

ISSN No. 0972-8503



INDIAN JOURNAL OF BIOLOGICAL SCIENCES

VOLUME 45

2009

INDIAN JOURNAL OF BIOLOGICAL SCIENCES

Vol. 15

ISSN 0972-8503

2009

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AN ERGONOMIC STUDY ON SUITABILITY OF CHRONOTYOLOGY QUESTIONNAIRES ON BENGALEE (INDIAN) POPULATION

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ABSTRACT ■ Many researchers believe that adjustment to rotating shift work is greatly influenced by morningness-eveningness orientation or chronotypology. The characteristics of morningness-eveningness are one of the strongest predictors of adjustment to shift work. Various self reported questionnaire were developed by different researchers; among them Morningness-eveningness Questionnaire (MEQ), Circadian Typology Questionnaire (CTQ) and Composite Morningness Questionnaire (CMQ) were widely used throughout the world. The Bengali (local language of West Bengal) versions of these questionnaires were applied on personnel working in rapidly rotating shift and general duty in health care units of west Bengal. From the results, it was observed that among the three chronotypology questionnaire, the CMQ, after it is suitably modified is the best morningness-eveningness orientation predictor for Bengalee (Indian) population. The modified CMQ is short, easily understandable and has adequate psychometric properties. CMQ is well correlated with external factors like waking time, bed time, etc.

Keywords : Chronotypology, CMQ, MEQ, CTQ, Bengali version

INTRODUCTION :

Individual differences in various parameters of circadian rhythms are related to the ease of adjustment to and tolerance of the night or rotating shifts (Moog, 1987). It was noticed that each individual is different in the pattern of circadian rhythms of his temporal responses (Baehr, Revelle and Eastman, 2000). The popular terms 'early bird' and 'night owls' indicate the extreme types of the spectrum of individual variations in the activity timing performance. The impact of these individual differences in circadian phase positions on physiological as well as cognitive variables has received increasing interest in

research work during recent years (Reviewed by Cavallera and Giudici, 2008). Several self reporting questionnaires have been developed to measure "diurnal type" or distinction between "larks" and "owls" (Horne and Ostberg, 1976, 1977, Folkard et al., 1979; Torsvall and Akerstedt, 1980; Moog, 1981; Smith et al., 1989). People who express performances for activities at the morning time of the 24 hours' day are termed as morning chronotype and people with other extreme as evening chronotype. These two types of people behave according to those performances and show reliable biological differences in circadian functioning. So, these

questionnaire techniques have increasing importance in pre-selection, placement and counseling of personnel engaged in rotating shift work or night work. Among the questionnaire, Morningness-eveningness Questionnaire (MEQ) of Horne and Ostberg (1976), Circadian Typology Questionnaire (CTQ) of Folkard et al., (1979) and Composite Morningness Questionnaire (CMQ) of Smith et al., (1989) were widely used and well accepted. Brief depictions of these three questionnaires are given below:

MEQ of Horne and Ostberg (1976) :

A modified form of Ostberg's Swedish-language questionnaire of morningness was used as the framework for this English language version. The final questionnaire consist of 19 questions, most of the answers (of 14 questions) were designed to be forced preference among four options. In the other 5 questions, preferences are given in time scales. Each answer was scored and the scores are added together, a high score indicating definite 'morningness' and a low score indicating definite 'eveningness'. People are classified into five chronotypes, namely 'Definite morning' type, 'Moderate morning' type, 'Neither' type, 'Moderate evening' type and 'Definite evening' type according to total score. Horne and Ostberg (1976) administered English version of this questionnaire to 150 adults, within the age group of 18-32 years. Oral temperature was taken as external validation criteria. Results indicated that the temperature rhythm of those individuals classified as 'morning' type were qualitatively and quantitatively different from the temperature rhythms of those classified as 'evening' type.

CTQ of Folkard, Monk and Lobban (1979) :

A total of 19 questions were given, each being associated with a 10 cm visual analog rating scale. Analysis of the scale based on three factors, i.e., rigidity (or flexibility) of sleeping habit (high scores on factor indicate inability to sleep at unusual time and a preference for regular sleeping times); vigorosity (or languidity) of sleep (people with high score relatively unaffected by lack of sleep and wake up easily in unusual time) and morningness (high score in this factors indicate 'morning' type and low scores 'evening' type). A sample of 48 night nurses responded to the items, which were re-administered in a slightly altered format 28 days later. Validity of the above mentioned three factors were measured by correlating factor scores with a variety of measures of adjustments (e.g., oral temperature, subjective alertness, etc.). Although the rigidity and vigor dimensions correlated with some of these variables, few significant relationships between the adjustment measures and the morningness dimensions were found.

CMQ of Smith, Reilly and Midkiff (1989):

This questionnaire is a compromise between MEQ of Horne Ostberg (1976) and Diurnal Type Scale (DTS) of Torsvall and Akerstedt (1980). After extensive psychometric analysis, 9 questions from MEQ and 4 questions from DTS were extracted to formulate CMQ, consisting of 13 questions. Answers are scored and according to total score people are divided into three chronotypes namely, 'morning', 'intermediate' and 'evening'. Smith et al.

(1989) administered the original English version of the questionnaire to 501 undergraduate university students. Several subjective parameters (self report of length of sleep, time of perceived peak alertness, etc.) were used for external criteria to validate the study.

Smith et al. (1989), reported population mean differences between American and Japanese responses on MEQ. Japanese respondents were found to be more morning oriented than typical American. Smith et al. (2002) have published a normative cross cultural data in this regard after comparing data regarding morningness in six countries. Unfortunately, no other comparative or normative cross cultural data available. In India very few attempts were made to test the suitability of this circadian questionnaire (Sen and Sahu, 1993; 1995; Sahu, 1997).

The present study aimed at ascertaining the relevance these three different questionnaire techniques in Indian context specific to Bengali spoken people and to find out the questionnaire best fit to the people of West Bengal (Bengalee).

Materials and Methods :

The English version of these three questionnaires was translated in to Bengali and applied on 50 pilot sample and their difficulty and other aspects were recorded. In the final version, 5 questions out of 19 questions of MEQ, 4 questions out of the 19 questions of CTQ and 4 questions out of 13 questions of CMQ, were slightly modified, since the language of a few of those questions were complex and not easily understood by the people of West Bengal and the response scale of some of the questions were extended

to the morning hours to make it suitable for Bengalee. But the meaning of the questions or scoring was not altered. The Bengali versions (the common language spoken in West Bengal, an eastern province in India; Latitude 27 deg 6½ to 20 deg 47½) of these modified questionnaires were applied on 382 persons working in rotating shifts in health care units (hospitals and nursing homes) and on 494 non-shift workers engaged in the same field. The shift workers are engaged in rapidly rotating shifts, i.e., worker spend two or three successive days in same shift duty and then they rotate to another shift. The morning shift in health care units is from 07:00 or 08:00 to 13:00 or 14:00 hours, the afternoon shift is form 13: 00 or 14:00 hours to 19:00 or 20:00 hours and the night shift is from 19:00 or 20:00 hours to 07:00 or 8:00 hours. The non-shift workers engaged in general shift from 09:00 hours to 17:00 hours. Among them 300 shift workers and 400 non shift control subjects completed all the questionnaires. Actual and preferred wake up time, bed times in holidays and working days, sleeping hours, daily habits, life style and other personal profiles were also noted. The nature of the study was explained to all subjects and the Bengali versions of the three questionnaires were administered in three consecutive days randomly one questionnaire at a time.

For statistical analysis, Pearson's product moment correlation coefficients were calculated and significant levels were studied by 't' test (Das and Das, 1993).

Results and Discussions :

Indian subjects felt difficulties to answer CTQ. In this questionnaire visual analog scale was used for response, two ends of the scale

Table 1: Time taken by the Bengalese to answer different questionnaires

Group	Time taken in minutes		
	MEQ	CTQ	CMQ
Shift workers (N = 300)	10.7 ± 3.06 (6 - 16)	16.4 ± 4.93 (12 - 30)	9.2 ± 2.06 (5 - 12)
Non-shift workers (N = 400)	9.3 ± 2.98 (6 - 15)	14.4 ± 3.73 (10 - 20)	8.4 ± 2.28 (7 - 11)

Values: Mean ± SD (Figures in the parenthesis indicate the range)

Table 2 : Percentage of Bengalee people classified in to different chronotypes by different questionnaires (Using original score and classification)

Chronotyp	MEQ		CTQ		CMQ	
	Shift Workers (N = 300)	Non-Shift Workers (N=400)	Shift Workers (N=300)	Non-Shift Workers (N=400)	Shift Workers (N=300)	Non-Shift Workers (N=400)
Definite Morning	12.0	10.25				
Morning	(67.33)	(44.00)	68.00	69.75	37.67	31.25
Moderate Morning	55.33	33.75				
Neither	31.67	54.00				
Intermediate	(31.67)	(54.00)	27.33	25.25	61.00	63.75
Moderate Evening	1.0	2.00				
Evening	(1.0)	(2.0)	4.67	5.00	1.33	1.25
Definite Evening	0.0	0.0				

(Figures in the parentheses are calculated values for comparison)

indicate two extreme responses. subjects (shift working and control) take long time to response to that type scale. One of the important aspects of the effectiveness of the questionnaire in the field study is its length. The CMQ is shorter and easier to answer for the Bengalese than MEQ and CTQ. The time taken by subjects to answer each questionnaire is given in Table-1.

From the table, it was observed that average time taken to answer CMQ by the Bengalee subjects is least compared to other two questionnaires. Shift workers take long time (mean 16.4 minutes) to answer CTQ.

Horne and Ostberg (1976), classified people into five chronotypes i.e., 'Definite morning', 'Moderate morning', 'Neither', 'Moderate evening' and 'Definite evening'. But Folkard

Table 3 : Percentage of Bengalee population classified in different questionnaires (Using original score and modified classification)

Chronotyp	MEQ		CTQ		CMQ	
	Shift Workers (N = 300)	Non-Shift Workers (N=400)	Shift Workers (N=300)	Non-Shift Workers (N=400)	Shift Workers (N=300)	Non-Shift Workers (N=400)
Morning	15.33	14.25	13.33	20.25	14.67	16.25
Intermediate	68.00	66.75	67.67	62.25	71.00	70.50
Evening	16.67	19.00	19.00	17.50	14.33	13.25

et al. (1979), and Smith et al. (1989), classified people into three chronotypes, i.e., 'Morning', 'Intermediate' and 'Evening'. The results obtained with the original classification for the chronotypes of people by different questionnaire techniques are given in table 2.

From table 2, it was observed that when the original classifications of Smith et al., (1989), was applied on Bengalee people (both the shift working group and non shift workers), most of them belonged to classify either as 'morning' or as 'intermediate' chronotypes. Only of 1.25% of non shift workers and 1.33% shift workers was identified as the 'evening' chronotypes. When the original scoring of Horne and Ostberg was applied, none of the shift working personnel was identified as the 'definite evening' chronotypes. A few control subjects (2.0%) were identified as 'evening' chronotypes. In the classification of Folkard et al., only a few shift working personnel were identified as 'evening' chronotypes.

There are differences between socio-economic, habitual, cultural and other conditions between Indian and Westerners in cold countries. Indians prefer to rise earlier in the morning and go to bed earlier. Since,

the classification of different chronotypes of people by different questionnaires were based on the studies on Western people, the original classification may not be suitable in Indian context. When it was applied on people of warm countries like India, morning orientation was observed. The questionnaires on individual variations in activity timing and other behaviors should be classified relative to the Indian conditions. So, the original scoring procedures of all the questionnaires were followed but the method of classifications of chronotypes was redesigned. In this modified method, the personnel whose score were more or less than the value of one standard deviation from the mean of the sample were classified as 'morning' and 'evening' chronotypes respectively. The personnel with the score in between classified as 'intermediate' type. The results with these modified classifications are given in Table 3.

It was observed from the Table 3, that when modified classification of MEQ was used 16.67% of the shift workers and 19.00% of non-shift workers were found to be 'morning' chronotypes. Similar types of results were obtained from modified classification methods. From these results, it may be concluded that every morningness

questionnaire should be reclassified according to the specific context of the country.

To evaluate psychometric properties of these three circadian questionnaires, inter-item correlation coefficients of these questionnaires and frequency distribution of total scores in Bengalee population were studied. Inter-item correlation coefficients of MEQ are presented in Table 4.

From the Table 4, it was observed that most of the coefficients were moderate and positive, ranged from -0.020 to + 0.618, and out of 171 coefficients, 97 coefficients were statistically significant ($p < 0.05$), among which 43 correlations were highly significant ($p < 0.001$).

Inter-item correlation coefficients of CTQ are presented in Table 5; Folkard *et al.* (1979) analyzed CTQ in terms of these separate components (i.e., rigidity, vigourisity and morningness). In the present study both the total 19 items scale and three sub scales were examined according to the method of Smith *et al.* (1989).

From the Table 5 it was observed that many of the inter-item correlations were low, often very close to zero, and many were negative. The coefficients range from -0.246 to +0.688, and out of 171 inter-item correlation coefficients only 39 coefficients were statistically significant ($p < 0.05$), among which 12 were highly significant ($p < 0.001$). The results indicated that the items were not sufficiently internally consistent. Inter-item correlation coefficients of CMQ in Indian population are presented in Table 6.

From the Table 6, it was observed that all the coefficients were positive with range from +0.138 to 0.706. Most of the coefficients

were moderate and statistically significant. Out of 78 coefficients, 73 were significant ($p < 0.05$) and 57 coefficients were highly significant ($p < 0.001$). This indicated that among the three questionnaires, CMQ showed best internal consistency.

Correlation coefficient of total scores of MEQ and CTQ was +0.349; and that of CTQ and MEQ was +0.397. But correlation coefficient between MEQ and CMQ was +0.829. As CMQ consists of 9 items of MEQ so, it is expected that high correlation exists between these two questionnaires.

It was observed that coefficients alpha of CMQ was very high (0.86). Correlation coefficients (r) with other factors like preferred arise time ($r = -0.72$), preferred bed time ($r = -0.66$), normal arise time ($r = -0.74$) and normal bed time ($r = 0.59$) were high and statistically significant ($p < 0.05$). These revealed that reliable differences existed between morning and evening oriented personnel on actual and preferred arise and bed time. That is, these results indicated that the more the morning coefficient of a person (indicated by high score in CMQ), the more the preference of the subjects to rise and go to bed early.

