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Circulating levels of the neuropeptide hormone somatostatin may determine hepatic fibrosis in *Schistosoma mansoni* infections

Shyama Chatterjee [a,*], Amadou Mbaye [b], Agaicha T. Alfidja [c], Joost Weyler [d], Janet T. Scott [e], Pierre Van Damme [d], Koen Van De Vijver [a], André Deelder [f], Eric A.E. Van Marck [a]

[a] *Pathology Unit, Department of Medicine, University of Antwerp, Universiteitsplein 1, B 2610 Antwerp, Belgium*
[b] *Région Médicale de Saint-Louis, ESPOIR, BP 394, Saint-Louis, Senegal*
[c] *Service de Radiologie Générale, CHU Fann, BP 5035, Dakar, Senegal*
[d] *Epidemiology and Social Health Unit, University of Antwerp, Antwerp, Belgium*
[e] *Institute of Tropical Medicine, Antwerp, Belgium*
[f] *Leiden University Medical Center, Leiden, The Netherlands*

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Abstract

The neuropeptide hormone somatostatin reduces fibrosis and *Schistosoma*-caused clinical morbidity in the rodent model. In our study we aimed to delineate an association between fibrosis and the inability to generate critical levels of endogenous somatostatin in *S. mansoni* infected subjects. In June 2001, 85 subjects from the district dispensary at Richard Toll in the Medical Region of Saint-Louis, Senegal, were selected. Fifty-seven subjects were infected with *S. mansoni* of whom 32 were suffering from severe morbidity (SM). Twenty-eight subjects showed an inactive disease status with no evident infection at the actual time of study. All subjects were classified according to age, sex, occupation, height, weight, and parasite eggs per gram. All 85 participated in a water contact and morbidity questionnaire, underwent a clinical examination and donated 5 ml of peripheral blood for detecting plasma levels of somatostatin. Ultrasonography detected fibrosis grade in all the subjects. To address whether inherent somatostatin levels determined clinically evident disease severity (epg, hepatomegaly, splenomegaly, hematemesis, ascites), the mean somatostatin values of the inactive disease status group and severe morbidity group were compared. Low somatostatin levels were depicted in subjects with severe morbidity symptoms associated with schistosomiasis as compared to exposed but inactive disease status subjects residing in the same region. Logistic regression analysis indicated that with decreasing somatostatin values the probability of severe morbidity increased with age being a confounding factor. To address whether inherent somatostatin levels determined fibrosis and if this association was significant, plasma somatostatin levels of non-fibrotics (ultrasonographic grading A), and fibrotics (ultrasonographic grading B–E) were compared. In all age groups as well as in adults alone, mean somatostatin levels were higher in the non-fibrotic group as compared to the fibrotics group, the difference being significant. The group B comprised of borderline fibrotic cases, therefore a separate analysis was done between groups A (non-fibrotics) and groups C, D (confirmed fibrotics). Mean somatostatin values were higher in the non-fibrotic group as compared to the fibrotics group, the difference being borderline significant.

* Corresponding author. Tel.: +323-820-25-46; fax: +323-820-25-32.
E-mail address: shyama@uia.ua.ac.be (S. Chatterjee).

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In schistosomiasis patients, circulating levels of somatostatin by binding to hepatic stellate cells (HSC) may modulate fibrosis. This phenomenon is regulated by age whereas gender and prior treatment have no effect on this association. Host specific somatostatin levels may create a 'preset environment' status that can determine progression to severe fibrosis.

Keywords: Neuropeptide; Somatostatin; Periportal fibrosis; Schistosomiasis; Morbidity

1. Introduction

In the study of host–parasite interactions caused by human schistosomiasis, neuropeptides have been reported to have an important role (Weinstock and Elliott, 1998, 2000). In man these neurokines include substance P, somatostatin and VIP. Somatostatin, a 14-aminoacid polypeptide originally isolated from the hypothalamus as a growth hormone inhibitor, is now known to be a multifunctional peptide with a wide anatomical distribution (Bloom and Polak, 1987; Scarpignato, 1999; Schusdziarra, 1996). Somatostatin receptors are present in the central and peripheral nervous system, pituitary, gastrointestinal (GI) tract, spleen, pancreas, heart, adrenals, kidneys and hepatic stellate cells (HSC). In recent years this peptide is reported to demonstrate anti fibrotic effects. In mice infected with *Schistosoma mansoni*, somatostatin induced a reduction in the degree of hepatic fibrosis, the portal pressure, weight of the spleen and the liver, the liver egg load and granuloma size and cellularity (Mansy et al., 1998).

It has been observed that in most endemic areas, prevalence and intensities of *Schistosoma* infection are higher in children than in adults (Warren, 1973). This has lead to a debate on the epidemiological contribution of transmission patterns (such as age-specific water contact), and host-related factors (such as acquired immunity) to age dependent infection patterns (Bradley, 1972). Studies in Kenya and the Gambia support the predominant role of acquired immunity in older children and adults in reducing their parasite burden (Butterworth et al., 1987; Butterworth and Hagan, 1987; Hagan, 1992). Yet this theory could not be confirmed by more recent data from Senegal (Stelma et al., 1993; Stelma, 1997). In a community recently exposed to intense transmission (new focus of infection), prevalence increased quickly and remained virtually 100% in all age groups above 5 years. However there was a marked reduction in worm load in all age groups above 20. Thus even in a new focus, there was a difference in the intensity of infection in children as compared to adults.

