline tuberculoid (19 patients) and multibacillary, (51 patients). The patients displayed varying degrees of anatomical deformities and body wasting.

Seventy age and sex-matched apparently healthy subjects, 42 men and 28 women, were used as controls. They were recruited from the immediate community and had no connection whatsoever with the Hospital. Physical examinations revealed no signs that could be remotely suspected to be leprosy.

The study protocol was approved by the Ethic Committee of the University of Nigeria Teaching Hospital Enugu, Enugu State, where the laboratory analyses were also carried out. The purpose and procedure for the study was thoroughly explained to the patients in the language they understood and consent obtained before their biodata; age, sex, height and weight, waist and hip circumference and blood sample, were obtained using standard methods. Body mass index was calculated as weight in kg divided by the square of height in meters (kg/m^2). FN was assayed in citrated plasma by the ELISA method using Quantimatrix Human Fibronectin$^{(R)}$ kit, a product of Chemicon International USA; catalogue No ECM 300. All samples were analyzed within 24 hours of collection and storage was at -20°C. Data were grouped into patients and control and further sub-grouped according to sex, age and drug treatment. Student t test, analysis of variance, (ANOVA) and Pearson correlation statistics were used to compare mean values using GraphPad Prism Version 11 statistical package.

RESULTS

The mean plasma fibronectin level of the leprosy patients was 76.5 ± 17.8 µg/ml (range, 59.1 to 94.3). This is very much lower than that of the control, 286.2 ± 130 µg/ml, (range, 156.2 to 416.2), ($p < 0.001$). Sex-dependent significant differences, ($p< 0.001$,) were recorded in the mean plasma fibronectin levels of the patients and control with the male values being greater than the female values. The patients on multiple drug therapy had mean value, (71.3 ± 13.8 µg/ml), that is significantly lower than that of on-colony patients, (85.3 ± 20.5 µg/ml), ($p < 0.01$) (Table 1).

The plasma fibronectin levels of the control subjects differed with age, ($F = 2.76$, $p < 0.05$), and was highest in the 40 to 49 years age bracket. Those of the leprosy patients also differed inversely with age, ($F = 2.14$; $p < 0.05$). Highest levels were recorded in the 20 to 29 years age range and lowest values in the 60 to 70 years age range. The mean body mass index (range, 15.1 to 24 8), and waist circumference (range, 33.7 to 43.7), of the patients, differed from those of the control, (range, 22.7 to 31.4) and (range, 38.2 to 53.0) respectively, ($p < 0.001$). While the waist circumference of the control subjects showed male-female difference, ($p < 0.05$), there were no male-female differences in the anthropometric parameters of the patients. The parameters showed no significant correlation with each other.

DISCUSSIONS

There is hypofibronectinaemia in leprosy in this study. Raised plasma fibronectin levels have been reported in untreated leprosy patients relative to controls by Vaishnavi et al. (1991). This study investigated plasma fibronectin levels in patients receiving drug treatment and those with stable treated leprosy. Fibronectin levels in the patients on drugs were lower than those of patients not on drugs. This may suggest an effect of drugs on plasma FN and account for the difference between the report by Viashnavi et al. (1991) and this study. Hypofibronectinaemia has been reported in early infection and in sepsis and the levels were said to be related to the severity of infection, (Thompson, 1989; Brodin, 1986). In situations of multiple wound as in

Table 1. Variations in mean plasma fibronectin values (µg/ml) in relation to sex and drug treatment of leprosy patients and control subjects

Control subjects (N = 70)			Leprosy patients (N = 70)					p-value
ALL	Men (n=42)	Women (n=28)	ALL	Men (n=42)	Women (n=28)	On-colony* (n=26)	MDT† (n=44)	
286.2 ± 130 (156.2-416.2)			76.5 ± 17.8 (59.1-94.3)					0.001
	395.5 ± 114.5 (281-509)	208.9 ± 71.7 (137.2-280.6)						0.001
				85.4±18 (67.4-103.4)	63.2 ± 3.6 (59.6-66.8)			0.001
	395.5 ± 114.5 (281-509)			85.4±18 (67.4-103.4)				0.001
		208.9 ± 71.7 (137.2-280.6)			63.2 ± 3.6 (59.6-66.8)			0.001
						85.3 ± 20.5 (64.8-105.8)	71.3 ± 13.8 (57.5-85.2)	0.01

