Original Article

Immunological response to antioxidant vitamin supplementation in rural Bangladeshi school children with group A streptococcal infection

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Group A beta haemolytic streptococcal (GABHS) infection induce an abnormal immune response in a susceptible host. Micronutrient deficiency may affect the immune response of an individual. The aim of this study was to determine whether antioxidant vitamins could improve the abnormal immune response in GABHS infected children in rural Bangladesh. A total of 516 GABHS infected school children aged 5 to 15 years were randomly assigned to two groups. Group 1 (*N*=258) was treated with phenoxymethyl penicillin V and group 2 (*N*=258) was treated with penicillin V plus antioxidant vitamins (beta carotene, alpha tocopherol and ascorbic acid). From each group two blood samples were drawn; the first sample at the beginning of the study and another one after eight weeks. Streptococcal antibodies and immunoglobulin levels were compared between the two samples. The mean age of the study population was 10.6 years. Equal number of boys and girls were included in both groups. After treatment, antistreptolysin O (ASO) and antideoxyribonuclease B (ADNase B) titres were decreased in both groups. Serum alpha tocopherol and beta-carotene levels were increased significantly in group 2. In group 1 immunoglobulin M and A levels decreased significantly (P = 0.0001) whereas immunoglobulin G showed no change. To the contrary, concentration of three immunoglobulins decreased significantly (P =0.0001) in group 2. Least-square means of between-group differences showed highly significant results for ASO, ADNase B, immunoglobulins M, A and G (P =0.0001). Our data indicate that treatment by antioxidant vitamins plus penicillin is more effective in decreasing immunological abnormalities in GABHS infected children then penicillin alone.

Key Words: Beta carotene, alpha tocopherol, immunoglobulins, streptococcal antibody, immunity, antioxidant supplements, infections, school children, Bangladesh.

Introduction

Group A beta haemolytic streptococcal (GABHS) infections are most common in children between 5 to 22 years of age in the developing countries, especially in Bangladesh,[1] although the infection may occur at any age. Its public health importance lies not only in the morbidity it causes but also in the fact that it is a precursor of serious non-suppurative sequelae: acute rheumatic fever (ARF) and acute glomerulonephritis.[2] The risk of an attack of rheumatic fever (RF) occurring following a symptomatic upper respiratory tract infection caused by GABHS varies from 0.3-3%[3,4] and it is estimated that only 20% of all streptococcal upper respiratory tract infections produce symptoms.[5] A large proportion of RF patients do not visit a physician for a streptococcal throat infection because of mild or no symptoms.[4,6]

The pathogenic mechanism of RF is widely believed to be related to an exaggerated immune response in a susceptible host to one or more streptococcal antigens.[7-,9] Many investigators have reported that due to hyper-sensitivity of the host [9,10] the mean antibody response is higher in patients who have developed RF than in patients in the same epidemic who have uncomplicated GABHS infections. In the economically deprived populations, such as Bangladeshi children, malnutrition in early life is quite common, and susceptible children could be hyper sensitised by immune imbalance.[11] Deficiency of micronutrients may affect immune competence of an individual. Among the different micronutrients, anti-oxidant vitamins are particularly effective in modulating immune functions and the host defence against micro-organisms or other invasive processes. This involves antigen specific

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humoral reactions of the immune system as well as non-specific inflammatory processes.[12] If antioxidant vitamins play an important role, as previously proposed, in the regulation of immune reaction then it may improve immunological abnormalities produced by GABHS infection. In this study, we examine whether antioxidant vitamins are capable of decreasing abnormal immune response in GABHS infected children.

Subjects and methods

Materials

The study was carried out on school children in a rural community of Bangladesh. Initially, written permission was secured from school heads. Management committees, consisting of representatives of parents, teachers and government officers, run the schools. A meeting was held between the investigators, parents and management committee. Consent was obtained from the parents. Then class teachers explained the purpose of the study to the students and requested them to participate in the study. The review committees of the National Centre for Control of Rheumatic Fever and Heart Diseases and the University of Dhaka have approved this study.