This finding indicates that the resistance to schistosomiasis may be regulated by inherent age related factors—one of them being the neuro-endocrinological interactions with the ability to induce specific or non-specific immune responses.

Thirty years ago, Brazeau showed that a cyclic 14 amino acid peptide secreted by the hypothalamus potently inhibited growth hormone released from the pituitary gland (Brazeau et al., 1973). They called this peptide somatostatin. Somatostatin is a ubiquitous peptide hormone that displays many specific and selective actions most of which are inhibitory ones. The peptide has earned itself the nickname 'endocrine cyanide' because of its effects on the secretion of many if not all hormones. Besides the regulation of growth hormone secretion it also inhibits the secretion of many other compounds such as insulin, glucagon, gastrin, growth factors, cytokines, and also endocrine and exocrine secretions. As a result somatostatin slows down a number of biological processes in the body and regulates the activity of many physiological processes.

Schistosoma egg-caused granuloma reactions in the liver result in hepatomegaly, the severity of morbidity depending on the intensity of infection. As inflammation continues repeated attempts to repair hepatic injury leads to fibrosis. Complications resulting from hepatic fibrosis are the principal cause of death in *S. mansoni* infected patients. These complications include portal hypertension and variceal bleeding. In such patients portal hypertension leads to the formation of portal-systemic collaterals specifically gastro-oesophageal collaterals (varices). Bleeding of these oesophageal varices can occur depending on the severity of fibrosis, the varice size and wall tension.

Despite extensive research in this field, the biological basis for the apparent selective manner in which *S. mansoni* infection leads to severe fibrosis

remains obscure. Reports have claimed that the down-regulation of fibroblast stimulating factors and fibrogenic cytokines in certain patients is responsible for the inability to develop severe fibrosis (Chen and Mott, 1988; Jacobs, 1998). However there is no clear information as to how the above determine fibrotic conditions. There may be a direct inter-relationship between granuloma formation and fibrosis and some author claim that the immunomodulatory network at play within the granulomas regulates the grade of fibrosis (Wyler, 1992a,b). Yet there is no definite proof for that either. Cytokines may determine the level of fibrosis but to date no definitively protective cytokine has been identified.

In such a situation we have focussed on the host specific factors of hormonal regulation that may have an intrinsic effect on fibrosis development. The levels of endogenous hormones secreted by the host may regulate fibrosis, which in itself is a host protective process against the parasite presence.

The main objective of this study was to delineate if intrinsic host levels of somatostatin modulated the morbidity caused by schistosomiasis. In endemic regions at any given time, only a fraction of infected patients develop severe hepatic fibrosis. Our hypothesis is that there may be an association between circulating levels of somatostatin in the infected host, and severe morbidity (SM) induced by *S. mansoni* (Chatterjee and Van Marck, 2001; Chatterjee et al., 2001a,b, 2003). The aim of this study therefore is to delineate the role of somatostatin in *S. mansoni*-caused pathogenesis, by studying host levels of somatostatin in the peripheral blood of *S. mansoni* infected individuals. Our objectives were to study in *S. mansoni* infected patients, the role of endogenous somatostatin in determining *S. mansoni*-caused clinical morbidity; the influence of age, gender and prior treatment on the above relationship; and to identify association between endogenous somatostatin levels and the degree of periportal fibrosis.

2. Design and methodology

2.1. Study group

To address these objectives a short study was carried out in June 2001 on subjects attending the district dispensary at Richard Toll, in the Région Médicale of Saint-Louis, Senegal. Eighty-five subjects were enrolled for this study. All 85 participated in a water contact and morbidity questionnaire (Table 1) and underwent clinical examination. Based on these and their parasite load, three study groups were identified.

1. Group 1: subjects with active infection and clinically evident severe morbidity—hepatomegaly, splenomegaly, haematemesis, ascites ($n = 32$).
2. Group 2: subjects with an inactive disease status at the time of study ($n = 28$).
3. Group 3: subjects with an active infection and no clinically evident severe morbidity ($n = 25$).

All subjects were examined by ultrasonography for the detection of periportal fibrosis and donated 5 ml of peripheral blood for measuring circulating somatostatin. The severe morbidity factors screened for by clinical examination and morbidity questionnaire were haematemesis, hepatomegaly, splenomegaly, and ascites formation. These symptoms were observed only in group 1. All patients were classified according to age, sex, occupation, height, weight and intensity of infection (epg = eggs per gram). Intestinal schistosomiasis represented by abdominal pain, diarrhea (bloody in SM group) and nausea were observed in all the three groups. Water contact questionnaire determined the specific daily activities with water from the Senegal River, the branch of it providing villages like N'Dombo, or from pools. Praziquantel treatment regimens followed by these patients in the last years were also determined. All procedures followed were in accordance with the ethical standards of the committee on human experimentation of the University of Antwerp and in accordance with the declaration of Helsinki.