In a multinational study, Smith *et al.* (2002) observed that Indian people are more morning oriented than those of cold western country, like, England, Holland, Spain and USA. The possible explanation for the extreme morning orientation of Indian subjects may be due to the cultural and geographical area of inhabitancy. As they live in warm tropical climate, where the environmental temperature is very high during the noon time, especially during the summer

**Table 4: Inter- item correlation coefficients of MEQ
(in Bengalee population)(N = 700)**

ITEM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1																				
2	0.457*																			
3	0.234*	0.168																		
4	0.385**	0.242*	0.259*																	
5	0.298**	0.259*	0.194*	0.436**																
6	0.187*	0.159	0.095	0.186*	0.248*															
7	0.365**	0.208*	0.129	0.512**	0.618**	0.218*														
8	0.246*	0.234*	0.156	0.126	0.185	0.048	0.097													
9	0.347**	0.229*	0.256*	0.385**	0.284**	0.137	0.242*	0.154												
10	0.248*	0.376**	0.072	0.134	0.142	0.068	0.172*	0.133	0.164											
11	0.481**	0.261*	0.148	0.184*	0.317**	0.156	0.320**	0.136	0.311**	0.252*										
12	0.124	0.284**	0.071	0.029	0.061	-0.009	-0.020	0.249	0.101	0.314**	0.168									
13	0.331**	0.161	0.322**	0.287**	0.184*	0.016	0.197*	0.172*	0.281**	0.094	0.253*	0.038								
14	0.274**	0.264*	0.094	0.181*	0.179	0.075	0.194*	0.164	0.156	0.191*	0.242*	0.121	0.198*							
15	0.289**	0.178*	0.103	0.270*	0.234*	0.004	0.226*	0.106	0.337**	0.132	0.321*	0.082	0.187*	0.174*						
16	0.128	0.184*	0.039	0.137	0.083	0.105	0.082	-0.015	0.007	0.208*	0.137	0.141	0.071	0.143	0.026					
17	0.348**	0.186*	0.041	0.256*	0.182*	0.107	0.220*	0.084	0.236*	0.174*	0.349**	0.078	0.179*	0.142	0.245*	0.085				
18	0.274**	0.286**	0.117	0.210*	0.116	0.105	0.236*	0.143	0.152	0.124	0.317**	0.074	0.136	0.138	0.223*	0.102	0.204*			
19	0.494**	0.421**	0.441**	0.412**	0.402**	0.154	0.384**	0.168	0.374**	0.298**	0.451**	0.456**	0.186*	0.306**	0.342**	0.358**	0.218*	0.296**		

**Table 5: Inter item correlation coefficients of CTQ
(in Bengalee population)(N=700)**

ITEM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1																				
2	0.238*																			
3	0.124	0.092																		
4	-0.001	0.002	0.353**																	
5	0.688**	0.238*	0.185*	0.211*																
6	0.179**	0.285**	-0.087	-0.234*	0.192*															
7	-0.019	-0.012	-0.072	-0.001	0.008	0.228*														
8	0.067	0.164	-0.184*	0.181*	0.046	0.307**	0.049													
9	-0.153	-0.093	-0.069	0.068	-0.072	0.113	0.038	-0.036												
10	-0.091	0.026	-0.023	0.001	-0.056	-0.045	0.063	-0.062	0.205											
11	-0.030	0.003	-0.037	-0.003	0.051	0.114	0.135	-0.073	0.382**	0.172*										
12	-0.153	-0.198*	0.039	0.164*	-0.037	-0.212	0.031	-0.246*	0.285**	0.212*	0.211*									
13	-0.112	-0.121	-0.053	0.059	-0.062	0.127	-0.059	-0.090	0.276**	0.179*	0.180*	0.291**								
14	0.041	0.063	-0.033	-0.112	0.086	0.131	0.051	0.071	0.165	-0.158	0.232*	0.092	-0.065							
15	0.076	0.041	-0.051	-0.091	0.096	0.168	0.161	0.036	0.112	0.058	0.523**	0.062	0.007	0.281**						
16	0.008	0.135	0.028	0.021	-0.045	-0.043	0.121	0.042	-0.104	0.014	-0.129	-0.164	-0.008	0.219*	-0.178*					
17	0.110	0.296**	0.050	-0.089	0.176*	0.218*	0.029	0.128	-0.115	-0.004	-0.065	-0.184*	-0.235*	0.021	0.064	0.006				
18	0.067	0.142	-0.021	0.039	0.084	0.178*	-0.040	0.029	0.168	-0.022	-0.092	-0.221	-0.213	0.089	0.058	0.076	0.189*			
19	0.009	0.148	-0.059	-0.024	0.091	0.183	0.020	0.131	-0.009	0.015	-0.009	0.182	-0.134	0.012	0.076	-0.027	0.476**	0.087		

Table 6: Inter-item correlation coefficients of CMQ (In Bengalee population) (N=700)

ITEM	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0.450**												
3	0.385**	0.245*											
4	0.298**	0.259*	0.435*										
5	0.365**	0.218*	0.512**	0.619**									
6	0.347**	0.229*	0.385**	0.284**	0.242*								
7	0.248*	0.376**	0.138	0.141	0.173*	0.167							
8	0.481**	0.265**	0.148	0.317**	0.320**	0.311**	0.252*						
9	0.494**	0.421**	0.597**	0.412**	0.384**	0.374**	0.298**	0.451**					
10	0.552**	0.276**	0.339**	0.278**	0.283**	0.382**	0.194*	0.386**	0.224*				
11	0.426**	0.284*	0.421**	0.372**	0.331**	0.418**	0.142	0.364**	0.474**	0.519**			
12	0.259**	0.219*	0.423**	0.542**	0.438**	0.282**	0.169*	0.334**	0.281**	0.504**	0.307**		
13	0.475**	0.421**	0.448**	0.346*	0.364**	0.327**	0.247*	0.426**	0.706**	0.527**	0.424**	0.299**	

season, they prefer to work in the morning with habits to go to bed early.

CONCLUSION :

Among the three chronotypology questionnaires (MEQ, CTQ and CMQ), the translated version of CMQ was very useful as it is easily understandable by the Bengalee (Indian) people. It was found to be short and easy to get quick and reliable response. This questionnaire has better psychometric properties as shown by inter item correlation coefficient in Bengalee responses. But, its classification procedure should be modified according to Indian response for evaluation of morningness in Indian subject. CMQ was also well correlated with some external factors (like waking time, bed time, etc.). So, it can be used after having language translation and context specific modifications for the use of heterogeneous language and work culture population groups in India.

ACKNOWLEDGEMENT :

The author wish to convey deep gratitude to all the subjects, who volunteered for the study and gladly given consent.

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CONTROL OF *EICHHORNIA CRASSIPES* THROUGH APPLICATION OF GRAMOXONE 24% S.L. PARAQUAT-DICHLORIDE AND ITS IMPACT ON FISH

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ABSTRACT ■ The present work deals with the control of aquatic weed (*Eichhornia crassipes*) through application of Gramoxone 24% s.l. paraquat dichloride with the help of non-ionic surfactant and its effect on fish production. The non-ionic surfactants do not have an electrical charge, which makes them resistant to water hardness deactivation. The most commonly used non-ionic surfactants are ethers of fatty alcohols. The efficacy and potential toxicity to non target organisms are to be assessed for a well administered management programme for aquatic weed control. The menace of aquatic weed is reaching alarming proportion in many parts of the world especially in tropical and subtropical zone where warm weather supports the growth of aquatic plants. So it is very much alarming to us and demands necessary control measures to check their growth as well as new area coverage. Optimum dose for field treatment was 9 ml Gramoxone (24% paraquat dichloride) and 1 ml surfactant per 1000 ml of water. It was not only effective in controlling 90% weed growth but also did not cause harm to non-target organisms. Outcome of this experiment would help in controlling the excessive growth of *Eichhornia crassipes*.

Keywords : Gramaxone, Surfactant, Fish production, *Eichhornia crassipes*.

INTRODUCTION :

Water is one of the most important natural resources. With its dissolved nutrient and gases, it forms a wonderful medium for the growth of plants. The presence of plants in water bodies is very important. Aquatic plants are the main source of biomass production in aquatic ecosystem

(Esteves, 1998). They conserve solar energy as chemical energy for the development of food chain of aquatic fauna. Furthermore aquatic vegetation helps in reducing the pollution load of water bodies by absorbing several pollutants. But the excessive growth of these plants may cause a number of inconveniences related

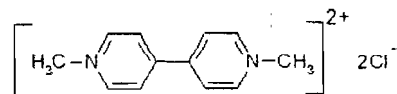
to water supply, transportation, fishing, energy production and proliferation of disease among others from environmental point of view. Some fast growing species may suppress other less aggressive species desirable to maintain diversity, or may negatively modify some of the physical characteristics of water (Cardoso *et. al.*, 2003).

The extent of aquatic weed problem in India can be realized from the fact that water hyacinth [*Eichhornia crassipes* (Mart.) Solms] alone accounts for 0.5 million hectare. At present water hyacinth has spread over 200000 ha of water surface in India. All these facts necessitate immediate control of aquatic weed. Several control measures are there like manual, biological, chemical etc. Manual control is very costly and biological control, though eco-friendly, takes longer time. Use of weedicide is effective and economical. However in summer time, India receives 22 inches of rainfall per month and the weedicides are washed away. Again the weedicides do not spread uniformly due to different topography of leaves. The use of surfactant may reduce the negative influence presented by these leaf structures. Lastly, efficacy and potential toxicity to non-target organisms are to be assessed for a well administered management programme for aquatic weed control.

Materials and Methods :

The experimental work was carried out at 0.8028 ha pond of East Kolkata Fishermen Co-operative Society, Kolkata, West Bengal, India and this study focused on to find out the efficacy of surfactants

and its effect on fish. The chemical structure of Gramoxone 24% s.l. paraquat dichloride is:



And chemical name is : (1,1'-dimethyl-4,4'-bipyridiumion) dichloride anion. It is a colourless crystal, water soluble and breaks down at 300 °C. The most commonly used non-ionic surfactants are ethers of fatty alcohols and they do not have an electrical charge, which makes them resistant to water hardness deactivation.

The chemical structure is:

Non Ionic Surfactants



The experimental fish species was *Cirrhinus mrigala* (common name mrigal). The chemical parameters of pond water like pH, temperature, transparency, dissolved oxygen, free carbon di-oxide and alkalinity were determined using the methods developed by APHA, 2005.

The spraying solution of weedicide was prepared by mixing of gramaxone S.I. paraquat dichloride and water at the ratio of 0.009: 1 i.e. 9ml weedicide mixed with 1 litre water and sprayed with the help of KNAPSACK SPRAYER. The capacity of sprayer was determined by spraying in a small area. Five litre water was sprayed with a KNAPSACK SPRAYER in 100 sq. m area. Rate of spraying was 30 metre/min and width of sprayer was 1 meter. Therefore, in 1 ha pond, 400 litre water could be sprayed.

Table - 1

Date and Time	Dose / Ratio	Period of drying of <i>Eichhornia crassipes</i>	Effect on fish
12/04/09 4.00 pm	20 ml of weedicide only with hand sprayer	<i>E. crassipes</i> started drying from next day and completely dried within 7 days	3 mrigal species was introduced and all were found dead next morning.
20/04/09 4.00 pm	9.1 ratio with hand sprayer	<i>E. crassipes</i> dried within 3 days and started decomposing from 6th day.	Out of 3 mrigal species introduced one was dead and the other two died after 1 day.
5/05/09 4.00 pm	5.5 ratio with hand sprayer	<i>E. crassipes</i> dried from next day and completely dried within 5 days	Out of three mrigal species introduced three died within next afternoon

Table-2

Date	Air Temperature °C	Water Temperature °C	pH	Transparency	DO ppm	Alkalinity ppm
5/5/09	33.5	31.0	8.2	21	3.0	88.0
12/5/09	35.0	31.0	8.2	22	4.2	88.0
19/5/09	33.0	31.0	8.0	22	3.6	86.0
26/5/09	33.5	31.0	8.1	20	3.3	88.0
31/5/09	32.5	31.0	8.1	21	4.1	85.0
5/6/09	33.0	32.0	8.2	20	4.8	87.0

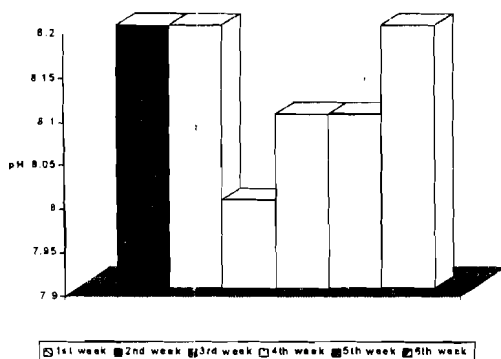


Figure-1: Variation of pH with 7 days interval



Figure-2: Variation of dissolved oxygen with 7 days interval

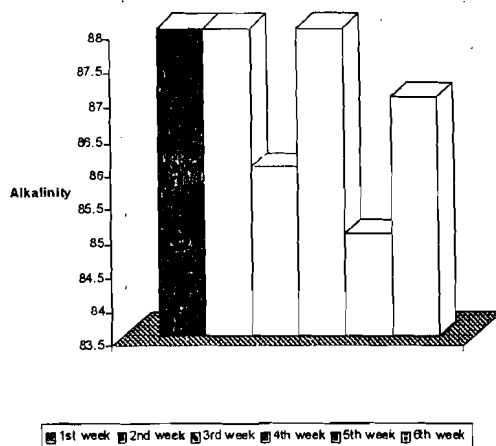


Figure-3: Variation of alkalinity with with 7 days interval

In 0.8028 ha pond, $(400 \times 0.8028) = 321.12$ litre of water was sprayed. Since the concentration of weedicide in water is 0.009 : 1, the quantity of weedicide required can be calculated using following formula:

Total spraying solution x Concentration of active ingredient in spraying solution

= -----

Concentration of active ingredient in weedicide formulation.

$$= \frac{321.12 \times 0.009}{24} = 0.120 \text{ litre.}$$

24

Therefore, to obtain 0.009% active ingredient in spraying solution, 120.42 ml of 24% S.I. Gramoxone should be mixed in water [since the general average threshold toxicity to fish is 10ppm]. The exact proportion of weedicide and surfactant was determined by trial and error method. The growth of experimental fish was observed through length weight basis.

RESULTS :

The correct proportions of weedicide and surfactant, the trial and error method in a big size cemented cistern are presented in table-1.

From the above result it was indicated that high dose of weedicide or surfactant application was causing rapid fish death. But a lethal proportionate dose of 9:1 is not causing rapid mortality. Since in the cemented cistern the concentration of surfactant mixture sprayed is high, and the water volume and depth are low, it is obvious for fish to die. But when it is applied in a 0.8028 ha pond where the surface area is 86400 sq.ft, the mortality of fish did not take place due to dilution factor. So 9:1 dose can be recommended for pond to control overgrown *Eichhornia crassipes* for mass control.

The physico-chemical parameters of water during the experimental trial is presented in table-2. From the results, it is observed that air and water temperature, pH, transparency, dissolved oxygen and

alkalinity did not vary significantly.

The diagrammatic presentation of pH, dissolved oxygen and alkalinity of experimental pond is given in figure-1,2 and 3 respectively. These parameters showed moderate deviation with time.

The planktonic communities found in the pond during the experiment were green algae : Volvox sp., Chlamydomonas spp. Spirogyra sp.; Diatoms : Nitzschia spp, Cyclotella sp.; Bluegreen algae: Anabaena spp.; copepods: Cyclops cladocera: Daphnia spp.