Study population

A total of 3750 children were sampled from the same socio-economic groups aged 5 to 15 years, who were students of grades one to ten. Students who attended the school on the day of clinical examination were considered as eligible. The study period was from January to December 1999. Inclusion for clinical screening was limited to students who fulfilled the following criteria: presence of sore throat, especially on swallowing and presence of redness of the tonsils or pharynx. Fever was not a prerequisite for inclusion. All children had no antibiotic treatment for at least 4 weeks and had no history of RF or RHD. Children under five years of age were excluded from the screening because it was difficult to obtain reliable information about the presence of soreness of the throat on swallowing, and this age group is not especially prone to RF.[1]

After clinical screening 1275 children underwent blood examination. Finally 516 children were selected for intervention therapy. High (≥300 IU/ml) ASO[13], high (≥340 IU/ml) ADNase B[13] and high (≥6mg/dl) CRP were the criteria for final selection. Result of the throat swab culture was not included in the study because a positive culture does not always indicate the presence of acute infection. Some children may be carriers of GABHS in their throat. Throat swab culture was done to exclude other common bacterial infections. ASO is the most commonly used and best-standardised antibody test for confirming a recent GABHS infection. Another important extracellular antigen produced by group A strains is deoxyribonuclease B. To specify group A streptococcal infection more precisely, ADNase B antibody should be measured. Although non-specific, CRP is the most commonly employed test to determine acute phase response status.

Intervention

The 516 subjects of both sexes selected for intervention were randomly divided into two groups. Group 1 (*N*=258) was treated with phenoxymethyl penicillin V and group 2 (*N*=258) with phenoxymethyl penicillin V plus antioxidant vitamins (beta-carotene, alpha-tocopherol and ascorbic acid). The dose of oral antibiotic phenoxymethyl penicillin V was 250 mg 6 hourly for 10 days and then 250 mg 12 hourly for 60 days for both groups. Antioxidant vitamins (beta-carotene 6 mg, vitamin E 50 mg, and vitamin C 200 mg) were given once in a single tablet daily for 60 days. Square Pharmaceuticals Limited, Dhaka, Bangladesh, supplied antioxidant vitamin (Rex™) tablets.

Ascertainment of variables

Sociodemographic: The following variables were obtained by interview of the subject or parent using a questionnaire: age, sex, number of siblings in the family, mother's occupation.

Anthropometric: Measurements of height for all subjects were done without shoes (using a mounted wooden scale). Height for age was calculated in the standard manner using the US National Health and Nutrition Examination Survey (NHANES) data. This index was chosen because it indicates long-term nutritional status.

Laboratory methods

Two blood samples were drawn from both groups for investigations, one at the beginning of the study and one after eight weeks. ASO, ADNase B, CRP, total protein, albumin, globulin, immunoglobulin M, A and G, beta-carotene and alpha-tocopherol values were obtained. Blood sample collection and preservation: A 5ml blood sample was collected aseptically by venipuncture into a sterile, dry screw capped test tube. The blood was placed immediately in an aluminum foil pack to protect from light for 1 hour at room temperature to allow clot formation. It was then centrifuged at 4000 rpm for 15 minutes in a refrigerated centrifuge. After separation, serum was distributed in several aliquots in Eppen drof tubes, levelled properly and preserved in deep freeze at -20^{0} C until tests were performed.

Serological and biochemical analysis

Sera were tested for ASO, ADNase B titre by latex agglutination slide and microtitre method respectively. CRP, total protein, albumin and immunoglobulins were determined by turbidometric method and antioxidant vitamins by colorimetric method. All tests were done in the laboratory of the National Centre for Control of Rheumatic Fever and Heart Disease, Dhaka. The tests were performed with commercially available kits and the manufacturer instructions were adhered in performing the tests. Technical blinding was maintained during the test performance.

Table 1. Background information

Variables	Total (*N* = 516)	*Intervention 1 (*N* = 258)	*Intervention 2 (*N* =258)	*P* value
Age, year, mean (SD)	10.6 (2.0)	10.5 (1.9)	10.7 (1.8)	0.15
Number of family members, mean (SD)	6.2 (1.8)	6.1 (1.7)	6.3 (1.9)	0.35
Number of siblings, mean (SD)	3.2 (1.9)	3.1 (1.8)	3.3 (2.0)	0.29
Working mother, n (%)	84 (13.7)	38 (11.4)	46 (16.0)	0.21
Height for age (%), mean (SD)	91.6 (5.3)	91.7 (5.4)	91.5 (5.2)	0.31
C- reactive protein, mg/dL, mean (SD)	31.9 (4.7)	31.3 (4.3)	32.5 (5.0)	0.62

*****Intervention 1 includes subjects who were treated with phenoxymethyl penicillin V; intervention 2, subjects who were treated with phenoxymethyl penicillin V plus antioxidant vitamins (alpha tocopherol, beta carotene and ascorbic acid).