2.2. Blood collection

The blood collected from all subjects in chilled syringes, was transferred into polypropylene tubes containing EDTA (1 mg/ml of blood) and Trasylol or Aprotinin (Bayer) (500 KIU/ml of blood). Samples were cooled immediately. Blood samples were centrifuged at $1600 \times g$ for 15 min at 0 °C, to separate out the plasma. The plasma was transferred to polypropylene tubes, and stored at −70 °C. Repeated freezing and thawing was avoided (EDTA; anticoagulant; Aprotinin/Trasylol: protease inhibitor).

Table 1
Questionnaire: water contact/severe morbidity

No.	Name							
Date	Village							
Age	Sex							
Height	Weight							
Occupation	EPG							
Hour of blood collection								
Activities with river water	Freq. yesterday	Freq. per week	Hour		Contact site			Obs.
			10–17 h	Other	River	Branch	Pool	
Washing hands, feet and face								
Collect water with recipient								
Bathing								
Washing clothes								
Washing vessels								
Fishing								
Gardening								
Rice culture								
Washing domestic pets								
Other activities with river water								
Clinical questionnaire								
	No.	Moderate (<3 per week) freq. per day	Intense (>3 per week) freq. per day					
Abdominal pain								
Nausea								
Diarrhea								
Clinical anemia								
Clinical examination								
Region medical of Saint-Louis								
District of Richard Toll								
Study of severe morbidity								
Date								
No.								
Bloody diarrhea in the last 2 months								
Hematemesis								
Clinical examination								
Conclusion	Normal	Malade						
Parasitological result: KATO	Positive	Negative						
Treatment								
Treated with praziquantel in the last 5 years								
Yes/no								
If yes how many times								
Note								

2.3. Somatostatin detection

An enzyme immunoassay method (EIAS-8001) was utilized at the Immunotechnology Core, International Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, to detect somatostatin levels in the human plasma. This simple three-step assay is based on an extraction free principle allowing for the direct assay of somatostatin in patient plasma, has a sensitivity of 0.06–0.08 ng/ml,

a range of 0–25 ng/ml, and does not cross-react with other neuropeptides.

2.4. Periportal fibrosis detection

Sonography has been proven to be as reliable as liver biopsy in detecting periportal fibrosis. In October 1996, in Niamey, Niger, a practical guide to the standardized use of ultrasonography, for the assessment of schistosomiasis-related morbidity was approved by the WHO (WHO Workshop Report, 2000). Using a portable device (PHILIPPS SDR 2200, The Netherlands) with a 3.5 MHz transducer, ultrasonography was performed in all the subjects according to the WHO (1993) approved Niamey–Belo Horizonte protocol for the qualitative assessment of periportal fibrosis. Liver texture was assessed, compared to predefined image patterns (A to F) (Niamey protocol), and a pattern score PT score was established (0–8) (Chatterjee et al., 2002).

When no abnormality was detected in the liver (pattern A), an image pattern score (IP score) of 0 was given. Borderline abnormalities (pattern B) suspicious for possible incipient periportal fibrosis ("starry sky" aspect with diffuse echogenic foci) were graded IP score 1. Pattern C (highly echogenic 'ring echoes' or 'pipe stems') depicting a peripheral periportal fibrosis like aspect, was graded IP score 3. Central periportal fibrosis like aspects in pattern D (highly echogenic 'ruff' around portal bifurcation and main stem), pattern E (highly echogenic 'patches' extending from the main portal vein and branches into the parenchyma), and pattern F (highly echogenic 'bands' and 'streaks'), extending from the main portal vein and its bifurcation to the liver surface, where they retract the organ surface) were, respectively, graded IP scores 4, 6, and 8.

Patterns indicating a different pathology isolated or associated with periportal fibrosis such as fatty liver-like texture and cirrhosis-like texture were marked, respectively, as patterns Y and X and were not included in the IP scores.

2.5. Prevalence of hepatitis infections

The region of Richard Toll is endemic for hepatitis B infections. All the 85 plasma samples were screened by the ELISA technique for reactivity to hepatitis B (HB) and hepatitis C. The HB surface antigen (HBs Ag) was detected with the ETI-MAK-4 (DiaSorin). Antibodies to HB core antigen (anti-HBc) were detected with the ETI-AB-COREK-2 (DiaSorin).

To detect hepatitis C (HCV), a third generation anti-HCV test (Ortho HCV 3.0 ELISA) was used to detect hepatitis C virus encoded antigen.

2.6. Statistical analysis

For basic statistical analysis a Graph-Pad Prism Program was used. Continuous data were tested for normality by the Shapiro–Wilkinson test. Normally distributed continuous data were compared by a Student's *t*-test. *P*-values of less than 0.05 were considered to be significant. Using SPSS a multiple logistic regression analysis was done in order to control for confounding by other determinants of the outcome (covariates).