DISCUSSION :

Many research works have been conducted over a long period of time for controlling aquatic weeds with chemical treatment. Chemicals have proved their efficiency but their effects on non-target organisms have not been assessed properly. Gramoxone [24% S.I. paraquat dichloride] is one of such chemicals on which research works are going on. Patnaik (1971) worked with Gramoxone to control Pistia stratiotes and concluded that 0.1-0.2 kg / ha i.e. with a dilution of 450 litre water / ha and addition of 0.1% detergent gave effective clearance. Again Esteves (1998) suggested that paraquat is non-toxic to fish at concentration even much greater than what is recommended for aquatic weed control. Analysis of results of this investigation revealed that application of Gramoxone along with surfactant is very much effective in controlling Eichhornia crassipes. The optimum dose selected for the field trial (i.e. 9ml Gramoxone and 1 ml surfactant /1000 ml of water) is not only

effective in controlling 90% weed growth but also does not show any signs of harm to the experimental fish species (*Cirrhinus mrigala*). Attalah and Salib in 1996 observed 75% mortality of *Eichhornia crassipes* using Gramoxone (20% S.I. paraquat dichloride) @360 gm a.i. / feddan. While performing field trial in pond of East Kolkata Fishermen Cooperative Society, where Gramoxone was mixed with non-ionic surfactant, 90% mortality of weed was recorded. Thus it can be concluded that addition of surfactant is capable of enhancing the efficacy of Gramoxone. Matuo and Nakamura (1989), Kirkwood (1993), Stock and Holloway (1993) have worked on surfactants. But little work has been done on their effects on fish. During standardizing the dose of surfactant in pond, haemoglobin level in fish blood was measured. Hematological studies revealed that when surfactant quantity increased there was rapid reduction in hemoglobin level. High dose of weedicide killed all fish, while lower dose killed lesser number of fish. However at low dose application, no fish death occurred. The water parameters were not affected by the application of surfactant. The results of physico-chemical parameters of water indicated that the Gramoxone application did not affect water quality.

ACKNOWLEDGEMENTS :

The authors are highly indebted to the members of East Kolkata Fishermen Co-operative Society, Kolkata, West Bengal, India for extending their full support to carry out the experimental work in their pond.

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A CROSS SECTIONAL STUDY OF PHYSICAL GROWTH AMONG THE GADA CHILDREN (5-12 YEARS) IN AN URBAN SLUM AREA OF CHHATTISGARH, INDIA

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ABSTRACT ■ Objective. The present study was conducted to evaluate the pattern of physical growth among the healthy Gada children in Chhattisgarh, India.

Methods. In this cross sectional study, sample consists of 342 children (176 boys and 166 girls), aged 5 to 12 years. The sample of the present study was collected from an urban slum area of Raipur W Chhattisgarh, India. Standard anthropometric measurements including linear, curvilinear and skinfold thickness were collected from each subject. Percentile curves were used to determine the pattern of growth.

Results. The nature of distance curves and percentile curves of the body measurements among the Gada children (boys and girls) showed increasing trends from 5 to 12 years of age with least sexual dimorphism. Utmost significant differences between sexes were observed in 11 years. The 50th percentile curves (median) of height and weight of Gada children were observed to be placed between 50th percentile curves of Indian and American children. The children in the current study were found to be significantly ($p < 0.01$) taller and heavier than Indian as well as Kamar tribal children of Chhattisgarh. Surprisingly, though the present children were shorter and lighter than American children but the differences were not significant in most of the age groups.

Conclusion. The growth pattern observed among Gada children in urban slum situation show better growth pattern compared to contemporary Indian children with respect to some anthropometric traits and in conformity with the growth pattern of socioeconomically higher urban children.

Key Words : Growth; Percentile; Gada; Chhattisgarh; India.

INTRODUCTION :

The young children are major segment of our community as they are potential parents in recent future. In this period of rapid growth, if we neglect their proper nourishment, it will not only influence their present state of health but also continue to

influence throughout their life¹. A deficiency or lack of required nutrients has now been identified as one of the key determinants of low birth weight (LBW) babies when the girls reach adulthood.² In order to resolve this problem, Government of India launched Integrated Child Development Services

(ICDS) programme since 30 years for early child development and nutrition intervention, which has expanded steadily across the country.³ Therefore, monitoring the pattern of physical growth of young children across a variety of populations and at different periods is undertaken to assess the present state of nutritional status, health and socioeconomic conditions in addition to understand the outcome of ICDS programme.

The assessment of pattern of growth is based on a set of standard physical or anthropometric measurements.⁴ Such studies have been done in different parts of India,⁵⁻⁸ where the pattern of growth is basically attributed to the impact of better living conditions, better nutrition and improved medical facilities as well as early biological maturation among young children.⁹⁻¹¹ However, the present study is concentrated among the Gada children.

The Gada is a scheduled caste (*Dalit*) community in central India. They are living in different slums of Raipur City of Chhattisgarh. Though they are living in so called "Urban Slum" area but their socioeconomic condition are much better compared to other neighbouring communities. In order to understand this situation, physical growth study among young children may reveal this condition. Therefore, the aim of the present study is to evaluate physical growth pattern among the healthy Gada children in Chhattisgarh.

MATERIALS AND METHODS :

Area and subjects :

The sample of the present cross sectional study was collected from an urban slum area of Raipur, Chhattisgarh, India. They get the benefit of urban amenities of better accessibility to health and awareness

of disease prevention. This should reflect in their physical growth and health status. We have undertaken a comprehensive health status and medical check up of the children by a team consists of Doctor and Anthropologist. The anthropometric part of the study describing the growth pattern of the children is reported here.

The sample consists of 342 children (176 boys and 166 girls), aged 5 to 12 years. They are belonged to low caste group (scheduled caste) of the Gada community from Raipur. Assessment of age is most essential for conducting growth studies. The accurate age of the children was recorded from the *Anganbari and School* registration books and family sources.

Anthropometric measurements :

Altogether 11 anthropometric measurements were for each children participant of the study as per the IBP recommendations.¹² These surements include: height, weight, sitting height, biacromial breadth, biilliocrystal breadth, chest circumference, arm circumference, head circumference, thigh circumference and skinfold thickness of triceps and subscapular.

Portable weighing machine and anthropometer, tape, spreading caliper and GPM skinfold caliper were used for various standard anthropometric measurements.

Statistical analysis :

Descriptive statistics like mean, standard deviation and percentile were used to understand the age-wise growth patterns and t-test was used to understand the significant differences of anthropometric measures between sexes and from other standards.

RESULTS :

The mean anthropometric traits of height, weight, sitting height, biacromial breadth, biilliocristal breadth, circumferences of chest, arm, head, and thigh as well as skinfold thickness of triceps and subscapular of the Gada children are shown in tables 1 and 2. The distance curves of height and weight showed a gradual increase along with increasing age. Similar pattern was observed in case of biacromial and biilliocristal breadths as well as different circumference measurements. On the other hand, triceps, and subscapular skinfolds however, show fluctuating growth patterns. Sex - wise differences of anthropometric measurements are reported in table 3. It was observed that the Gada boys have higher anthropometric values compared to girls in all through the ages except few cases. However there was lowest significant differences existed between sexes. Highest significant differences between sexes were observed in 11 years, whereas highest significant differences were found in case of thigh circumference between sexes.

Percentile growth curves

Figures 1 to 4 show a steady increase of height and weight (percentile) in different age groups among the Gada boys and girls. Comparison of 5th through 95th percentile height curves of boys (figure 1) indicate that about 90 percent of the 5 year old boys attained height between 107.32 cm and 112.78 cm while 90 percent of those at 12 years of age attained height ranges between 148.10 cm and 157.43 cm. Similarly, comparison of 5th through 95th percentile height curves of girls (figure 3) indicate that about 90 percent of the 5 year old girls attained height between 107.15 cm and 112.39 cm while 90 percent of those at 12

years of age attained height ranges between 147.18 cm and 154.37 cm.

In case of weight percentile comparison between different age groups, the 5th and 95th percentile of 5-year-old boys (Figure 2) showed that about 90 percent of their weights vary between 16.50 kg and 20.30 kg. It was evident from the percentile extremes that the increase in the 5th percentile between 5-12 years is 16.50 kg to 39.13 kg. While during the same age period the increase in 95th percentile was 20.30 kg to 45.69 kg. This indicated a gradual winding of the range variation with the increase of age from 5-12 years of age. Figure 4 also shows a steady increase of body weight percentile values from 5 to 12 years of age among the girls. The comparison of 5th through 95th indicated that 90 percent of 5-year-old girls had attained weight between 16.20 kg to 20.59 kg. This range goes up to 38.23 kg to 45.25 kg in 12 years of age.

Comparison of growth patterns among the Gada children and other standards

The height and weight data of the Gada children from an urban slum in the present study was compared with Indian¹³, American (NCHS)¹⁴ and with Kamar tribal children from the same region (tables 4, 5, 6 and 7).¹⁵ The 50th percentiles of height and weight (Figures 5,6, 7 and 8) of the Gada children (both boys and girls), when compared with the data for Indian children, was found to be higher in all the age groups whereas results were opposite, when compared with the data for American children, which was found to be lower in all the age groups. There was significant differences existed in height and weight between current studied children and Indian as well as Kamar children in all through the

TABLE 1. Means and standard deviations (SD) for anthropometric measurements of Gada boys by age and number

Age (Yrs)	n	Height (cm)		Weight (kg)		Setting height (cm)		Biacromial breadth (cm)		Billioicristal breadth (cm)		Chest circumference (cm)		Arm circumference (cm)		Head circumference (cm)		Thigh circumference (cm)		Skinfolds (mm)			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5+	23	110.20	1.89	18.69	1.20	55.43	1.17	23.20	2.06	18.77	0.71	54.86	1.25	15.45	0.58	49.79	0.86	27.46	0.69	9.07	1.62	7.33	1.42
6+	20	115.96	1.83	21.99	1.18	58.14	1.22	25.01	0.92	20.35	1.18	57.18	1.98	16.33	0.66	49.92	0.64	29.46	0.85	9.30	1.49	7.60	0.93
7+	21	122.17	2.26	24.55	1.33	61.91	2.43	26.44	0.78	22.21	0.98	60.77	1.37	17.99	0.87	50.17	0.71	31.65	1.07	9.43	1.43	7.93	1.12
8+	22	128.20	2.18	27.28	1.13	64.20	1.29	27.99	0.95	23.46	1.19	63.61	1.18	19.09	0.80	50.26	0.59	33.20	0.93	9.08	1.26	7.81	1.14
9+	22	134.30	1.97	30.45	1.26	67.11	1.04	29.92	0.98	25.46	1.27	66.65	1.42	19.96	0.98	50.55	0.60	33.69	0.96	8.81	1.45	7.93	1.33
10+	23	140.01	2.04	33.83	1.31	70.16	1.18	30.95	1.27	26.43	0.93	69.53	1.32	20.09	1.77	50.23	0.85	36.08	1.17	8.91	1.44	8.42	1.51
11+	23	145.72	2.29	37.03	1.95	73.63	1.23	33.03	1.29	28.60	1.37	70.75	0.98	20.54	1.44	50.43	1.01	38.17	1.13	9.13	1.34	7.82	1.04
12+	22	151.83	2.83	42.35	2.09	75.73	1.13	33.85	1.25	29.40	1.08	72.93	1.22	20.78	1.09	50.33	0.90	39.38	1.79	8.54	1.05	7.91	1.00

TABLE 2. Means and Standard deviations (SD) for anthropometric measurements of Gada girls by age and number

Age (Yrs)	n	Height (cm)		Weight (kg)		Setting height (cm)		Biacromial breadth (cm)		Billioicristal breadth (cm)		Chest circumference (cm)		Arm circumference (cm)		Head circumference (cm)		Thigh circumference (cm)		Skinfolds (mm)			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5+	20	110.14	1.53	18.14	1.32	55.32	0.82	23.45	1.15	19.14	1.16	54.14	1.68	15.45	0.95	48.87	1.22	25.93	1.77	8.36	1.04	7.25	1.37
6+	21	115.54	1.89	21.28	1.31	57.62	1.02	23.91	1.86	19.31	1.93	57.71	1.27	16.11	1.21	50.12	0.71	28.57	1.58	7.98	1.23	7.71	1.08
7+	20	121.74	2.31	23.85	1.81	60.98	1.25	25.96	1.21	21.42	1.01	59.78	3.27	17.26	1.49	49.74	0.76	27.56	1.70	8.28	1.27	7.69	0.98
8+	21	128.15	2.27	26.61	2.15	63.57	1.78	27.50	1.62	23.09	1.94	62.73	1.81	18.67	1.80	49.54	0.94	29.09	1.34	8.60	1.34	7.99	1.22
9+	22	133.69	2.26	30.05	1.80	66.82	1.21	29.18	1.73	24.92	1.96	65.68	2.52	19.57	1.88	50.91	0.81	30.29	1.42	9.59	1.18	8.50	1.07
10+	22	139.08	2.51	32.29	1.56	69.81	1.82	30.06	1.15	25.91	1.19	69.15	2.28	19.77	1.40	50.31	1.08	31.77	2.76	9.18	1.27	8.39	1.67
11+	20	143.06	2.22	35.11	2.34	72.16	1.23	32.67	1.69	28.69	1.46	70.36	1.78	19.52	1.71	50.13	1.69	32.03	2.74	8.26	1.03	8.28	1.12
12+	20	151.35	2.00	41.65	1.78	75.47	0.98	33.23	1.43	28.89	1.23	71.12	2.05	20.08	2.32	51.21	1.04	31.72	2.91	9.19	1.36	8.25	1.26

TABLE 3. Test of significance of various anthropometric measurements between the Gada boys and girls

Age (Yrs)	Height	Weight	Setting height	Biacromial breadth	Billiocrystal breadth	Chest circumference	Arm circumference	Head circumference	Thigh circumference	Triceps	Subscapular
5+	0.12	1.44	0.36	-0.48	-1.28	1.61	-0.01	2.90**	3.84**	1.69	0.22
6+	1.22	1.92	1.67	2.46*	2.21*	-0.98	0.89	-0.97	2.36*	3.17**	-0.43
7+	0.60	1.41	1.54	1.52	2.55*	1.28	1.93	1.90	9.28**	2.72*	0.73
8+	0.07	1.27	1.32	1.22	0.77	1.90	1.01	3.04**	11.70**	1.19	-0.52
9+	0.95	0.84	0.84	1.73	1.07	1.57	0.85	-1.69	9.27**	-1.81	-1.57
10+	1.36	3.59**	0.76	2.46*	1.62	0.70	0.67	-0.32	6.85**	-0.65	0.09
11+	3.86**	2.92**	3.92**	0.79	-0.21	0.91	2.14*	0.74	9.84**	2.38*	-1.41
12+	0.64	1.17	0.82	1.50	1.44	3.50**	1.27	-2.92**	10.37**	-1.72	-0.96