* Patients with stable treated leprosy and living on the colony. † Patients on multiple drug therapy (rifampicin, dapsone and clofazimine); Mean ±SD (Range of values).

leprosy, sepsis cannot be ruled out completely and may contribute to the hypofibronectinaemia seen in this study. Hypofibronectinaemia has also been reported in chronic wound and was said to be due to the elevation of the plasma level of serine proteases which degrade fibronectin. (Sottile and Hocking, 2002; Brodin, 1986). There were no reports on the level of these enzymes in leprosy but the condition is definitely characterized by the presence of multiple wounds. According to Vuento et al. (1988), plasma FN binds to the exposed sub-endothelial collagen of wound and participates in wound healing and in the final stage of blood coagulation. It also binds to antigen 85 secreted by mycobacteria, (Stuart et al., 1999). Conceivably, the plasma FN is used up in these processes (Babizhyev and Brodskaya, 1989), and it may be one of the reasons for the low plasma fibronectin values in leprosy. Since lipocytes produce fibronectin (An-Fei et al., 2004), individuals with lower body mass index may have low fibronectin concentrations than those with higher body mass index. The leprosy patients had low body mass index, mean 19.9 kg/m^2 (range 15.1 to 24.8) compared with that of the control group, mean 27.2 kg/m^2 (range 22.7 to 31. 4), ($p < 0.001$). This may yet account for hypofibronectinaemia in leprosy. Yet another reason may be the fact that plasma FN is sensitive to nutritional repletion and depletion, (Kirby, 1985), and these patients, understandably may not be as well fed as the control.

The very low levels of FN in on-colony patients not taking any drugs when compared with the controls may not justify such a speculation. However, the on-colony patients had mean body mass index which did not differ significantly from that of patients on drugs. And they definitely were not as well fed as the control. These can account for the low FN in the on-colony patients. However, the on-colony patient had mean FN level significantly higher than that of the patients on drug. This may be explained by the fact that the former may have fewer wounds than the later. The presence of wounds may still explain the difference between the FN levels of those on drugs and on-colony patients. Nevertheless it may not be presumptive to rule out completely adverse effect of drugs on plasma FN without further studies.

The disposition of plasma fibronectin as regards

age in the subjects, especially the controls, also poses problems of interpretation. Since in the patients the concentrations of plasma fibronectin decreased consistently with age, it may be related to the length of time of disease and drug treatment, since it is reasonable to think that the older patients may have had the disease and received drug treatment for a longer period of time than the younger ones. The highest FN values were recorded in the first decade of the age range of the patients, 20 to 29 years, corresponding to the third decade of life when they may not have had as much drug therapy or wounds as those in the last decade, 60 to 70 years, where the least values were recorded. With the passing of years after infection, period of drug therapy increases and the likelihood of developing wounds also become higher especially in those who reported late to the hospital. It therefore means that the age of the patient at the point of infection, time of initiation of treatment after infection as well as length of time of therapy may affect the level of plasma fibronectin in the leprosy patients.

The reference range of plasma fibronectin was quoted as 350 to 450 µg/ml, (300 mg/l) (Liotta et al., 1983). The mean value recorded in this study, 286.2 ± 130 µg/ml, for the control subjects is outside this range. In Saudi Arabia, Dhafir et al. (1996) reported a range of 180 - 622 µg/ml in an adult apparently healthy population. This is similar to the range of 156.2 to 416.2 µg/ml recorded in this study. In this report also, male control subjects and male patients had higher mean levels than female subjects and female patients, ($p < 0.05$). This agrees with the results of the work by Dhafir et al. (1996), in Saudi Arabia which recorded the following figures; males 180 to 622 µg/ml and females 135 - 547 µg/ml. Peak levels were recorded in the fourth decade of life and thereafter there was a steady decline in value of plasma fibronectin. The physiological significance of this is not certain.

The results of this study pose a number of questions to be answered in further researches. Firstly, are the levels of enzymes that destroy fibronectin elevated in leprosy and if so what about the levels of their inhibitors? Secondly, what is the effect of drugs on plasma fibronectin in leprosy? Thirdly what role does concurrent infection play in the hypofibronectinaemia of leprosy? Fourthly, why are the fibronectin levels of on-colony patients still so low? Fifthly, does the age of the subject at the point of infection with “Micobacterium leprae” or the time lag between infection and initiation of treatment determine the prevalent level of plasma fibronectin? However, there is marked plasma hypofronectinaemia in leprosy which may be due to one or a combination of these above factors.

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