3. Results

3.1. Prevalence of hepatosplenic and intestinal morbidity in the three study groups

The subject population consisted of individuals who were on record as having visited the district dispensary over the last years. They all belonged to the Richard Toll district and were residents of the following villages (Clinically evident severe morbidity patients (group 1) from Gadalkhout, Richard Toll (Escale), Khouma, N'Dombo Diop, Embranchement, M'bane, Savoigne, Loug deymiss, Rosso, Keur M'Baye, Thiago, Campement, Rosso Senegal, N'Dombo Alaiba, Khouma Panth; inactive disease status subjects (group 2) from Campement, Souloulou, N'Dombo, Gae H; subjects with active infection but no severe morbidity evident by clinical examination (group 3) from N'Dombo). The individuals attended the district dispensary at Richard Toll, where they were examined and screened for schistosomiasis. The age range varied from years 2 to 75. The inactive disease status group (group 2) comprised of subjects who tested egg negative at the time of study as tested by the Kato–Katz test done on two stool samples. Most of the subjects were residents of N'Dombo, a small village along the banks of the branch of the Senegal River that has become well known ever since *S. mansoni* infections

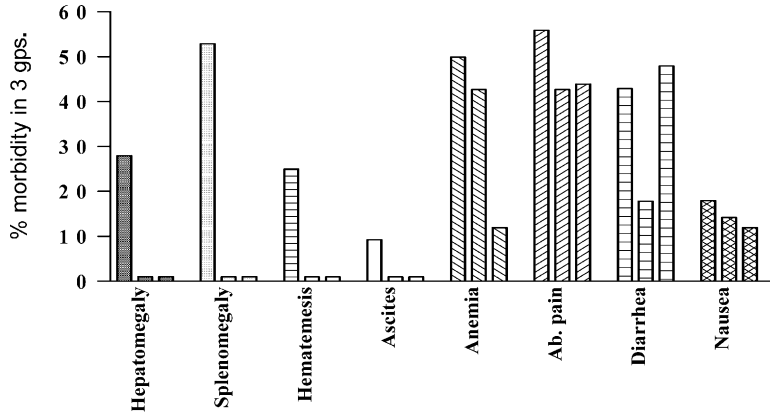

Fig. 1. Clinically evident severe morbidity and intestinal morbidity observed in the three groups of subjects. For each morbidity factor the percentage values in the three groups (group 1: active disease status subjects with severe morbidity; group 2: inactive disease status subjects; group 3: active disease status subjects) are depicted consecutively.

were first reported in northern Senegal. Fig. 1 depicts the hepatomegaly, splenomegaly, haematemesis and ascites values for group1, whereas values for anemia and intestinal schistosomiasis symptoms are depicted for all the three groups. Cases of abdominal pain were equally prevalent in all the three groups, so were symptoms of nausea. Several cases of diarrhoea were recorded in the groups 1 and 3 of individuals with the former group subjects suffering predominantly from bloody diarrhoea. Equivalent numbers were much less in the group 2.

3.2. Age distribution in the study groups

Fig. 2 presents the age distribution in the three subject groups. Median values observed were 14.5 years (group 1), 39 years (group 2) and 18 years (group 3). This gave proof that severe morbidity due to schistosomiasis does set in at an early age. Severe morbidity patterns were increasingly evident in young individuals, with the age group as low as 4–11 years being affected as well.

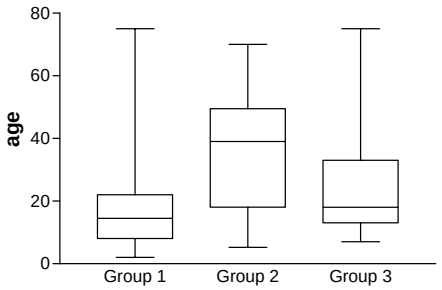

Fig. 2. Age distribution in the three groups (group 1: with severe morbidity; group 2: inactive disease status; group 3: active disease status).

3.3. Parasite load and treatment doses in the study groups

Fig. 3 represents the parasite load (epg) distribution in the three groups. Median values were zero both for the severe morbidity group (1), as well as for the inactive disease status group (2) of subjects. In the active disease status group (3), median epg values were 420. Maximum values obtained were zero for the group (2), 5280 for the group (1) and 1610 for the group (3). In the severe morbidity group, there was a big variation

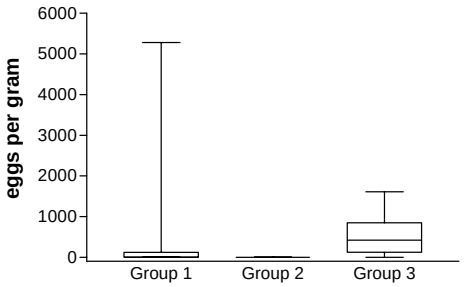

Fig. 3. The parasite load distribution in the three groups of subject.