*p<0.05, **p<0.01

TABLE 4. Comparison of height of Gada boys with other data

Age (Yrs.)	A Present Study		B Indian boys (ICMR, 1972)		t- test (A and B)	C NCHS boys (Frisaache, 1999)		t- test (A and C)	D Kamar tribe (boys) (Mitra, 2002)		t- test (A and D)
	n	Mean	n	Mean		n	Mean		n	Mean	
5+	23	110.2	3484	102.1	4.85**	675	112.6	2.17*	40	98.8	10.93**
6+	20	116.0	3816	108.5	5.09**	298	119.2	2.35*	23	105.0	8.86**
7+	21	122.1	3711	113.9	4.37**	312	125.1	2.39*	23	108.5	9.12**
8+	22	128.2	3957	119.3	5.72**	296	129.8	1.19	22	114.7	9.14**
9+	22	134.3	3873	123.7	5.40**	322	135.8	1.21	25	120.6	10.26**
10+	23	140.0	4065	124.4	7.56**	334	140.9	0.62	24	127.1	12.84**
11+	23	145.7	3879	133.4	6.08**	324	146.4	0.45	20	129.4	15.96**
12+	22	151.8	4052	138.3	6.27**	349	152.2	0.23	26	132.5	22.46**

*p<0.05, **p<0.01

TABLE 5. Comparison of height of Gada girls with other data

Age (Yrs.)	A Present Study		B Indian girls (ICMR, 1972)		t- test (A and B)	C NCHS girls (Frisancho, 1990)		t- test (A and C)	D Kamar tribe (girls) (Mitra, 2002)		t- test (A and D)
	n	Mean	n	Mean		n	Mean		n	Mean	
5+	20	110.1	3221	101.4	5.33**	673	112.0	1.57	31	97.6	13.34**
6+	21	115.5	3665	107.4	4.32**	296	118.3	2.28*	20	103.7	9.03**
7+	20	121.7	3603	112.8	4.74**	331	124.2	1.85	23	108.0	11.25**
8+	21	128.1	3487	118.2	5.89**	276	129.8	1.29	19	112.6	15.10**
9+	22	133.6	3556	122.9	6.69**	322	135.7	1.36	22	115.9	17.51**
10+	22	139.0	3646	128.4	6.21**	330	141.5	1.58	21	121.4	14.63**
11+	20	143.0	3442	133.6	4.47**	303	148.1	2.77**	20	126.9	11.79**
12+	20	151.3	3426	139.2	5.30**	324	154.6	2.04*	29	130.6	20.45**

*p<0.05, **p<0.01

TABLE 6. Comparison of weight of Gada boys with other data

Age (Yrs.)	A Present Study		B Indian boys (ICMR, 1972)		t- test (A and B)	C NCHS boys (Frisancho, 1990)		t- test (A and C)	D Kamar tribe (boys) (Mitra, 2002)		t- test (A and D)
	n	Mean	n	Mean		n	Mean		n	Mean	
5+	23	18.6	3484	14.8	8.28**	676	19.9	2.07*	40	10.5	21.08**
6+	20	22.0	3816	16.3	10.04**	298	22.6	0.74	23	13.1	16.72**
7+	21	24.5	3711	18.0	9.92**	312	25.1	0.65	23	15.9	16.15**
8+	22	27.2	3957	19.7	10.66**	296	27.7	0.45	22	17.1	17.94**
9+	22	30.4	3873	21.5	9.48**	322	31.3	0.67	25	19.3	15.77**
10+	23	33.8	4065	23.5	9.49**	334	35.4	0.98	24	20.0	21.51**
11+	23	37.0	3879	25.9	8.45**	324	39.8	1.33	20	22.8	17.19**
12+	22	42.3	4052	28.5	10.61**	349	44.2	0.80	26	24.8	23.88**

*p<0.05, **p<0.01

TABLE 7. Comparison of weight of Gada girls with other data

Age (Yrs.)	A Present Study		B Indian girls (ICMR, 1972)		t- test (A and B)	C NCHS girls (Frisancho, 1990)		t- test (A and C)	D Kamar tribe (girls) (Mitra, 2002)		t- test (A and D)
	n	Mean	n	Mean		n	Mean		n	Mean	
5+	20	18.1	3221	14.5	6.99**	674	19.5	1.95	31	11.1	22.74**
6+	21	21.2	3665	16.0	9.15**	296	21.8	0.76	20	13.5	18.26**
7+	20	23.8	3603	17.6	9.55**	331	24.7	0.89	23	16.0	16.92**
8+	21	26.6	3487	19.4	10.29**	276	28.1	1.08	19	16.8	18.19**
9+	22	30.0	3556	21.3	11.02**	322	32.0	2.07*	22	19.1	19.53**
10+	22	32.2	3646	23.6	7.07**	330	35.7	1.95	21	20.5	21.10**
11+	20	35.1	3442	26.4	7.07**	303	41.8	2.72**	20	22.1	19.07**
12+	20	41.6	3446	29.8	7.99**	324	47.1	2.29*	29	23.6	21.28**

*p<0.05, **p<0.01

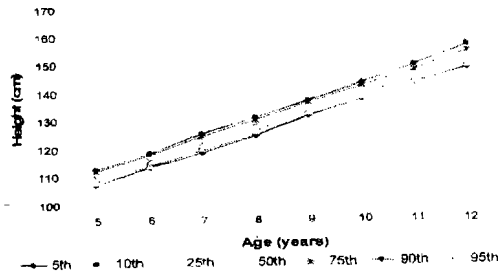


Fig.1. Percentile curve of height (cm) among Gada boys

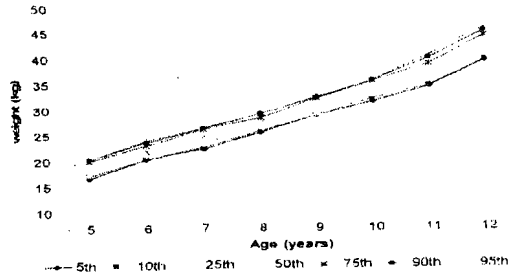


Fig.2. Percentile curve of weight (kg) among Gada boys

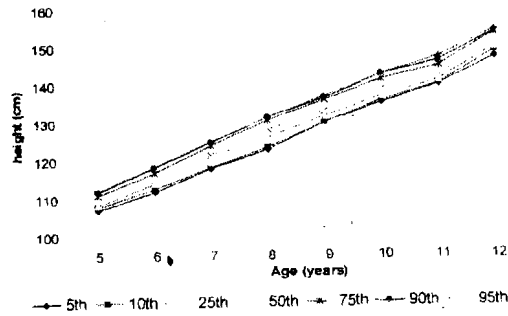


Fig.3. Percentile curve of height (cm) among Gada girls

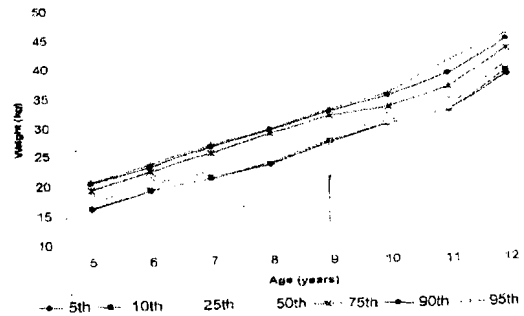


Fig.4. Percentile curve of weight (kg) among Gada girls

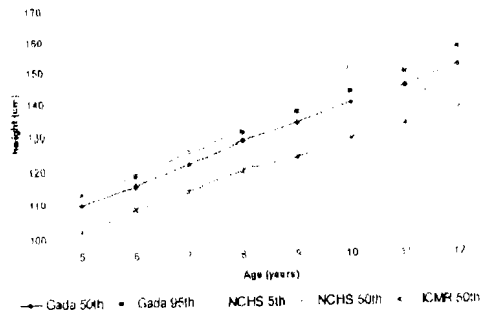


Fig. 5. Comparison of height (cm) of Gada boys with other standards

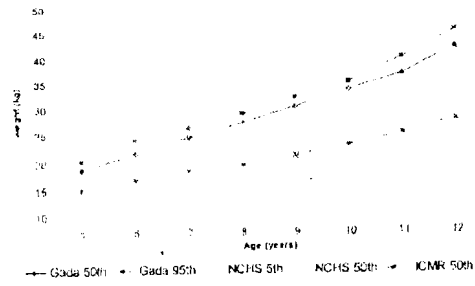


Fig. 6. Comparison of weight (kg) of Gada boys with other standards

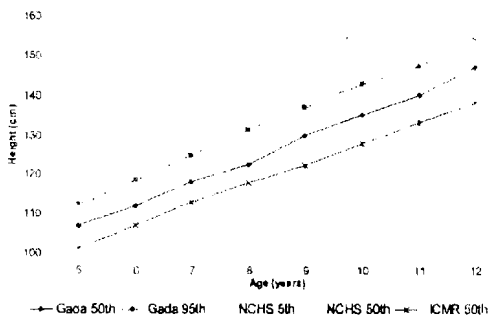


Fig. 7. Comparison of height (cm) of Gada girls with other standards

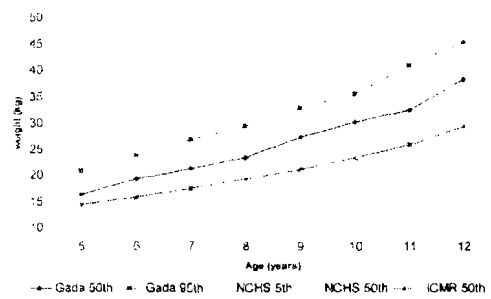


Fig. 8. Comparison of weight (kg) of Gada girls with other standards

ages. Surprisingly, though the present children have lower values of height and weight than American children but the differences were not significant in most of the age groups.

DISCUSSION :

Analysis of the various anthropometric measurements of growth status among the Gada children living in an urban slum of Raipur City from Chhattisgarh and nature of distance curves revealed that the mean values of different body measurements represent more or less an increasing trend with the advancement of age. The rate of increase was however, not uniform for all the characteristics. Whatever the genetic component of these characteristics, the environment has an important influence, particularly in weight and skin-fold thickness characteristics.¹⁶ Interestingly, there was lowest sexual dimorphism of the anthropometric measurements and highest significant differences between sexes were observed in 11 years. This mainly corresponded to the stage of adolescent growth spurt, where boys were taller and heavier compared to girls. The percentile values of height and weight among the Gada children showed increasing trends with increasing age which also corresponds to other children of rural lower and middle class family.¹⁷ The 50th percentile curves (median values) of height and weight of Gada children were observed to be placed between 50th percentile curves of Indian and American children. However, tallness and heaviness of the current studied children was much closer to American children compared to Indian children. It may be attributed to the impact of better living conditions, better nutrition and improved medical facilities as well as early biological

maturation among young children⁹⁻¹¹, and changes in environmental and socioeconomic factors as reflected in the form of secular trend^{11,16, 18-20}, which may be initiated with the help of ICDS programme in the studied urban slum areas. The children in the current study were found to be significantly ($p < 0.01$) taller and heavier than Indian as well as Kamar tribal children of Chhattisgarh. Surprisingly, though the present children were shorter and lighter than American children with lowest significant differences.

CONCLUSION :

The results suggest that the growth pattern observed among Gada children in urban slum situation show better growth pattern compared to contemporary Indian children with respect to some anthropometric traits and in conformity with the growth pattern of socioeconomically higher urban children. It will be interesting to investigate after 30 years of intervention of ICDS programme whether similar results are observed in urban slum situation in other parts of India.

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Author's contributions :

MM, GPD were responsible to formulate study design and collected the data and SC and PB were responsible to analyze and formulate the first draft and final version of the paper.

Funding source : None

MENOPAUSE : A STUDY ON THE PERI AND POSTMENOPAUSAL WOMEN OF URBAN WEST BENGAL

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ABSTRACT ■ The present study is an attempt to find out variations in some typical menopausal symptoms, and in the perception and attitude towards menopause between perimenopausal and postmenopausal women. Data were collected from 200 Bengali speaking Hindu women (postmenopausal=100, perimenopausal=100) of West Bengal. A structured schedule was used to collect data on sociodemographic aspect, menopausal symptoms and perception and attitude of the participants towards menopause. Results show that significant differences exist between women of differential menopausal status in the incidence of two typical menopausal symptoms (vasomotor and vaginal) but the perception and attitude of these women towards menopause are largely similar.

Key words : Menopause, Urban, Women.

INTRODUCTION :

In the first International Congress on Menopause (Campbell, 1976), there was a consensus that climacteric is a stage when women move from the reproductive to non-reproductive phase. The World Health Organization (WHO) (1996) refers this stage of a woman's life as perimenopausal, a phase immediately prior to menopause, when the menstrual cycle becomes erratic. This period generally lasts for 2-3 years after which a woman stops menstruating for ever. A woman is considered to have attained menopause when this non-menstruating phase continues consecutively for a period of 12 months.

Both the perimenopausal and postmenopausal stages of women's life are typified by the prevalence of some menopausal symptoms. Findings from different studies show that the prevalence of these symptoms is not similar between perimenopausal and postmenopausal women (Tang, 1994; Nagar and Dave, 2005; Sharma, 2007). Moreover, irrespective of the menopausal status, these problems differ both in their types and magnitude among various sociocultural and ethnic groups (Bernis & Reher, 2007; Damodaran et al., 2000; Dennerstein et al., 2002; Frackiewicz & Cutler, 2000; Gelfand, 2000; Malacara et al., 2002). For example, the frequency of vasomotor, vaginal and psychosomatic

problems is high among the postmenopausal women of Mexico and Greece (Malacara et al., 2002; Beyne, 1986) compared to that of the Japanese women (Gold et al., 2004). Again, another study on perimenopausal women of Japan revealed that the incidence of psychosomatic, vasomotor and psychological problems is high in this population (Lock and Kaufert, 2001). Similar findings related to vasomotor problems have also been reported from Turkish women who are in the transitional state (Uncu et al., 2007)

A number of factors that are inherent in the prevailing sociocultural paradigm play a role in the experience of menopause. Several studies have reflected the association of culturally influenced behaviors (diet, reproductive performance, smoking), people's attitude towards and expectation about the menopause (Martin, 1988; Avis and McKinlay, 1991; Sommer *et al.*, 1999), marital status (Avis et al., 2004), education (Avis et al., 1997) and meanings assigned to menopause (e.g., natural, deviant or illness (Estok and O'Toole, 1991) with menopausal health. In addition to it, woman's role in the society (which is shaped by the cultural context) sets the condition of responding to menopause (Beyne, 1986).

Studies also show that the perception of women towards menopause varies widely. There are some sociodemographic and sociocultural variables associated with the perception and attitude of women towards menopause (Qzumba et al., 2004; Uncu et al., 2007). For example, studies on Nigerian postmenopausal women reveal that they have a negative attitude towards menopause due to increased frequency of vasomotor problem and low family support (Qzumba et al., 2004). However, another study from Nigeria reflects that women perceive

menopause positively as it is a freedom from menstrual bleeding (Adequene, 2000). On the other hand, women of some Asian countries and of Jordan view menopause positively since this is liberation from reproduction (Maoz et al., 1970; Mahadeen et al., 2008). In contrast, the Iranian women have a negative attitude towards menopause since their society gives value to fertility (Khademi and Cooke, 2003). Women in developing countries tend to view menopause and related symptoms as a natural process and unaware about health related issue of menopause (Defey et al. 1996; Mashiloane, 2001; Wasti et al., 1993).