Statistical analysis

Separate analysis for boys and girls were done using SAS package (Release 6.11, SAS Institute Inc., Cary, NC). Combined results are also presented. Paired 't' tests were done to examine statistical difference between pre- and post-intervention levels of the continuous variables as values were from the same subject and there was no major deviation from normality. Then ANCOVA was done to compare between-group differences in dependent variables (antibodies and immunoglobulins) adjusted for age, number of siblings, mother's occupation, number of family members, height for age and CRP with F tests computed from type III sum of squares. This form of sum of squares applies to unbalanced study designs and reports the effect of an independent variable after adjustment for all other variables included in the model. *P* values of less than 0.05 were considered statistically significant.

Results

There were 516 subjects including both sexes. Mean age was 10.6 years. There was no significant difference in mean age between groups. Background information of the two groups, which included age, number of family members, number of siblings, mother's occupation, height for age and CRP, are shown in Table 1. Streptococcal antibodies were markedly elevated in both groups initially. After treatment, serum levels were decreased in both groups. Reduction of ASO titre was similar in both sexes, but ADNase B was more prominent in boys. ASO and ADNase B titres were decreased in both intervention groups, but reduction of ADNase B level was more prominent in case of group 2 as compared with group 1 (Table 2).

After treatment, serum levels of alpha tocopherol and beta-carotene were increased significantly in both boys and girls of group 2. Differences were statistically significant in boys and girls. Alpha tocopherol and beta-carotene level showed no change in group 1 (Table 2). Initially, serum concentrations of all three immunoglobulins were high. After treatment serum concentration of immunoglobulins M and A were decreased significantly (P = 0.0001) in group 1 in both boys and girls, but immunoglobulin G levels remained almost similar in both sexes. In contrast, concentration of immunoglobulins M, A and G all decreased significantly (P =0.0001) in both boys and girls in group 2 (Table 2). Finally we examined differences (post minus pre-intervention) between the groups in antibodies and imunoglobulins by ANCOVA. Least-square means of differences (Table 3) showed highly significant results (P = 0.0001) for ASO, ADNase B, IgM, IgA and IgG.

Discussion

This report represents a trial of antioxidant vitamins on school children with abnormal immune response produced by GABHS infection. Our working concept was to reduce immunological abnormalities in children produced by GABHS infection. To our knowledge this study is the first one with this hypothesis. However, our study has a weakness in the randomisation of inter-vention. To overcome the weakness we have done ANCOVA to compare between-group differences in dependent variables (antibodies and immunoglobulins) adjusted for age, number of siblings, mother's occupation, family size, height for age and CRP (Table 3).

Antibody production is increased in a hypersensitive host due to frequent streptococcal infections. Increased antibody production was observed in our children in the initial stage. We have observed that serum levels of immunoglobulins were above normal in both groups in our study children. After treatment three, immunoglobulin levels were reduced significantly in the antioxidant vitamins group, but in the penicillin group reduction was limited to only two immunoglobulins (IgM and IgA). In the penicillin group, IgG levels remained unchanged, even after treatment. This elevated level of IgG may be due to persistence of continuous inflammatory process set by streptococcal infection. In the antioxidant group all immunoglobulins levels were reduced, possibly due to the antioxidant action of the vitamins, because serum level of antioxidant vitamins was raised in this group after treatment. It is interesting to note that the majority, but not all of the treated subjects showed a correction of immune response, towards normal level. The quantitative changes of the immunoglobulins were variable depending on the individual responder.

Table 2. Streptococcal antibodies, antioxidant vitamins and immunoglobulins in intervention groups before and after treatment