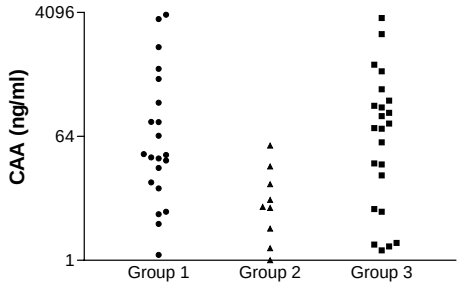

Fig. 4. CAA levels in the three groups of subjects. The circulating antigen levels are depicted in log 2 scale.

in the epg values (0–5280). The absence of parasite egg stages in the inactive disease status group confirms their status.

Fig. 4 gives the circulating anodic antigen (CAA) levels of all subjects. In patients of groups 1 and 3, CAA levels rose to 3802 and 3409 ng/ml, respectively, in contrast to group 2 where the highest value was 46 ng/ml. We consider this value as the cut off point for negligible antigen count. Median CAA values were 12.45 ng/ml for group 1, and 82.9 ng/ml for group 3. Mann–Whitney test to compare the median values of groups 1 and 3 showed no significant differences ($P = 0.0633$). Significant differences in median values existed between groups 1 and 2 ($P = 0.0008$), and between groups 2 and 3 ($P < 0.0001$).

Fig. 5 represents the treatment schedules of all subjects. Between none and six treatments with Praziquantel were given to the subjects. Median values were a single treatment for the severe morbidity group (1), three doses for the group (2), and two doses for the subjects of group (3).

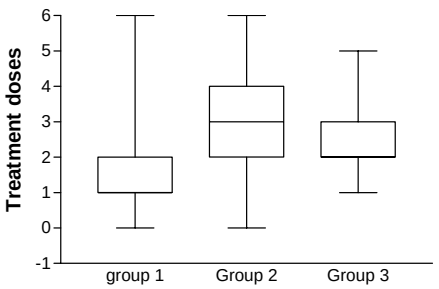

Fig. 5. Treatment doses in the three groups of subjects.

3.4. Circulating somatostatin levels in the study groups

Fig. 6 represents a box plot of somatostatin levels in the three groups. The questions addressed here were whether inherent somatostatin levels determined clinically evident disease severity (epg, hepatomegaly, splenomegaly, hematemesis, ascites), and if this association was significant. Moreover did factors like age, gender or prior treatment influence this association. Following basic statistics and tables, the mean somatostatin values of the inactive disease status group (4.363) and severe morbidity group (2.172) were compared. A t-value of 5.373 ($P = 0.000002$) was obtained, depicting significantly lower somatostatin levels in subjects with severe morbidity symptoms associated with schistosomiasis (group 1), as compared to exposed but inactive disease status subjects (group 2) residing in the same region. This group of individuals though uninfected at the time of blood collection have experienced *S. mansoni* infections in the past.

Logistic regression analysis indicated that with decreasing somatostatin values the probability of severe morbidity increased. To realize if variables like age, gender or prior treatment played any role in determining the association between disease severity and endogenous somatostatin levels, a multiple logistic regression analysis was performed. Based on a stepwise backward procedure the final model included only somatostatin ($B = -1.006$, sig. $= 0.003$, exp. (B) $= 0.366$) and age ($B = -0.046$, sig. $= 0.013$, exp. (B) $= 0.955$). The adjusted odds ratio was 0.366. This meant that with per unit decrease of somatostatin there is an increase of odds by three

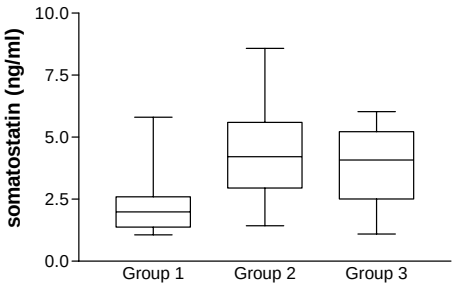

Fig. 6. Box plot of somatostatin levels in the severe morbidity (group 1), inactive disease status (group 2) and active disease status (group 3) subjects.

times for disease severity. A positive association is thus observed between severe clinical morbidity and the inability to generate required somatostatin levels.

Our results show a confounding effect of age on the regulatory role of somatostatin in determining severe morbidity in *S. mansoni* patients. Upon assessing this neuropeptide level in different age groups, somatostatin levels in uninfected children and adolescents were significantly lower in comparison to that of adults, infected adults bore significantly lower somatostatin levels than uninfected adults, furthermore somatostatin levels in infected children and adolescents were significantly lower as opposed to uninfected children and adolescents (Chatterjee et al., 2003).

3.5. Somatostatin levels in fibrotics

Our next objective was to identify possible correlation between individual hepatic fibrosis scores (regardless of initial grouping) and respective somatostatin blood levels. Results till now indicated an age dependent inverse correlation between endogenous somatostatin levels and severe morbidity in the Senegalese subjects. We wanted to study the specific correlation of intrinsic somatostatin levels with fibrosis. The fibrotic status of the subjects in the three groups was not directly evident from the clinical examinations, on the basis of which the initial groupings had been done. All subjects were next screened by ultrasound. The first impression was that sonographic morbidity globally correlated with the clinical and parasitologic status. Cases of fibrosis were detected in the group with hepatosplenomegaly, although some cases of hepatosplenomegaly could be due to other liver disease such as cirrhosis, or due to malaria. The active disease group (3) with no clinically evident severe morbidity had some cases of liver fibrosis, and a few cases were also found in the inactive disease status group (2), possibly generated by past infections.