There are hardly any studies carried out on perimenopausal and postmenopausal women of India in general and of the State of West Bengal in particular. The present study is an attempt to find out-

1. the menopausal problems of perimenopausal and postmenopausal women and
2. the perception and attitudes of perimenopausal and postmenopausal women towards menopause.

Materials and Methodology :

The present study has been conducted on three districts of West Bengal – Howrah, Hoogly, and North 24 Parganas. These three districts are closely located to the city of Kolkata, the state capital. For the purpose of the study a total number of 200 women (100 perimenopausal and 100 postmenopausal) belonging to Bengali speaking Hindu community were selected. The participants selected for the study were above 40 years of age, still living in wedlock, have at least one child and without a history of any major gynecological problem. The menopausal status of the women was determined following World Health Organization (1996).

All the participants were selected through network sampling method. A well-structured schedule was used for collecting data on sociodemographic variables, menopausal symptoms, and on perception and attitude of the participants towards menopause. Data on sociodemographic variables include educational level, employment status, monthly expenditure and number of family members. A number of studies have designed check-lists of menopausal symptoms (Chompootweep et al, 1993 ;Chirawatkul and Manderson, 1994;). But, barring some typical menopausal symptoms (vasomotor, vaginal and urinary), these check-lists vary from one study to the other. Thus, for the purpose of the present study, data have been collected on typical menopausal symptoms like, vasomotor (hot flushes and night sweat), urinary symptoms (dysuria, increased frequency of urination and urine leak during laugh and cough) and vaginal (vaginal atrophy and uterine prolapse). The participants were asked to report about their personal experience of any of these symptoms during the last one month period, prior to the date of interview. Data on perception and attitude toward menopause include - the understanding of the participants about menopause, sources of knowledge, husband's awareness of their menopausal state, perceived stress in life, self assessment of present state of menopausal health, intake of Soya product (per week), taking up physical exercise (per week) and administration of medicine. All data types were collected with the help of tested schedule.

Prior to the collection of data, the nature of research is explained to all the participants and verbal consent is taken from each of them. Data was analyzed using SPSS 11.0.

Results and Discussion :

The sociodemographic profile of the study participants (Table 1) in terms of educational level, employment status, monthly household expenditure and number of family members shows similarity between the two groups. For example, in both the groups, around 55% of the participants have attained education beyond higher secondary level, 60% of them are employed, 65% of the participants have a monthly household expenditure beyond Rs. 10,000 and almost 80% have families with <4 members.

The mean age of the participants is 45.00 ± 2.73 years (perimenopausal 43.16 ± 1.96 years and postmenopausal 46.85 ± 2.01 years).

Table 2 shows that both the types of vasomotor problems (hot flush and night sweats) are more frequent among perimenopausal women but, the incidence of hot flushes is significantly more among the perimenopausal group compared to its counterpart. The urinary problem types have been found to be distributed in similar frequency between these two groups. With respect to vaginal symptoms, the problem of 'vaginal atrophy' is significantly more among the perimenopausal participants compared to the postmenopausal group. However, the problem of 'uterine prolapse appears to be present in similar frequency between these two groups.

Table 3 shows that overwhelming majority of women, irrespective of their menopausal status perceive menopause as freedom from both menstrual nuisance and religious restriction. A considerable section of the participants of both the groups believe that menopausal status elevates the position of a woman in the family. Majority of the

participants gathered knowledge about menopause from elderly people, and the rest from friends and media. Around 50% and 40% of the perimenopausal and postmenopausal participants respectively have not disclosed their menopausal status to their husbands. A large section of the participants (70%) of both the groups perceive moderate stress and 25% rate their own health as poor in this reproductive phase of their life. More than one half of the participants of both the groups consumes Soya products in their diet and does physical exercise. But, mostly the frequency of both these life style behavior is occasional in a week. A large section of these women of both the groups have administered medicine for menopausal problems, but most of them do not take it regularly.

Thus, it appears from this study that significant differences exist between women of differential menopausal status in the incidence of two typical menopausal symptoms (vasomotor and vaginal) but the perception and attitude of these women towards menopause are largely similar. The present study corroborates with some earlier studies where the incidence of vasomotor symptoms has been found to be higher among women who are in perimenopausal state compared to the postmenopausal ones (Kaufert et al., 2000; Meschia et al., 2000). The Melbourne Women's Midlife Health Project (2005) reported that increased urine frequency and urine leak during cough and laugh were more often reported in perimenopausal women than in postmenopausal women. Similar findings have been reported in this study. The higher frequency of vaginal atrophy among the perimenopausal women than postmenopausal women conforms to the report of Oldenhave

et al., (1993).

Most of the studies reveal that women perceive menopause as freedom from menstrual hazards and reproduction, and as a natural physiological event (Beyene, 1986; Aaron *et al.*, 2002; Mazhar and Gul-e-Erum, 2003; Leon *et al.*, 2007). However, there are some contradictory views. For example, in Iran, menopause has been perceived as a negative event since this phase of life marks the end of fertility and brings in forth a number of menopausal problems (Khademi and Cooke, 2003 ; Qzumba *et al.*, 2004). Our study is in close agreement with the former findings. Additionally, in accordance with the Indian culture, the women of this research also consider this stage as elevation in status their social and family life.

Health of aging woman is a global concern (United Nations [UN], 1995a) because women have a more complex phase of old age than man because of the effects of physical and psychological changes related with menopause. Globally, including the country of India, menopausal women are increasing in number (World Health Report, 1998; WHO, 2000). In India, along with the numerical increase of this group, a recent trend of advancement in the menopausal age of Indian women has been observed (Syarnala, & Sivakami, 2005). If this trend continues, then in future, a large number of Indian women would be spending menopausal life for a long period, burdened with menopausal health problems. Unfortunately, since the implementation of the proposals laid down in the Cairo conference (UN, 1995b), the Indian government has done little to improve the reproductive health of the postmenopausal women.

Table1: Socio demographic features of participants

Socio demographic features of participants	Perimenopause	Postmenopause
	Number (%)	Number (%)
<i>Educational levels of participants</i>		
Higher secondary and below	58 (58.0)	53 (53.0)
Above higher secondary	42 (42.0)	47 (47.0)
<i>Employment status of participants</i>		
Working	42 (42.0)	39 (39.0)
Non working	58 (58.0)	61 (61.0)
<i>Monthly household expenditure (in rupees)</i>		
≤ 10000	35 (35.0)	33 (33.0)
>10000	65 (65.0)	67 (67.0)
<i>Number of family members</i>		
< 4	79 (79.0)	82 (82.0)
≥ 4	21 (21.0)	18 (18.0)

Table2: Prevalence of some typical menopausal symptoms

Menopausal symptoms		Perimenopausal (n=100)	Postmenopausal (n=100)	Chi square
<i>Vasomotor</i>	Hot flush	88 (88.0)	63 (63.0)	16.89***
	Night sweats	68 (68.0)	59 (59.0)	1.74
<i>Urinary</i>	Dysuria	60 (60.0)	63 (63.0)	0.190
	Increased frequency of urination	76 (76.0)	69 (69.0)	1.229
	Urine leak during laugh, cough	67 (67.0)	66 (66.0)	0.022
<i>Vaginal</i>	Vaginal atrophy	67 (67.0)	93 (93.0)	22.91 ***
	Uterine prolapse	61 (61.0)	60 (60.0)	0.21

***p<0.001

Table 3: Attitude and perception toward menopause

Attitude and perception of the participants towards menopause	Perimenopausal (n=100)	Postmenopausal (n=100)
Freedom from menstrual nuisance	84 (84.0)	83 (83.0)
Freedom from religious restriction	83 (83.0)	83 (83.0)
Elevated status in family	55 (55.0)	74 (74.0)
Onset of aging process	14 (14.0)	17 (17.0)
Diminishing activity	12 (12.0)	13 (13.0)
Loss of sexual desire	13 (13.0)	12 (12.0)
Loss of femininity	2 (2.00)	3 (3.00)
<i>Sources of knowledge about menopause</i>		
Elderly people	60 (60.0)	68 (68.0)
Friends	26 (26.0)	20 (20.0)
Media	14 (14.0)	12 (12.0)
<i>Husband's awareness about the menopausal status of their spouses</i>		
Yes	52 (52.0)	61 (61.0)
No	48 (48.0)	39 (39.0)
<i>Perceived stress in life</i>		
Moderate	71 (71.0)	69 (69.0)
Serious	18 (18.0)	31 (31.0)
<i>Self assessment of present state of menopausal health</i>		
Poor	26 (26.0)	26 (26.0)
Fair	64 (64.0)	74 (74.0)
Good	9 (9.0)	-
Excellent	1 (1.0)	-
<i>Intake of soya products (per week)</i>		
Occasionally	34 (34.0)	27 (27.0)
Regularly	16 (16.0)	26 (26.0)
<i>Taking up physical exercise (per week)</i>		
Occasionally	32 (32.0)	44 (44.0)
Regularly	17 (17.0)	17 (17.0)
<i>Administration of medicine</i>		
Occasionally	57 (57.0)	45 (45.0)
Regularly	24 (24.0)	36 (36.0)

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REPEATED MULTIPLE PATHOGEN INFECTION ENHANCES ANTI-OXIDANT RESPONSE IN HYPERCHOLESTEROLEMIC MICE CORRELATING CARDIOVASCULAR INFLAMMATION

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ABSTRACT ■ Repeated *Escherichia coli* and *Staphylococcus aureus* infections in conjunction with hypercholesterolemia could lead to development of oxidative stress that may affect the progression of atherosclerosis in hypercholesterolemic mice. Male Swiss albino mice (4 weeks old) were randomly assigned to high fat diet (HFD) or normal laboratory diet (NLD) groups. At 10 weeks of age, mice were inoculated intraperitoneally with viable *E. coli* and *S. aureus* cells or vehicle for 5 weeks. Serum cholesterol, low density lipoprotein, high density lipoprotein, uric acid levels, and selective antioxidant enzyme activity in murine aorta, heart and liver during hypercholesterolemia, were examined. Serum cholesterol level, low density lipoprotein (LDL) levels were elevated and high density lipoprotein (HDL) level was decreased in HFD-fed mice, compared to NLD. The activities of catalase and glutathione peroxidase (GPX) were elevated in HFD fed-infected groups as compared to NLD fed-infected group with respect to vehicle treated group except superoxide dismutase (SOD). The myeloperoxidase content of aortic tissue was significantly higher in all groups infected with *E. coli* and *S. aureus*. Our study suggests that during hypercholesterolemia, repeated multiple pathogen infection induces an endogenous antioxidant response that serves to increase vascular inflammation leading to cardiovascular diseases and atherosclerosis.

Key Words : hypercholesterolemia, multiple pathogen infection, inflammation, anti-oxidant enzyme, cardiovascular disease

INTRODUCTION :

The role of microbial infection in the development and progression of cardiovascular diseases (CVD) had been under consideration for more than a century, with the earliest study reported way back in 1889 (Gilbert and Lion 1889). Since then many investigators had suggested the role of microbial infection in the development and progression of cardiovascular inflammation

leading to CVD. It had been speculated that bacterial infection might have a direct effect on the vascular wall or that could act indirectly through the induction of an autoimmune inflammatory response involving mechanisms such as molecular mimicry and epitope spreading to generate atherosclerosis (Ludewig et al. 2004). All studies focusing on the role of microbial infection in atherosclerosis had examined a single

pathogen. But if infection does play a role in atherogenesis, it would be unlikely that any one of these was the causal agent. Recent studies have suggested that the impact of infection on atherogenesis was related to the aggregate number of pathogens with which an individual was infected, a concept referred to as pathogen burden (Zhu *et al.*, 2000). We therefore postulated that multiple pathogens might causally involve, and that CVD risk relating to the aggregated pathogen load (i.e. pathogen burden). Long-term exposure to proinflammatory, toxic, or transforming microbes and their products might be possible mechanism for infection-related atherosclerotic CVD. As emphasized at a 1998 National Institutes of Health workshop, research on associations between infection and atherosclerotic CVD must consider the co-influence of multiple infectious agents (Taylor 1999).

It was suggested that LDL does not normally trigger immune responses in its native state; however in oxidized state (ox-LDL), it could initiate inflammation-leading to atherosclerosis. Ox-LDL particles induced macrophage foam cell formation, smooth muscle cell migration and proliferation, and, in addition, stimulated the secretion of inflammatory cytokines (Olofsson and Boren 2005). Ox-LDL induced the expression of adhesion molecules promoting endothelial cell dysfunction and leukocyte extravasation (Groyer *et al.* 2006).

Evidences indicated that oxidative stress play a major role in the initiation and progression of cardiovascular dysfunction associated with hyperlipidemia. An imbalance in the generation of reactive oxygen species

(ROS) and production of antioxidants could play both physiological and pathophysiological roles (Taniyama and Griendling 2003). Considerable experimental and clinical data indicated that intracellular oxidant milieu was also involved in several redox-sensitive cellular signaling pathways such as ion transport systems, protein phosphorylation, and gene expression and thus also played important roles as modulator of vascular cell functions such as cell growth, apoptosis, migration, angiogenesis and cell adhesion. Overproduction of ROS under pathophysiological conditions was integral in the development of cardiovascular diseases (Rojas *et al.*, 2006). Increased production of ROS, in particular, superoxide and radicals derived from superoxide, had been associated with endothelial dysfunction in animal models of disease, and there was increasing evidence correlating oxidative stress and endothelial dysfunction in humans (Droge 2002). Superoxide dismutase, glutathione peroxidase and catalase were considered to be important members of these enzyme systems. In addition, serum uric acid was also a powerful free-radical scavenger and increased in response to acute oxidative stress (MacKinnon 1999).

E.coli and *S.aureus* were chosen because they are one of the most abundant Gram-negative and Gram-positive bacteria respectively found in the Indian subcontinent. The aim of our study was to investigate the role of repeated multiple infections with *E.coli* and *S.aureus* during hypercholesterolemia, and whether it could lead to the development of oxidative stress as depicted by the alterations in the antioxidant enzyme activities.

Materials and Methods :

Experimental animals :

All animal experimentations were approved by the departmental animal ethical committee. Four weeks old male Swiss albino mice were used for all experimental procedures. All the animals were kept in well-ventilated, departmental animal house facility, within rooms having temperature regulated to approximately 20°C, relative humidity of 55±10%, and a 12-h light dark cycle. After a 7-day quarantine period, the mice were randomly assigned to two groups viz. a normal lab diet fed group and a high fat diet fed group (NLD and HFD respectively). NLD groups were fed on rodent's normal lab diet (wheat flower 22.5%, roasted bengalgram flour 60.0%, skim milk powder 5.0%, casein 4.0%, refined groundnut oil 4.0%, salt mixture 4.0%, vitamin mixture 0.5%; National centre for laboratory animal sciences, National Institute of Nutrition, Hyderabad) where as the HFD groups were fed on rodent's diet containing 2% cholesterol and 1% sodium cholate (Plump 1992). A pair-feeding method was adopted for the experimental animals, wherein the amount of food provided to the infected group animals was equal to the amount delivered to the control animals so that there was a qualitative, quantitative and chronological correctness in the feeding of the animal groups. Any amount of unconsumed food was discarded before providing fresh diet. At 10 weeks of age, the NLD-fed and HFD-fed animals were further divided into two groups, each group containing six animals. One group of either diet fed animals was injected intraperitoneally with viable *E. coli* and *S. aureus* cells. These were considered as the "Infected" groups.

