NEUROHUMORAL ALTERATIONS AND THEIR ROLE IN AMOEBIASIS

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ABSTRACT

Amoebiasis is world wide in distribution and continues to be an important Public health problem. Intestinal amoebiasis may be present as dysentery, diarrhea or may stimulate other intra abdominal conditions. Clinical symptoms may not be seen in majority of patients, while amebic cysts are passed in the stool. This single-celled parasite is transmitted to humans via contaminated water and food. Amoebic dysentery can be accompanied by amebic infection of the liver and other organs. The present study was carried out to evaluate the changes in the circulating levels of neurohumors, their metabolizing enzymes and cortisol in these patients both before and after one month of chemotherapy. In the patients of amoebiasis the circulating acetylcholine (ACh), histamine, histaminase, cortisol, 5-Hydroxy tryptamine (serotonin) levels were significantly enhanced with no change in the Dopamine-beta-hydroxylase (DBH) activity, while the activities of erythrocyte acetylcholinesterase (AChE) and plasma Monoamine oxidase (MAO) were found decreased in comparison to normal healthy controls. After one month of treatment all the parameters reverted towards their control values, while the level of plasma histaminase remained still significantly high. The normal DBH activity reflects that there is no alteration in the circulating catecholamine levels, while the alteration in the levels of histamine, serotonin and cortisol may be due to the nonspecific response of the body to the stress of the disease and the parasitic infestation.

KEY WORDS

Amoebiasis, Acetylcholine, Cortisol, histamine, Histaminase.

INTRODUCTION

W.H.O. Expert committee (1969) defined amoebiasis as the condition in the human associated with harboring of *Entamoeba histolytica* with or without clinical manifestations. The protozoan parasite *E. histolytica* is the cause of amoebic dysentery and amoebic liver abscess.

Amoebiasis is world wide in distribution and continues to be an important public health problem (2). This parasite is transmitted to humans via contaminated water and food. Intestinal amoebiasis may be present as dysentery (inflammation of the intestine) with ulcers in the colon, diarrhea or may stimulate other intra-abdominal conditions like amoebic infection of the liver and other organs (3). Clinical symptoms are not seen in majority of patients, while amoebic cysts are

passed in the stool (4). Some times amoebiasis may also stimulate idiopathic ulcerative colitis (5).

The diagnosis of amoebiasis is often difficult and time consuming. The presence of serotonin in *E. histolytica* is known (6), thus its assay is suggested to provide valuable diagnostic information.

Controversial reports are available as regard to cortisone and related drug therapies in amoebiasis. Some postulate an enhancing effect on the virulence, multiplication and invasion of *E. histolytica* (7), while others did not find any significant difference in number of lesions of amoebic infections (8). The two conditions which amoebiasis most closely mimics are ulcerative colitis and carcinoma of the caecum (9).

In the present study an attempt has been made to gather information on alteration in the circulating levels, and role played by the neurohumors such as acetylcholine (ACh), histamine, 5-hydroxy tryptamine (5-HT), their metabolizing enzymes acetylcholinesterase (AChE), histaminase, enzymes of catecholamine metabolism dopamine-beta-hydroxylase (DBH) and monoamine oxidase (MAO) and cortisol in the patients suffering from amoebiasis both before and after therapy.

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MATERIALS AND METHODS

Thirty five cases of amoebiasis attending the Gastroenterology Unit of J.N. Medical College of A.M.U., Aligarh were included in this study. They were of both sexes and their ages ranged between 20 to 45 years. The diagnosis of these patients was established on the basis of thorough clinical examination, stool culture and sigmoidoscopy. In all these cases stool samples were found positive for *Entamoeba histolytica*. These cases were treated with antimicrobials such as sulphonamide or amoebicide along with Kaolin or chalk mixture. Normal healthy volunteers of similar age and sex were also included as controls. All the chemicals were of analytical grades and were purchased from commercial sources.

Fasting blood samples were collected from each patient before and after one month of treatment. Heparinized blood samples were centrifuged in cold at 10,000 rpm for 15 minutes. The plasma samples were subjected for the estimation of cortisol, 5-HT, DBH, MAO and histaminase, while whole blood was utilized for the determination of histamine. The erythrocytes were washed with n-saline and subjected for the estimation of acetylcholine (10) and Acetylcholinesterase by the method of Ellman et.al. using 0.075 M acetylthiocholine iodide as substrate (11). The reaction mixture contained washed diluted RBC (1:600), 25 µl substrate and 20 µl of 0.01M dithio bis nitrobenzoate (DTNB) to give yellow color, the absorbance of which was measured at 412 nm. All enzyme assays were performed at 37°C in a final volume of 1 ml, using a spectrophotometer (Beckman DU-7500). Plasma levels of histamine (12), histaminase (13), Cortisol (14), 5-HT (15), DBH (16), and MAO (17) were determined by standardized procedures. Briefly,

histaminase activity was measured by taking 15 µl of 0.2 M histamine diphosphate as substrate, 15 µl peroxidase and 15 µl of O-diansidine (1 mg/ml), the conversion of which to a colored product was read at 470 nm. For measuring the activity of MAO 8 nM benzylamine was taken as substrate and the product benzaldehyde was extracted in the organic layer and measured spectrophotometrically at 242 nm (17). The measurement of plasma DBH activity was based on the enzymatic conversion of 0.4 mol tyramine to octopamine and the photometric assay of p-hydroxybenzaldehyde after the oxidation of octopamine with periodate. The endogenous inhibitors that interfere with the assay of DBH *in vitro* were inactivated by adding N-ethylmaleimide (0.2 mol) (16).