The distribution of fibrotics in *S. mansoni*-exposed subjects with: pattern A (non-fibrotics), pattern B (borderline abnormalities), patterns CDE (periportal fibrosis-like aspects) and other patterns Y (fatty liver-like) and X (cirrhosis-like) are depicted in Fig. 7. Due to the high prevalence of hepatitis B infection in this region, and its influence on liver pathology, it was important to note the number of subjects with chronic hepatitis complications possibly due to hepatitis B. Three cases of cirrhosis-like echostructure (pattern X) were detected by ultrasonography, all in the severe morbidity group. Cirrhosis of the liver is an often-fatal complication that develops about 10–15 years after chronic hepatitis infection. In our observations the actual cirrhotic subjects were few, although several of the study group subjects displayed reactivity to hepatitis B (surface and core antigens) and to hepatitis C viruses (Fig. 8).

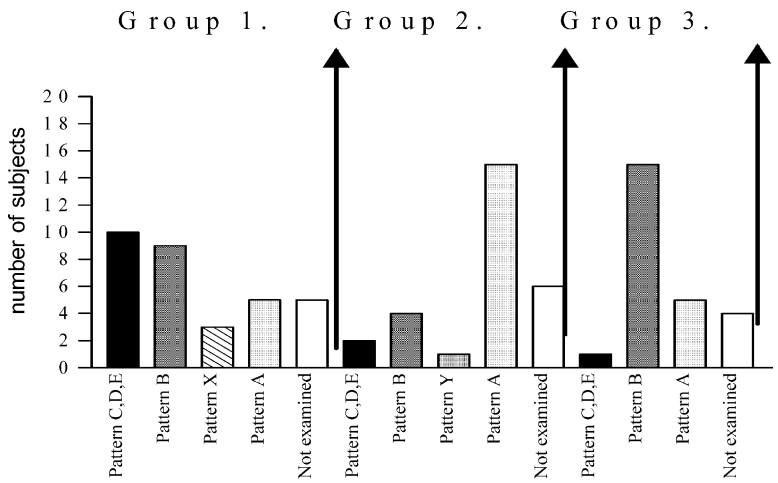

Fig. 7. Prevalence of schistosoma-caused fibrosis-like and cirrhosis-like patterns in study subjects. Fibrotics number maximum in the severe morbidity (group 1), and are also present in the inactive disease status (group 2) and the active disease status (group 3).

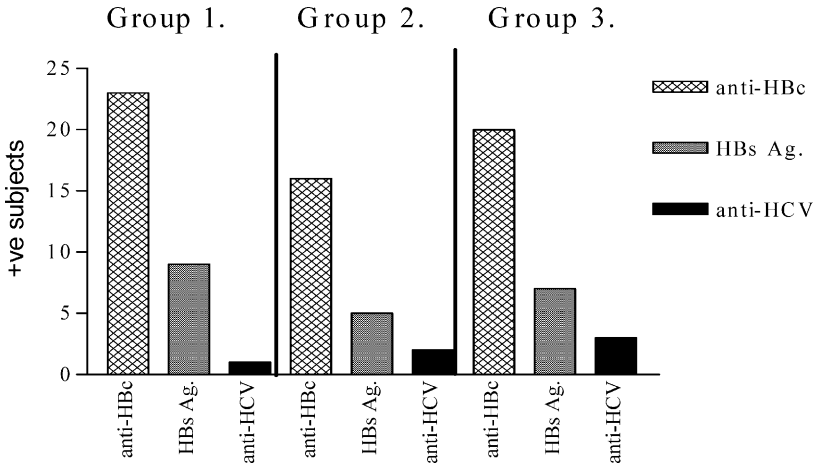

Fig. 8. The serological determination of hepatitis B and C prevalence in the three groups of subjects. Low prevalence rates of hepatitis C infection are observed in comparison to hepatitis B.

The question addressed here was whether inherent somatostatin levels determined fibrosis and if this association was significant. Moreover did factors like age, gender or prior treatment influence this association.

Fig. 9a shows the distribution of somatostatin levels in the plasma of non-fibrotics (ultrasonographic grading A) and fibrotics (due to schistosomiasis, ultrasonographic grading B–E) amongst the *S. mansoni* exposed subjects. Mean somatostatin levels were higher in the non-fibrotic group ($n = 22$, mean = 4.190) as compared to the fibrotics group ($n = 29$, mean = 2.824), the difference being significant (t-value = 2.825, P-value = 0.006819). The group B comprised of borderline fibrotic cases therefore a separate analysis was done between groups A (non-fibrotics) and groups C, D (confirmed fibrotics). Mean somatostatin values were higher in the non-fibrotic group ($n = 22$, mean = 4.190) as compared to the fibrotics group ($n = 10$, mean = 2.807), the difference being borderline significant (t-value = 1.962, P-value = 0.059129). Results indicated that in the borderline fibrotic cases, somatostatin levels were comparable to that observed in the fibrotic cases (Fig. 9b).