To the either diet fed remaining groups of animals, dilute sterile phosphate buffered saline was administered, and they were counted as "Control" groups.

Preparation of *E. coli* :

E. coli cells were cultured in nutrient broth. Freshly prepared culture was centrifuged so as collect bacterial cells from mid-logarithmic growth phase. The cell pellets were then resuspended with sterile phosphate buffered saline (PBS) under laminar flow. This suspension was diluted and their concentration estimated by spectrophotometry at A_{660} on the basis of the relationship: $A_{660} 0.2 = 5 \times 10^7 / \text{ml}$ (Hiemstra *et. al.*, 1996). 100 μl of this bacterial cell suspension was then injected intraperitoneally to the infected group animals.

Preparation of *S. aureus* :

To obtain *S. aureus* (ICH - 757) in the mid-logarithmic phase 100 μl of an overnight culture made in nutrient broth was added to 10 ml of nutrient broth and incubated for 2-5 h at 37°C with orbital shaking. The bacteria were washed in 10 mM sodium phosphate buffer (pH 7.4) and their concentration estimated by spectrophotometry at A_{620} on the basis of the relationship: $A_{620} 0.2 = 5 \times 10^7 / \text{ml}$. 100 μl of this suspension was used to infect the mice intraperitoneally (Yao *et. al.*, 1997).

Mode of *in vivo* infection :

Each mice belonging to infected group was infected separately once in a week with either *E. coli* or *S. aureus* (5×10^6 cells) with an interval of three days between each doses and continued for 5 weeks. The vehicle groups were injected sterile saline during the period.

Blood collection and separation of serum :

Blood was collected by cardiac puncture after anesthetizing the animals with diethyl ether (anesthetic ether). The blood was transferred to microcentrifuge tubes and centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant pale yellow colored serum was pipetted out carefully with the help of micropipettes into fresh microcentrifuge tubes, labeled and stored at -80°C for further analysis.

Preparation of tissue homogenates :

After perfusing the heart with butylated hydroxyl toluene (BHT) buffer containing 20 imole/L butylated hydroxyl toluene and 2 micromole/L ethylene diamine tetra acetic acid (EDTA) in Dulbecco's phosphate buffered saline (DPBS)-1X (pH 7.2), liver, heart and aorta were dissected out from each animal and kept separately at -20°C. Then tissues were homogenized using a polytron homogenizer. The supernatants obtained after centrifugation of crude homogenates were used for assay.

Estimation of serum cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) :

Serum cholesterol, LDL level and HDL level were quantitatively estimated using biochemical kit (Labkit, Chemelex, S.A., Spain) according to the manufacturers' instruction. (Burtis *et al.*, 2006).

Estimation of serum uric acid :

Protein free filtrate from the serum of different groups was allowed to react with the uric acid reagent containing phospho-

tungstic acid which upon reaction with the uric acid gives blue colored complex in presence of sodium carbonate solution. The coloured product formation is due to reduction of phosphotungstic acid by uric acid in alkaline medium. The reaction of uric acid in alkaline solution with phosphotungstic acid reagent oxidizes uric acid to allantoin and itself is reduced to tungsten blue which was measured at 620nm (Oser 1976).

Assay of antioxidant enzyme activity from tissue homogenates :*Catalase activity*

Catalase activity in the cell free homogenate and serum was determined spectrophotometrically by measuring the decrease in H₂O₂ concentration at 240 nm. At time zero, 1.8 ml of each homogenate was mixed with 0.2 ml of phosphate buffer containing 10 mM H₂O₂. One ml of the mixture was immediately added to a cuvette and placed into a spectrophotometer. Catalase activity was observed via degradation of hydrogen peroxide as determined by a decrease in UV light absorbance over time. Measurement of absorbance was taken at 20 s interval after addition of the homogenate to hydrogen peroxide buffer (Aebi 1984).

Superoxide dismutase (SOD) activity

100 µl of tissue homogenate or serum was mixed with 1.5 ml of a Tris-EDTA-HCl buffer (pH 8.5), then 100 µl of 7.2 mmol/L pyrogallol was added and the reaction mixture was incubated at 25°C for 10 min. The reaction was terminated by the addition of 50 µl of 1M HCl and measured at 420 nm. One unit was determined as the amount of

enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as U/mg protein (Lee et al. 2003).

Glutathione peroxidase (GPX) activity

100 µl of tissue homogenate or serum was incubated at 37°C for 5 min with a reaction mixture containing 0.1 mol/L Tris-HCl buffer, 30 mmol/L glutathione and 6 mmol/L NADPH. The reaction was initiated by the addition of 100 µl of 7.5 mol/L H₂O₂ and change in absorbance was measured at 340 nm spectrophotometrically. The activity was expressed as nmole/min.mg of tissue protein (Paglia and Valentine 1967).

Myeloperoxidase (MPO) enzyme activity: an index of aortic tissue neutrophil content

MPO enzyme activity was assayed spectrophotometrically. For the assay, 150 µl of tissue homogenate was added to a reaction mixture containing 0.8 mM H₂O₂, 10 mM KH₂PO₄ (pH 6.0) and 0.4 mM O-dianisidine dihydrochloride. The reaction was performed at 37°C and absorbance was measured spectrophotometrically at 460 nm every 20 s for 4 min, and the linear portions of the tracing was used for analysis. H₂O₂ dependent MPO activity was expressed as change in absorbance per min per mg tissue protein (Lapointe et al 1967).

Estimation of tissue protein :

Protein content of tissue homogenates and serum was estimated by dye binding technique according to Bradford (Bradford 1976).

Statistical analysis :

One-way model 1 ANOVA was

performed between the groups. In ANOVA observed variance is partitioned into components due to different explanatory variables. A level of P<0.05 was considered significant (Das and Das 2005).

RESULTS :

Effect of high fat diet administration on serum cholesterol level

The serum cholesterol level was increased significantly (P<0.05) in HFD fed-vehicle groups than the NLD fed- vehicle groups. The level was also increased significantly in HFD fed- infected group than the NLD fed- infected group (Table 1).

Effect of multiple pathogen infection during hypercholesterolemia on serum low density lipoprotein (LDL) and high density lipoprotein (HDL) level :

The serum LDL level was increased significantly (P<0.05) in HFD fed -vehicle group than the NLD fed vehicle group. LDL level was also significantly increased in HFD fed-infected group compared to NLD fed-infected group (Table 1). Serum HDL level was decreased in NLD fed-infected group than NLD fed vehicle group though the decrease was not significant. But serum HDL level was decreased significantly (P<0.05) in HFD fed-infected group compared to HFD fed-vehicle group. The reduction was also significant in HFD fed-infected group when it was compared to NLD fed-infected group (Table 1).

Table 1: Estimation of serum cholesterol, LDL, HDL and uric acid levels

GROUP	Serum cholesterol level (mg/dl) Mean \pm SD	Serum LDL level (mg/dl) Mean \pm SD	Serum HDL level (mg/dl) Mean \pm SD	Serum Uric acid level (mg/dl) Mean \pm SD
NLD + Vehicle	79.64 \pm 2.73	43.31 \pm 4.22	24.42 \pm 0.385	2.214 \pm 0.043
NLD + Multiple pathogen infection	85.08 \pm 1.96	51.43 \pm 1.19	22.09 \pm 0.665	3.94 \pm 0.051
HFD + Vehicle	142.41 \pm 3.56	105.19 \pm 2.57	19.78 \pm 0.62	2.58 \pm 0.033
HFD + Multiple pathogen infection	151.23 \pm 3.68	117.51 \pm 2.50	15.49 \pm 0.775	5.44 \pm 0.038

Statistical analysis was done using one way model 1 ANOVA and $P < 0.05$ was taken as level of significance.

Effect of multiple pathogen infection during hypercholesterolemia on serum uric acid level :

The serum uric acid level increased significantly ($P < 0.05$) in the NLD fed-infected group than the NLD fed-vehicle group. The activity also increased significantly ($p < 0.05$) in the HFD fed- infected group than the HFD fed-vehicle group. The level also increased significantly in the HFD fed-infected group than the NLD fed- infected group (Table 1).

Effect of hypercholesterolemic diet and multiple pathogen infection on

antioxidant enzyme activity

Catalase activity in tissues

After 5 weeks of multiple pathogen infection (*E. coli* and *S. aureus*), activities of catalase in the aortic tissue were significantly elevated as compared to vehicle treated groups, irrespective of the diet ($P < 0.05$). In cardiac homogenates, there was a significant increase ($P < 0.05$) in the catalase activity in the NLD-fed, bacteria infected group when compared to its control and the result was identical for the HFD fed mice. Hepatic catalase activity was increased after 5 weeks post infection in NLD-fed mice as

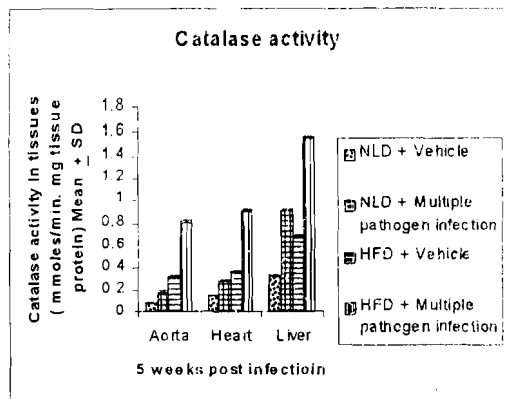


Fig 1: Catalase activity in tissues

compared to the vehicle treated group ($P < 0.05$). In case of HFD treatment, 5 weeks post infection mice showed increased hepatic catalase activity from the vehicle treated group ($P < 0.05$). For all three tissues, there was significant increase in the activity of catalase in HFD fed mice compared to NLD fed groups receiving multiple pathogens (Fig 1).

Superoxide dismutase (SOD) activity in tissues :

After 5 weeks infection with multiple pathogens, the activities of SOD in aortic tissue were significantly decreased as compared to vehicle treated groups, irrespective of the diet ($P < 0.05$). The activity of SOD was also decreased significantly in HFD fed-infected mice than the NLD fed-infected group. In cardiac tissue homogenates, the activity of SOD was decreased in the NLD fed-infected group than the NLD fed-vehicle group though the decrease was not significant. But the activity was decreased significantly ($P < 0.05$) in the HFD fed-infected group than the HFD fed-vehicle group. After 5 weeks post infection, the

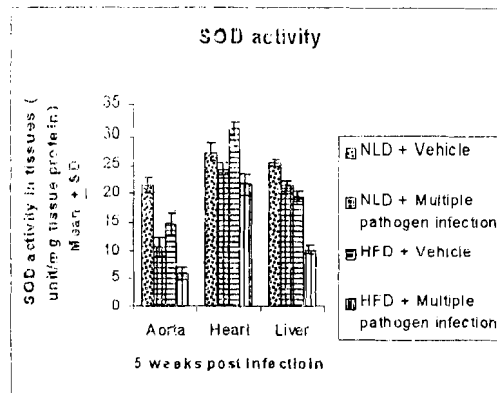


Fig 2: Superoxide dismutase (SOD) activity in tissues

hepatic SOD activity was significantly decreased than vehicle treated mice of either diet fed animals ($P < 0.05$). The activity was also decreased significantly in HFD fed-infected mice than the NLD fed-infected group (Fig 2).

Glutathione peroxidase (GPX) activity in tissues :

After 5 weeks of infection with multiple pathogens, the activities of GPX increased significantly ($P < 0.05$) in aortic tissue compared to their respective controls. The activity was also increased significantly in HFD fed-infected mice than the NLD fed-infected group. In cardiac tissues, 5 weeks of bacterial infection resulted in significant increase in the enzyme activity in both diet fed groups, with respect to their controls ($P < 0.05$). The activity was also increased significantly in HFD fed-infected group than the NLD fed-infected mice. In hepatic tissues the GPX activity was significantly higher in bacteria infected groups irrespective of the diet ($P < 0.05$) (Fig 3).

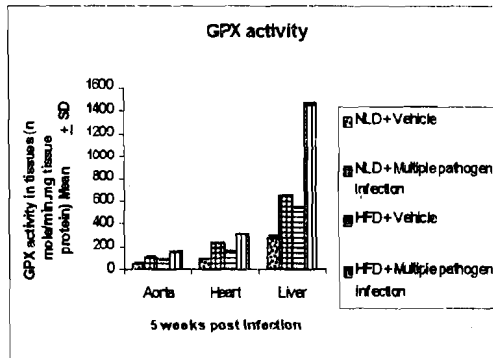


Fig 3: Glutathione peroxidase (GPX) activity in tissues

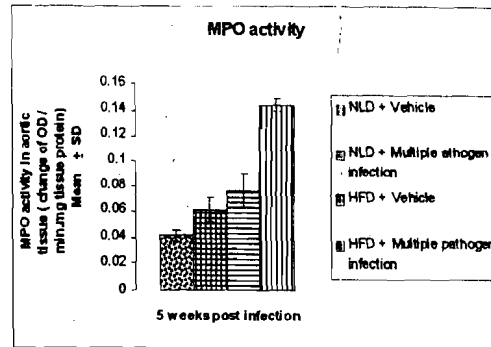


Fig 4: Determination of aortic tissue neutrophil infiltration after 24 week post infection quantified by tissues myeloperoxidase (MPO) activity

Effect of hypercholesterolemic diet and multiple pathogen infection on aortic myeloperoxidase (MPO) activities in mice:

The MPO activity was increased in aortic tissue in the NLD fed- infected group than the NLD fed- vehicle mice after 5 weeks of multiple pathogen infection. But the increase was not significant. Whereas the activity was increased significantly ($P < 0.05$) in the HFD fed- infected mice than the HFD fed vehicle group. The activity was also significantly higher ($P < 0.05$) in HFD fed infected mice compared to NLD fed infected group (Fig 4).

DISCUSSION :

Increased serum cholesterol level in the HFD fed mice than the NLD fed group indicated that hypercholesterolemia was successfully induced in the HFD fed animals. Since serum LDL level was significantly higher in HFD fed- multiple pathogen infected group than that of NLD fed-multiple pathogen infected group, it may be suggested that

infection by multiple pathogens caused an increase in serum LDL level and the effect was more prominent in the high fat diet fed mice. HDL is considered as good cholesterol. Decreased level of HDL in HFD-fed and infected animals indicated that multiple pathogen infection during hypercholesterolemia reduce the level of serum HDL, which might interfere with cholesterol transport and uptake and ultimately enhance the progression of atherosclerosis.