Variables	Intervention 1				Intervention 2			
	Before	After	Difference*	*P* value	Before	After	Difference*	*P* value
	Mean (SD)	Mean (SD)	Mean (SEM)		Mean (SD)	Mean (SD)	Mean (SEM)	
	(*N* =258)				(*N* =258)			
ASO**, IU/mL	505 (180)	386 (123)	-119 (10.5)	0.0001	495 (137)	237 (91)	-258 (8.8)	0.0001
ADNase-B**, IU/mL	450 (210)	391 (147)	-59 (14.6)	0.0001	487 (285)	287 (168)	-200 (17.5)	0.0001
Alpha tocopherol, mg/L	8.1 (0.2)	8.08 (0.2)	- 0.02 (0.02)	0.34	8.1 (0.2)	10.6 (0.5)	2.5 (0.03)	0.0001
Beta-carotene, mg/L	0.1 (0.02)	0.09 (0.02)	- 0.01 (0.01)	0.67	0.1 (0.02)	1.2 (0.4)	1.1 (0.02)	0.0001
Immunoglobulin M, mg/dL	278 (55)	216 (34)	-62 (3.6)	0.0001	288 (53)	143 (24)	-145 (3.4)	0.0001
Immunoglobulin A, mg/dL	329 (37)	221 (41)	-108 (3.1)	0.0001	364 (120)	232 (54)	-132 (7.6)	0.0001
Immunoglobulin G, mg/dL	2521 (265)	2545 (284)	24 (20.0)	0.23	2498 (274)	1819 (227)	-679(17.2)	0.0001

* Post intervention minus pre-intervention
**ASO indicates antistreptolysin O; ADNase B, antideoxyribonuclease B.

This variability of response could be partly due to the differences of serum concentration of antioxidant vitamins. Inflammatory process was not completely ceased in the penicillin treated children and their serum level of antioxidant vitamins remained low during the study period as they did not receive any antioxidant vitamin supplementation during this period. Antioxidant vitamins exert their beneficial effect on the host defence by preventing the infection induced anti-inflammatory process produced by tissue prostaglandin. During the immune response, the body secrets inflammatory substances, such as interleukin-6 and CRP, which intensify and help sustain the immune response. Vitamin E has emerged as a potent anti-inflammatory nutrient, protecting against inflammatory disorders. In our study we observed that initially in all children, serum IgG was elevated along with other two immunoglobulins and their serum levels of vitamin E and beta-carotene were low. Children treated with penicillin were unable to eliminate inflammatory process within a short period due to their low serum level of antioxidant vitamins.

Zaman *et al.*,[14] in a study in Bangladesh showed that RF patients and GABHS infected control subjects have similar levels of beta-carotene. We have observed low serum levels of beta-carotene in our subjects infected with GABHS. Beta-carotene levels correlated negatively with IgG levels, which indicates an indirect sign of inflammation.[15] Bendich *et al.*,[16] reported that beta carotene exerts its antibacterial effects by an immunomodulatory effect.

Ascorbic acid also plays an important role in the defence against infection. In a study Anderson *et al.*,[17] showed that ascorbic acid supplementation has no effect on serum IgGs but it reduced antibody titre significantly in streptococcal infection.

All immunological indices are reduced towards normal range by antioxidant vitamin treatment, whereas these indices are partly reduced by penicillin treatment. The partial correction of immune response indicates that the inflammatory process set by streptococcal infection to some extent is continuing while treating with penicillin. Penicillin treatment may need a long period for correction of immune response. Antioxidant vitamins along with penicillin in our study show quick correction of abnormal immune response. This indicates that penicillin therapy alone is not sufficient to correct or 'condition' immune

Table 3. Least-square means* of differences (post minus pre treatment) of antibodies and immunoglobulins in intervention groups*

Variables	Intervention 1	Intervention 2	df	F value	*P* value
Antistreptolysin O, IU/mL	-119	-258	1	44.4	0.0001
Antideoxyribonuclease B, IU/mL	-59	-200	1	31.0	0.0001
Immunoglobulin G, mg/dL	24	-679	1	445.7	0.0001
Immunoglobulin M, mg/dL	-62	-145	1	207.2	0.0001
Immunoglobulin A, mg/dL	-108	-132	1	11.5	0.0007

*Least-square means were obtained by adjusting for co variants: age, mother's occupations, number of siblings, family size, height for age and CRP.

response in children with micronutrient deficiency. Adjustment for multiple socio-demographic variables does not attenuate appreciably the relationship between serum antioxidant vitamin levels and indices of immune responses that we have observed. Antioxidant vitamins may therefore be considered necessary for improved immunological abnormalities.

Conclusion

The findings of our study indicate that immunological abnormalities in children produced by GABHS infection may respond better to combined treatment with anti-oxidant vitamins and penicillin than penicillin alone. The study has limitations and would be more informative if there were data about nutritional status before the illness, which is not possible with the current study design. Our intervention included beta-carotene, alpha tocopherol and ascorbic acid, with blood measurements of beta-carotene and alpha tocopherol only. ASO titre was performed by a semi-quantitative method; quantitative measurements would provide a more sensitive estimate of differences within and between groups.

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