In a third graph we also compared somatostatin levels in the adults only (Fig. 9c). Mean somatostatin levels were higher in the non-fibrotic group ($n = 15$, mean = 4.537) as compared to the fibrotics group ($n = 12$, mean = 2.831), the difference being significant (t-value = 2.535, P-value = 0.0179).

Thus low somatostatin values can be considered a marker for progression to fibrosis in chronic schistosomiasis patients, rather than a clinical offshoot of severe morbidity.

A positive association is thus observed between the development of periportal fibrosis and the inability to generate required somatostatin levels. Multiple logistic regression analysis was carried out next, to observe if variables age, gender and prior treatment played a confounding role in the fibrosis-somatostatin correlation. Based on a stepwise backward procedure, the final model included only somatostatin ($B = -0.460$, sig. = 0.012, exp. (B) = 0.631). The adjusted odds ratio was 0.631. This meant that with per unit decrease of somatostatin there is an increase of odds by 1.5 times for fibrosis. Results showed no confounding effect of any of the variables, and indicated that with decreasing somatostatin values, the probability of fibrosis increases.

4. Discussion

In recent reports we presented that the neuropeptide somatostatin may play a critical role in disrupting host–parasite relationships. Host somatostatin levels may be one of the factors determining pathology

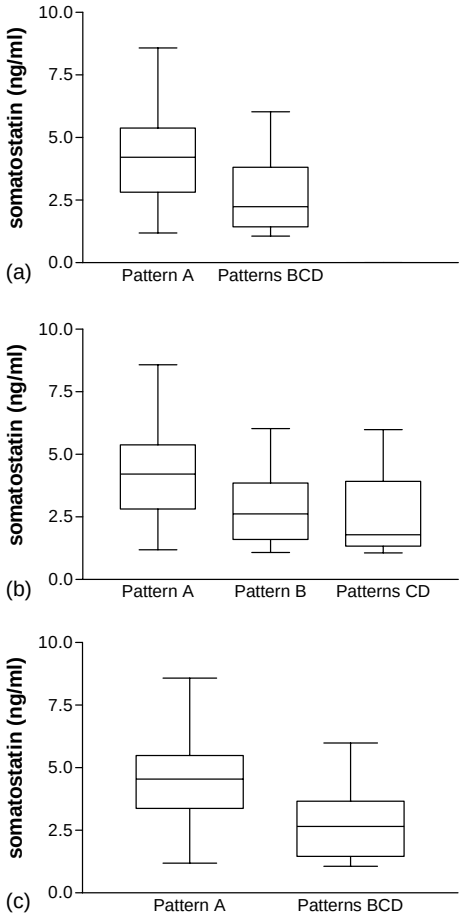

Fig. 9. The distribution of somatostatin in the study subjects comprising of fibrotics and non-fibrotics.

caused by *S. mansoni* specifically fibrosis. The murine model of *S. mansoni* has given some indications in this area. Upon in vivo administration of somatostatin in infected mice the hormone was effective in reducing hepatic fibrosis, granuloma size, liver egg load, portal pressure and weight of the spleen and liver in the host (Mansy et al., 1998). However the mechanism whereby somatostatin exerted its antifibrotic effect was unclear. The possible pathways could either be through anti-inflammatory effects associated with suppression of pro-inflammatory cytokines like IFN-gamma, or by a direct effect on hepatic stellate cells.

Literature shows extensive studies on the immunoregulation of granulomatous inflammation using murine schistosomiasis models (Blum et al., 1992). Using molecular, biochemical and immunological techniques, Drs. Weinstock and David Elliott have studied how neurokines like substance P, somatostatin and VIP regulate inflammation in the granulomas and mucosal surfaces (Elliott and Weinstock, 1996; Elliott et al., 1994, 1999). Somatostatin is produced endogenously by the granulomas in the murine model of *S. mansoni*-mice. This hormone has been shown to regulate the granulomatous inflammation—granuloma cells tightly and specifically bind somatostatin. Somatostatin regulates cell function by binding to cell surface receptors on granuloma CD4+ T cells, leading to the suppression of interferon gamma secretion by these T cells (Weinstock, 1990; Weinstock and Elliott, 1998, 2000). The lymphokine IFN-gamma controls multiple circuits of the immune system. Macrophage activation and antigen presentation are augmented by this lymphokine. T helper cell circuitry is tightly controlled by IFN-gamma. Moreover IFN-gamma augments IgG_{2a} isotype switching by B cells. Thus it is likely that somatostatin impinges on multiple aspects of the granulomatous response by suppressing intralesional IFN-gamma release. This reflects on the inflammatory response and may reduce subsequent fibrosis.

A second more plausible explanation for the activity of somatostatin has been via its activity over the hepatic stellate cells, which play a central role in the pathogenesis of hepatic fibrosis. Following acute or chronic liver tissue injury HSC undergo activation mediated by cytokines, extra cellular matrix components and reactive oxygen species. During chronic liver injury activated stellate cells are responsible for increased production of extra cellular matrix proteins, leading to inflammation and accumulation of extra cellular matrix.