Significant increase in serum uric acid level in hypercholesterolemic mice after multiple pathogen infection indicates the important role of uric acid in combating the oxidative stress of body. As uric acid is also a marker of inflammation, its increased value also indicates the ongoing process of inflammation.

Activities of anti-oxidant enzymes particularly catalase and GPX were elevated after multiple pathogen infection in HFD fed mice. It may be suggested that infection and HFD enhances the generation of ROS, which may induce oxidative effect on circulating

LDL. Increased amount of ROS production in these mice causes excess induction of tissue antioxidant enzymes to scavenge the free radicals formed. Higher GPX activity indicates that it may prevent the rise of lipid peroxides induced by high-cholesterol diets.

In the contrary, multiple pathogens infected and HFD-fed mice exhibited decreased SOD activity. It may be due to the down regulation of SOD gene after infection. This multiple pathogen induced down regulation might cause a mismatch between the requirement of SOD and its supply which leads to lowered SOD activity.

Since MPO activity in the aorta was significantly higher in HFD fed infected group compared to NLD fed infected mice but the increase was not significant for diet only i.e., when the infection was absent, it may be suggested that high fat diet itself might not have an inflammatory effect on aorta, but it induced inflammation when exposed to multiple pathogen infection. Although both the bacteria are introduced in equal amount at each time during multiple infections we did not measure the survival rate of both bacteria inside the mice peritoneal cavity. Increased bacterial burden in the peritoneum may dissipate to the aorta for inducing inflammation.

As the mice were infected with viable *S. aureus* and *E. coli*, antibody against those whole cell antigens might have generated, although we have not tested the antibody titre. Since the mice were chronically inoculated with *S. aureus* and *E. coli* through intraperitoneal route, numbers of bacteria may be present either in the intraperitoneal

fluid or blood, as well as may also be infiltrated in the peripheral lymphoid organs particularly in the spleen and lymph nodes which is going on in our laboratory.

In a recent study we have demonstrated that *E. coli* lipopolysaccharide administration alter antioxidant profile during hypercholesterolemia. (Dutta and Bishayi 2009). We have also reported that repeated systemic *E. coli* infection induces an endogenous antioxidant response that serves to modulate vascular inflammation leading to cardiovascular disease (Dutta et al 2009). This study provide evidences that infection with multiple pathogens including *E. coli* and *S. aureus* during hypercholesterolemia leads to the excess production of free radicals and which in turn modulate the body's own defense mechanism against ROS by inducing anti oxidant enzymes. But because of more production of ROS than its inactivation by the anti oxidant enzymes, the progression of cardiovascular inflammation takes place along with progression of inflammation in the aorta. Further experiments could be of interesting for making a comparison between single bacterium versus two bacterial infections using similar amount of bacteria introducing same period of time via the same route. Much more information is required before any standard recommendation can be made for the use of anti-oxidant treatment of pre-existing cardiovascular conditions.

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EVALUATION OF THINNESS AMONG URBAN BENGALEE ADOLESCENTS OF MIDNAPORE, INDIA : USING NEW INTERNATIONAL BMI CUTOFFS

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ABSTRACT ■ To evaluate the prevalence of thinness, a cross sectional study was undertaken in an urban area of Paschim Medinipur districts, West Bengal, India. Weight and height measurement was made following standard techniques. The thinness was assessed following the internationally accepted age and sex specific BMI cutoff values. A total of 1265 (640 boys and 625 girls) Bengalee adolescents aged 10-12 years were selected from lower-middle class socio-economic group following simple random sampling methods. The overall prevalence of thinness was 37.6%. The prevalence of thinness was significantly higher among boys (43.6%) than girls (31.4%). The boys had 1.69 times more chances to being thin compared with girls. Moreover, the prevalence of thinness and overweight including obesity were 7.8% (boys=10.0%, girls=5.6%) and 7.6% (boys=7.5%, girls=7.8%) based on Indian standards. In conclusion, the present study supports the evidence of high prevalence of adolescent undernutrition (thinness) in the developing countries belonging to the South Asia Region.

Keywords : Thinness, Overweight, Adolescent, Bengalee, India, Body Mass Index.

INTRODUCTION :

In a recent period anthropometric measurements have become a popular measure for the assessment of nutritional status among children and adolescents. Adolescents are defined as those individuals aged between 10 – 19 years. Approximately, 21% of the total population in India is belonging to adolescent age group. It is well established that among other anthropometric measures body mass index (BMI) is not only the single most appropriate, cost effective and non-invasive tool for the assessment of the

nutritional status of adolescents and adults (WHO 1995) but it is also the best indicator of thinness during adolescence (de Onis et al 2001). Numerous surveys conducted in rural and urban areas in the South-East Asia Region shows high prevalence of thinness among adolescents (WHO 2005). However, there is a paucity of data on the prevalence of thinness among urban adolescents in the West Bengal state of India (Das and Bisai 2009, de Onis et al 2001, Ghosh and Bhandhopadyay 2009). Given the context, the aim of the present study is to assess the

different grades of thinness by using the new international age and sex specific cut off values on BMI as proposed by Cole et al. (2007). Again the results were compared with the prevalence of thinness as assessed by Indian standards (Agarwal 2001).

Materials and Methods :

Present study was undertaken during November, 2008 – March 2009 in Midnapore town, West Bengal. Subjects were selected from Hindu Bengalee speaking adolescents of lower-middle socio-economic class. Socio-economic status was screened based on household income as proposed by Agarwal (2008). After interview and screen, guardian of the eligible household were informed about the objectives of the study and their consent was obtained. Information on age, sex, weight and height were recorded with the help of questionnaire. The minimum estimated sample size was calculated using standard formula found elsewhere (Bisai 2008), with 45% prevalence of thinness (Bose and Bisai 2008) and desire precision of 3%. The estimated sample size is 1100. An additional 15% added to make the sample more representative. Therefore, a total of 1265 (640 boys and 625 girls) healthy children aged 10 -12 years were measured randomly.

Anthropometric measurement like height and weight was measured following standard methods (Lohman et al. 1988) by using anthropometer rod and weighing scale to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was computed following internationally accepted standard equation (WHO 1995). Presence of thinness was evaluated using the cut-off values of

international survey as developed by Cole et al (2007). While age and sex specific Indian standards were utilized for the assessment of thinness and overweight including obesity (Agarwal 2001).

The distributions of the height, weight and BMI were not significantly skewed. Therefore, Student's t-tests and one-way analysis of ANOVA were performed to test for differences in mean anthropometric characteristics by sex and age of children. Odds ratio (OR) was calculated to measure the risk for being thinness between sexes. All statistical analyses were undertaken using the SPSS Statistical Package. Statistical significance was considered as $p < 0.05$.

RESULTS AND DISCUSSION :

Result revealed that boys were shorter and thinner at all ages compared with girls (results not shown). There was a significant increasing trend in height, weight and BMI with age except BMI among boys. Moreover, there existed significant sex differences in height, weight and BMI except BMI and height at age 10 and 12 years, respectively. Prevalence of different grades of thinness among the subjects is presented in **Table 1**. The overall prevalence of thinness was 37.6% based on international cutoffs. The prevalence of grade-I, grade-II, and grade-III thinness were higher among boys (27.7%, 11.2% and 4.7%) than their girls' counterparts (20.3%, 7.7% and 3.4%). The prevalence of total thinness was significantly higher among boys (43.6%) compared with girls (31.4%). The boys had 1.69 (OR=1.69; 95% CI: 1.34-2.13) times more chances to being thin compare with girls (table not shown). While,

Table 1: Prevalence of different grades of thinness among the adolescents.

Age (years)	Thinness									
	Boys					Girls				
	n	III	II	I	Total	n	III	II	I	Total
10	221	13 (5.9)	28 (12.7)	52 (23.5)	93 (42.1)	203	10 (4.9)	18 (8.9)	40 (19.7)	68 (33.5)
11	213	12 (5.6)	18 (8.5)	55 (25.8)	85 (39.9)	218	6 (2.8)	11 (5.0)	50 (22.9)	67 (30.7)
12	206	5 (2.4)	26 (12.6)	70 (34.0)	101 (49.0)	204	5 (2.5)	19 (9.3)	37 (18.1)	61 (29.9)
Total	640	30 (4.7)	72 (11.2)*	177 (27.7)*	279 (43.6)*	625	21 (3.4)	48 (7.7)*	127 (20.3)*	196 (31.4)*

Significant sex differences; $p < 0.05$. Values are percentages in parentheses.

age and sex specific Indian standard was employed for the assessment of thinness. results revealed that only 7.8% adolescents were found to be thin. The prevalence of thinness was significantly higher among boys than the girls (boys=10.0%, girls=5.6%; Chi-square = 8.49, $p < 0.05$).

Moreover, the prevalence of overweight including obesity was 7.6% based on Indian standards. Similar results were also reported from studies undertaken middle socio-economic group of people in India (Kaur *et al.*, 2008, Kaur and Kapil 2008, Kuriyan *et al.*, 2007). There was no significant sex difference existed in the rate of overweight including obesity (boys=7.50%, girls=7.84%). It is important to mention that the prevalence of overweight decreased with advancement of age for both sexes.

Table 2 shows prevalence of thinness among Indian children and adolescents. In the present study the prevalence of total thinness was 37.6%. Similar results were reported from other parts of urban West Bengal (Mukhopadhyay *et al* 2005). The prevalence of thinness was higher in the present study than those reported from Sudan (Awad and Enayat 2007) and Portuguese (Marques-Vidal *et al* 2008) as assessed by international criteria and other parts of West Bengal by using WHO (1995) recommended method (Bose and Bisai 2008, Bose *et al* 2009, Das *et al* 2007, Das and Bisai 2009, Ghosh and Bhandhopadyay 2009). In addition, prevalence of thinness in the present study was lower than those reported from other parts of India (Medhi *et al* 2007, Kurz 1996, Rao *et al* 2006, Venkaiah *et al* 2002, Mandot

Table 2 : Prevalence of thinness among Indian children and adolescents.

Area	District	State	Age group	n	Methods	Prevalence of thinness			References
						Boys	Girls	Total	
Rural	Paschim Medinipur	West Bengal	10-15	2016	WHO	51.7	36.9	44.5	Bose & Bisai (2008)
Rural	Paschim Medinipur	West Bengal	11-18	1094	WHO	41.8	25.2	35.3	Bose & Bisai (2008)
Rural	Bankura District	West Bengal	6-14	454	WHO	27.8	19.4	23.1	Bose et al (2009)
Rural	Dibrugarh district	Assam	10-18	605	WHO	59.5	41.3	50.2	Medhi et al (2007)
Rural	-	Nine states	10-17	12789	WHO	62.9	41.7	51.8	Rao et al (2006)
Rural	Sirohi	Rajasthan	5-16	2017	WHO	69.7	59.3	65.8	Mandot et al (2009)
Rural	Purba Medinipur	West Bengal	5-10	569	Cole	62.9	61.6	62.2	Chakraborty & Bose (2009)
Rural	Faridabad	Haryana	12-18	494	WHO	43.8	30.1	39.5	Anand et al (1999)
Rural	Wardha	Maharashtra	-	764	CDC	40.7	69.8	53.8	Deshmukh et al (2006)
Urban	Nalgonda	Andhra Pradesh	10-18	223	Indian standard	-	20.6	-	Prashant & Shaw (2009)
Urban	North 24 Parganas & Howrah	West Bengal	9-17	1153	WHO	28.4	16.9	21.8	Ghosh & Banhopadhyay (2009)
Urban	North 24 Parganas	West Bengal	10-16	502	WHO	37.7	-	-	Bose & Mukhopadhyay (2004)
Urban	Kolkata	West Bengal	9-16	6580	WHO	50.5	-	-	De Onis et al (2001)
Urban	Mumbai	Maharashtra	10-19	138	WHO	-	-	53.0	Kurz 1996
Urban	North 24 Parganas	West Bengal	11-14	559	WHO	41.1	30.6	36.5	Mukhopadhyay et al (2005)
Urban	Paschim Medinipur	West Bengal	9-20	930	WHO	37.6	19.4	28.6	Das & Bisai (2009)
Urban	Paschim Medinipur	West Bengal	10-12	1265	Cole	43.6	31.4	37.6	Present study

et al 2009, Deshmukh et al 2006) and neighboring countries (Sahabuddin et al 2000). In all studies except that of Deshmukh et al (2006), the rates of thinness were higher among boys as compare to girls. We also obtained a similar finding. The cause of this sex difference in rates of thinness needs to be investigated further. An earlier study conducted among Telaga adolescents in Kharagpur town has reported significantly lower rates of thinness as compared to our study (Das and Bisai 2009). More importantly the prevalence of thinness was lower as assessed by Indian standard than the international standards. The possible explanation is that there was significant ethnic variation in BMI (Rosner et al 1998) and ethnic differences in the rate of underweight and overweight (Tuan and Nicklas 2009). Therefore, there is need for BMI standards for diagnostic purposes of thinness and obesity for Indian children. Given the context, ethnic group specific standards are more appropriate for comparing health-compromised children, especially in our country where the problem is more pronounced for underweight than overweight (Agarwal 2001).

In conclusion, future investigations in India should utilize these new cut-off values as suggested by Cole et al (2007) to evaluate the thinness during childhood. Such studies will provide valuable data on prevalence of thinness which can be used for the formulation of effective nutritional intervention and public health policies. Moreover, they would also provide useful datasets for national and international comparisons.

ACKNOWLEDGEMENT :

Financial assistance of UGC-Dr. D. S. Kothari Post-Doctoral Fellowship Scheme (F. 4-2 / 2006 (BSR) / 13-51 / 2008 (BSR)) is gratefully acknowledged.

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HISTO-HEMATOLOGICAL CHANGES IN LUNG OF RAT FOLLOWING EXPOSURE TO WOOD SMOKE

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ABSTRACT ■ Smoking is the major cause of airway inflammation in chronic obstructive pulmonary disease. The exposure effect of wood smoke on the hematological parameters and histopathology of the lung of rats was studied. Rats were exposed to repeated wood smoke generate from combustion of wood dust and fire wood over a period of 45 days. First 15 days exposure caused mild hyperplasia and emphysema. These lesions progressed further during 30 and 45 days of exposure. Hematological studies show marginal alteration in hemoglobin levels, R.B.C and W.B.C but significant changes in eosinophils were recorded during 30 and 45 days. In the study, it was found that differences changes in blood parameters and cellular changes in lungs were time dependent.

Key words: Wood smoke, histopathology, hematology, lung and rat.

INTRODUCTION :

The cardiovascular system, the lungs from an integrated mechanism by means of which the constancy of the internal environment is maintained. The lungs may be considered as a dual organ whose function is to supply oxygen to the tissue and to remove carbondioxide. The general structure of the lung is to familiar to the reader to need recapitulation, but reference may be made to the bronchopulmonary segments, the musculature and the alveolar membranes (Boy *et al.*, 2002).

The structural changes in the lung produced by various disease processes. Smoking is the most important etiological factor in the development of chronic

obstructive respiratory diseases. Respiratory diseases are widely prevalent in developing countries including Bangladesh. Apart from principal environmental factors, smoke from biomass fuel emissions (like wood, manure and agricultural wastes), primarily used for cooking and heating is considered major factors for respiratory disorders (Malik, 1995).