Student's 't' test was utilized for statistical analysis and the results are expressed as Mean ± standard error of mean (SEM).

RESULTS

In the patients of amoebiasis the levels of erythrocyte ACh, blood histamine, plasma cortisol and 5-HT were raised significantly ($p < 0.001$) as compared to controls, while the activities of erythrocyte AChE and plasma MAO were significantly ($p < 0.05$) decreased. The activity of plasma DBH did not change while a significant rise was observed in the activity of plasma histaminase in these patients in comparison to controls ($p < 0.001$) (Table 1).

After one month of treatment the levels of all these parameters returned to their control values, with the exception of plasma histaminase which remained high although a reversion towards control value was observed.

Table 1. Alterations in circulating ACh, AChE, Histamine, Histaminase, DBH, MAO, Cortisol and 5-HT levels in Amebiasis before and after therapy. (Mean ± SEM)

	Normal (20)	Amoebiasis (35)	After therapy (25)
RBC ACh (µg/ml)	0.672 ± 0.020	0.950 ^b ± 0.086	0.701 ± 0.210
RBC AChE (µmoles/min/mg)	5.821 ± 0.174	4.842 ^a ± 0.395	4.950 ± 0.713
Blood histamine (PU/ml)	7.261 ± 0.524	12.120 ^d ± 0.317	7.893 ± 0.201
Plasma histaminase (PU/ml)	23.505 ± 0.508	41.061 ^d ± 3.174	27.532 ^a ± 2.209
Plasma DBH (nmoles/min/ml)	2.961 ± 0.106	3.142 ± 0.018	3.120 ± 0.094
Plasma MAO (PU/ml)	10.756 ± 0.384	8.628 ^b ± 0.962	9.753 ± 0.021
Plasma cortisol (µg /dL)	16.462 ± 0.534	20.549 ^d ± 0.701	16.531 ± 0.397
Plasma 5-HT (µg/ml)	0.087 ± 0.002	0.503 ^d ± 0.021	0.099 ± 0.015
a $p < 0.05$, b $p < 0.025$, d $p < 0.001$ as compared to controls.			

DISCUSSION

The significantly raised levels of ACh, histamine, 5-HT and cortisol as observed in the present study may be responsible for the frequently described pathologic lesions of amoebic dysentery i.e. ulcer and diffused inflammation (6). The irritation of intestinal mucosa, inflammation, edema, and focal hemorrhage from distended capillaries may be due to raised levels of histamine. *In vitro* studies have revealed an enhancement in pathogenicity of three isolates of *E.histolytica* in the presence of histamine. The Neal's caecal score of non-pathogenic strain NIH-200 were also found increased significantly in the presence of histamine (20 µg/ml) stepwise up to second culture and became stabilized at third generation with histamine (18). The enhanced level of histamine may be due to its release from the mast cells in response to increased serum IgE levels caused by the *E.histolytica* infestation (19). The increased level of histaminase the degrading enzyme of histamine may be due to body's natural response to degrade the excessive histamine.

The altered active electrolyte transport and diarrhea of rabbit ileum and rat colon induced by *E. histolytica* lysate was found similar to those caused by serotonin (19). Thus, the enhanced circulatory 5-HT level as seen in these patients may be both due to the stress of the disease (20) and *E. histolytica* infection, and responsible for the diarrhea seen in amoebiasis (19). Furthermore, the administration of 5-HT or its precursor L-tryptophan in infected animals is reported to significantly enhance the pathogenicity of various strains of *E.histolytica* (21). It is well recognized fact that the enzymes involved in metabolism of various neurohumors play an important role in the actual neurohumoral activity. Accordingly, the activity of these enzymes may considerably reflect the changes in neurohumoral levels in a particular situation. Thus, the decreased MAO activity (the degrading enzyme of 5-HT) as observed here might also be responsible for enhancement of 5-HT level. The increased level of 5-HT may also be due to body's natural response to decrease the increased motility of colon seen in amoebiasis. The decreased activity of MAO and near normal level of DBH shows that the level of circulating catecholamine is not altered in these patients.

The increase in the levels of these neurohumors may be due to the nonspecific response of the organism to the stress of the disease too. An alteration in the levels of these neurohumors has been reported earlier (22). The decreased activity of AChE is also reported in dysentery (23) which is a common feature of amoebiasis.

At least one month of therapy was found effective in reverting these altered biochemical parameters to their

control values. Similar to our finding the length of treatment of protozoan infection is suggested to be not less than one month by others too (24), while the Western text books suggest a three to seven days treatment.

It has been observed that corticosteroids initiate multiplication and invasion by amoeba (25). The enhanced level of cortisol as observed in the present study causes immunosuppression easily. Thus, it may be suggested that though the steroids did not increase the susceptibility to amoebic infection, but if once the infection is established, amoeba penetrate the mucosa easily in immunosuppressed animals (6).

Such an observation has a clinical implication as well. Therefore, once the immunity presumably cellular is depressed sub clinical or latent amoebic infections in human patients could become fulminating.

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