The ambiguity surrounding the working mechanism of somatostatin in vivo was cleared by recent studies that demonstrated the presence of somatostatin receptors on the surface of the hepatic stellate cells (Reynaert et al., 1999, 2001). Evidence is now given for the direct antifibrotic effects of somatostatin by modulating collagen I and III synthesis and alpha-smooth muscle actin (SMA) expression in activated hepatic stellate cells. Collagen I and III are fibril-forming collagens, which make up a significant part of scar tissue in liver fibrosis. Smooth muscle

alpha actin is a marker for HSC activation. Suppression of alpha-SMA was observed in a range of somatostatin concentrations used in the experiments. In vitro studies showed that somatostatin at a concentration of 10^{-9} mol/l induced a decrease in collagen I and III synthesis, as well as a decrease in alpha-SMA synthesis in activated rat HSC. This suggests that somatostatin in addition to its beneficial effects on portal pressure (Avgerinos et al., 1997), may also decrease fibrosis in chronic liver disease through a direct effect on stellate cells.

In the Richard Toll region in northern Senegal, following repeated treatment schedules, the parasite load has been controlled in most subjects in this region, however morbidity patterns (fibrosis, hepatosplenomegaly, ascite formation) are increasing. In recent reports, bloody diarrhea and clinical anemia are being used as predictive indicators of past/present *Schistosoma* infections, in situations where an immediate parasite count is either not possible or provides zero count. We observe intestinal schistosomiasis symptoms (diarrhea, abdominal cramps, nausea) in almost similar numbers amongst the subjects of the three groups. Furthermore, after ultrasonography fibrotic condition was observed not only in subjects belonging to group 1 (active disease with severe clinical morbidity), but also to group 2 (inactive disease status group) and group 3 (active disease status with no clinical morbidity). These reports indicate that parasite load and intestinal morbidity cannot be used alone as markers of disease severity.

Pathogenesis to schistosomiasis is regulated by inherent age related factors—one of them being neuro-endocrinological interactions. Our results show that the endogenous secretion of the neuropeptide somatostatin increases with age. Somatostatin levels are lower in children than in adults, moreover somatostatin levels are depressed in infected as compared to uninfected subjects. Thus endogenous somatostatin levels may determine severe morbidity due to schistosomiasis, this balance being regulated by host age.

Moreover there appears to be a positive association between the progressive development of periportal fibrosis and the inability to generate required somatostatin levels. The model proposed for this analysis is that critical levels of somatostatin will delay the onset of fibrosis (WHO Workshop Report, 2000). Such questions have been addressed by our sero-epidemiological study, carried out in the *S. mansoni* endemic region of Richard Toll, northern Senegal. Lower levels of somatostatin were detected in *S. mansoni* infected subjects that display severe morbidity patterns (both clinical severity + periportal fibrosis), as compared to inactive disease status subjects. In the group of subjects with active disease but with no severe morbidity, results on 10 subjects showed that the somatostatin levels in these few subjects were comparable to that observed in the inactive disease status group. These results point to the role of the neuropeptide somatostatin in determining morbidity due to *S. mansoni* infection. Circulating somatostatin levels in the host may disrupt the host–parasite relationship, irrespective of gender, prior treatment and parasite count. Age has a confounding effect on this inter-relationship; we have observed severe morbidity factors settling in, in young children.

Our paper suggests that circulating somatostatin levels differ in a host specific manner, thereby creating a ‘preset environment’ that plays a regulatory role in the binding to receptors on hepatic stellate cells and thereby controls the level of hepatic fibrosis induced by schistosomiasis. This argument is based on multiple logistic regression analysis showing that patients with significant hepatic pathology have lower circulating somatostatin levels as compared to patients with active or inactive infection with little or no fibrosis. Patients with ultrasonic evidence of ‘borderline’ fibrosis had higher levels than those with established fibrosis. Indirect arguments include the negative effect of age.

Alterations in somatostatin levels have been reported in several diseases exhibiting prominent cognitive dysfunction, including Alzheimer’s disease, major depression, Huntington’s chorea, multiple sclerosis, schizophrenia and Parkinson’s disease (Bissette and Myers, 1992). We have conducted a cross-sectional study, therefore the question whether low somatostatin levels determine fibrosis is still not fully answered. Only a longitudinal study will answer that, and we intend to do a follow up study in the coming year. Yet direct comparison of the effect of low somatostatin levels in Alzheimer’s disease patients (Bissette and Myers, 1992) adds credibility to our belief that in disease status decreases in somatostatin levels is a cause rather than a result of the disease. Further prospective research studying the evolution of somatostatin levels

in subjects exposed to *S. mansoni* infections, will confirm the favorable preset environment hypothesis. Our study taken together stresses the regulatory role of physiological somatostatin levels in determining schistosoma-caused morbidity, and points to the therapeutic possibility of this neuropeptide hormone in alleviating the pathology caused by this disease.

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