Epidemiologic studies have linked exposure to wood smoke with increased prevalence of respiratory illness in children and adults (Bruce *et al.*, 2000). Increased respiratory symptoms, decreased pulmonary function, and increased prevalence of chronic bronchitis have been associated with wood smoke exposure in Columbia

(Dennis *et al.*, 1996), Papua, New Guinea (Anderson, 1979), Washington (Koenig *et al.*, 1993), India (Behera and Jindal, 1991), Nepal (Pandey, 1984) and Kenya (Boleij *et al.*, 1989). Wood smoke can be an important contributor to particle and gaseous material in ambient air and can account for as much as 80% of the airborne particle concentrations during the winter heating season in some locations (Larson and Koenig, 1994). Indoor exposures to wood smoke can occur not only from infiltration of outdoor air, but also from non-airtight or improperly operated wood stoves and other wood-burning appliances. Some studies report wood smoke concentration as total suspended particulate matter from 2.7 to 25 mg/m³, much higher concentrations of particulate matter than those to which U.S.A populations are currently exposed (up to 1 mg/m³ on a 24 h. basis) (Larson and Koenig, 1993).

Although wood smoke is an important environmental air contaminant in several locations, the toxicological database on subchronic or chronic exposures to wood smoke is very small (Larson and Koenig, 1994). Very little information indicate that wood smoke emissions induce sister chromatid exchanges (Shivmany *et al.*, 1985), increase in the level of carboxyhemoglobin in blood (Behera *et al.*, 1988) and causes mild bronchiolitis, hyperplasia, edema, mild emphysema (Fick *et al.*, 1984) and asthma (Schwartz *et al.*, 1993). In vitro experiments have indicated increase in polymorphonuclear cells and alveolar macrophages with decreased

adherence and phagocytosis (Fick *et al.*, 1984).

In Bangladesh most of the people cook their food using firewood and biomass fuel. Biomass fuel is composed mainly of wood, animal dung, and crop residue. About 50% of the world population and 90% of rural communities in developing countries are using biomass as a single source of cooking fuel (WRI, 1998). Wood and biomass smoke emission might be play a vital role for the infection of lungs. Therefore, present studies have been designed to characterize and assess the events leading to hematological parameters of blood and histopathological changes in lung of rats following exposure to wood smoke.

Methods :

Albino rats rearing: For the purpose of this experiment, group of eight healthy and sexually mature albino rats, weight about 180 gm were collected from locally (Rajshahi) and reared in the Department of Zoology, Rajshahi University. Animals were kept in steel cages in the laboratory under constant conditions at room temperature (33 ± 40C) and feed on rice, wheat and tap water. These were reared for a total period of 45 days on systematic ways. Another group of four rats, supplied food along with above rats and were marked as control animal.

Wood smoke exposure system: Rates were exposed to smoke generated from combustion wood dust and fire wood, 15 minutes daily under dynamic exposure conditions over a period of 15, 30 and 45 days. Control groups were simultaneously

exposed to a current of compressed air only under identical experimental conditions.

Histological processing :

The animals of control and experimental groups were vivisected on 15th, 30th and 45th days respectively after exposure to wood smoke. The exposure lung was carefully removed. These organs were rinsed in 0.85% saline solution for three times to remove any blood and debris attached on the external surface. Then the tissues were cut into small pieces of approximately 2-4 mm and fixed in Bouin's fluid for 18 hours. After fixation, tissues were dehydrated through ascending grades of ethanol. There after, it was cleared in xylene and finally embedded in paraffin wax with a 58-600 C melting point. Paraffin sections were cut at 6 mm using a rotary microtome, the sections were mounted on clear slides and stained with haematoxylin and eosin. Observation was made using a binocular compound microscope and photographs were taken with a digital Motic advanced biological microscope (B1 series) and microphotographs were made by the help of motic image J.I. software in machintosh computer.

Hematological studies: The blood sample was collected from femoral vein by 5 ml needle-syringe and occasionally after sacrificed the animal in a double oxalated vials. Various parameters, viz. hemoglobin levels, total erythrocyte count (TEC), total leucocyte (TLC) and differential leucocyte count were analysed by routine produces in control and exposed group of an animals. The data obtained on hematological studies were subjected to statistically analysis using student's t test.

Results :

Bodyweight and mortality :

Food intake of animals was not altered during observations period but growth rate slowed down beyond 15 days. Mortality of animals during observation period was mainly due to congestion and blockage of air ways. However, there was no relationship between the mortality and duration of exposure.

Clinical symptoms :

Following exposure to wood smoke the exposed rats showed nasal and oral irritation perinasal and perioral wetness and uncomfortable movements in exposure chamber but they recovered quickly from such clinical symptoms as they were out of exposures. There was normal intake of food and water. Clinical symptoms remain same through out the exposure periods.

Histological findings :

During autopsy lung parenchyma of the exposed animals were reddish to brownish in colour, microscopically parenchymatous blood vessels had mild congestion, hyperplasia of the lymphoid follicles, mild hypertrophy and hyperplasia of the bronchiolar lining epithelial cells, hypertrophied alveolar lining cells mild thickening of the alveolar septa and mild emphysema (Plate 2) were by 30 and 45 days of post exposure noticed during 15 days of post exposure, the tissues revealed the similar type of edaema, emphysema and congestion but epithelial cellular lesions of bronchioles were enhanced by 30 days (Plate 3) and more so during 45 days of post exposure. During these intervals polymorphonuclear infiltrative cells were replaced by mononuclear cells,

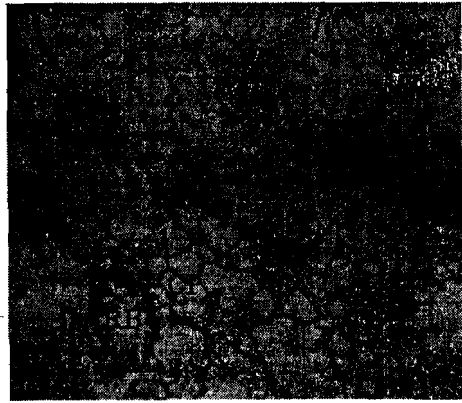


Plate 1. Section of lung of control rat showing normal architecture (RB=Respiratory branchiole, PA=Palmonary artery, CP=Ciliated epidermis, A=Alveoli

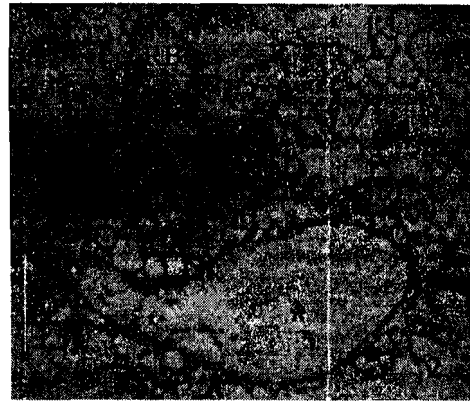


Plate 2. Section of lung showing hypertrophied and hyperplastic bronchiolar cells (*) after 15 days of post exposure to wood smoke.

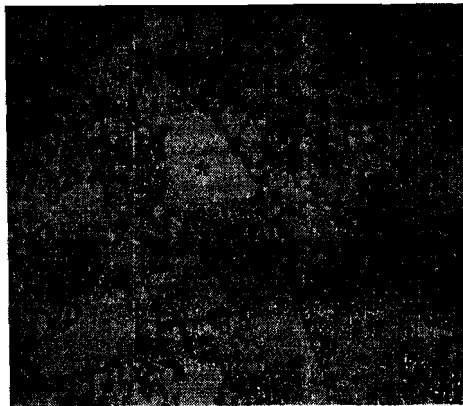


Plate 3. Section of lung showing more marked bronchiolar lesions (*) after 30 days of post exposure to wood smoke.

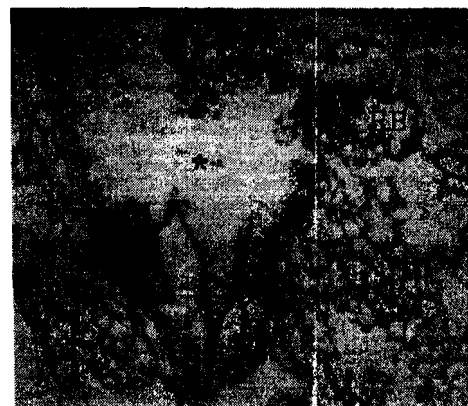


Plate 4. Section of lung showing extensive hypertrophy and hyperlasia of bronchiolar epithelial cells (*) after 45 days of wood smoke exposure.

**Table 1. Hematological parameters in Albino rats for various durations
(Control and Exposure groups)**

Types of animals	Hb (%)	Total RBC×10 ⁴ mill/ccm	Total WBC×10 ² th/ccm	Neutrophil	Lymphocytes	Eosinophil	Basophil	Monocyte
Control	9.2 ± 2.75	400.00 ± 0.57	77.56 ± 22.36	66.00 ± 24.33	28.00 ± 4.04	3.66 ± 0.38	0.21 ± 0.01	2.33 ± 0.35
Treatment	8.7 ± 3.29	364.66 ± 0.33***	76.00 ± 22.47	62.00 ± 25.71	29.66 ± 9.04	6.33 ± 1.83**	0.38 ± 0.06* **	3.00 ± 0
Control	8.3 ± 3.05	394.33 ± 0.46	76.66 ± 23.71	55.66 ± 12.89	35.66 ± 7.99	3.33 ± 0.19	0.30 ± 0	3.33 ± 1.34
Treatment	7.1 ± 2.36	401.00 ± 0.47***	75.50 ± 23.18	55.33 ± 19.11	31.66 ± 9.84	9.33 ± 2.08***	0.41 ± 0.6	3.33 ± 1.52
Control	9.96 ± 2.49	409.62 ± 0.62	79.70 ± 21.61	60.66 ± 17.70	34.33 ± 2.49	3.00 ± 0	0.35 ± 0.20	3.49 ± 1.35
Treatment	6.9 ± 2.25*	421.66*** ± 0.52	68.83 ± 24.17	52.33 ± 14.33	32.66 ± 9.36	10 ± 1.52	0.51 ± 0.12	3.33 ± 1.34

* P<0.05; **P<0.01; ***P<0.001

alveolar septa thickening was marked, rather lesions progressed further with subsequent exposure (Plate 4).

Hematological studies :

The values of various hematological parameters for control and exposed groups of animals have been given in Table 1. Following exposure to wood smoke, the hemoglobin levels, R.B.C and W.B.C and DC (differential count) showed changes. Exposure to smoke W.B.C was found to be decreased (non-significantly) on day 30 and 45 while R.B.C indicated a significant increase (p<0.001) on day 30 and 45.

The differential count of neutrophil and lymphocytes showed marginal changes in exposed group, where the eosinophils increase gradually. The eosinophils showed a significant increase on day 15 and 30

(p<0.01 or higher). Among the exposed group, on day 45, the eosinophil showed highest value (Table 1).

Discussion :

It has been well confirmed that wood smoke emission play a vital role for the infection of lungs. The present study shows that during autopsy lung parenchyma of the exposed animals were reddish to brownish in colour, microscopically parenchymatous blood vessels had mild congestion, hyperplasia of the lymphoid follicles, mild hypertrophy and hyperplasia of the bronchiolar lining epithelial cells, hypertrophied alveolar lining cells mild thickening of the alveolar septa and mild emphysema were noticed during exposure of wood smoke. Dasch(1982) and Kamens et al.(1984) also reported similar results of

hyperplasia of bronchiolar lining epithelial cells, in some bronchiolar lumens had necrosed and desquamated epithelial cells, mixed with loose polymorphonuclear cells, perivascular and peribronchiolar cuffing of polynuclear cells, mild peribronchial, perivascular and parenchymatous oedema were observed in rats lung after wood smoke exposure.

Smoke decreased WBC on day 30 and 45 while RBC indicated increase on day 30 and 45. Among the exposed group, on day 45, the eosinophil showed highest value. The villagers needs, allowing for their attitudes and traditions. The people have been accustomed to living in smoky houses for generations and the smoke has some positive effects, such as protecting wood and driving out mosquitoes. Alternative methods of wood preservation using cheap paints should be adopted. Efforts should be made to motivate the villagers to adopt the necessary changes in attitudes and behavior. To make all this possible effective community participation, health education, and a general raising of the educational and socioeconomic standards of the people are essential.

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EFFICACY OF *SPIRULINA* FEED IN PRAWN (*MACROBRACHIUM ROSENBERGII*, de MAN) CULTURE

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ABSTRACT ■ Microalgae feeds are currently used in relatively small amounts in aquaculture, mainly for the production of larvae and also rearing of the juveniles. The blue green alga *Spirulina* is used in substantial amounts (100 tons Y⁻¹) as a fish and shrimp feed. In shrimp ponds indigenous algal blooms supply a part of the dietary requirements of the animals, but it is difficult to maximize productivities there. A separate algal production system could feed shrimps and minimize the need for added feed.

In the present investigation feed trial experiments on juvenile prawn (*M. rosenbergii*) were conducted using *Spirulina* feed (pelleted) alone, *Spirulina* powder mixed with other conventional feed and conventional feed alone separately. The results indicate that *Spirulina* feed alone induce much faster weight increment after 7 days than other two trials and the rate of increment gradually falls with time.

Key words : *Spirulina*; aquaculture; feed trial experiment.

INTRODUCTION :

It is well evident that fish species require high protein diet for their better growth and thereby the yield. Over past two decades, fish meal protein diet was applied in most of the managed fisheries. Increasing cost of fish meal and also non-availability of the required quantum of such feed have necessitated a different line of research to find out alternative fish feed protein sources (Kitchell and Windell, 1970; Chakravorty et al. 1973; Mathavan et al. 1976; Pantastico and Baldia 1980; Raj and Kutty 1983; Raj 1989; Daniel and Sahayaraj 1990; Daniel and Kalavalli 1991). Microalgae are the natural foods of the bivalves and fishes and

thus form the basis of essentially all freshwater and marine food chains. Now, that for many aquatic species such as shrimps, carps, and many bivalves aquaculture techniques are relatively well developed, the provision of low cost feeds, including microalgae, are becoming of increasing interest.

Considering the high protein, - carotene and lipid value of *Spirulina* feed, several trials have been made using *Spirulina* feed in aquacultural practices. An estimated 100 tons of dried *Spirulina*, a blue green alga is used for ornamental Koi fish feed in Japan, where *Spirulina* is valuable in providing pigments. *Spirulina* is also used

in smaller amounts in feeding other fishes. (Hanson 1990; Daniel and Kalavalli 1991, 1992; Ayyappan 1992) and Juvenile shrimps (Cuzon et al. 1981). Various other types of conventional protein rich feeds viz. soyabean meal, groundnut meal, squid meal were used for rearing of juvenile prawns. So far, no attempt has been made for utilization of *Spirulina* as protein feed in cultivation of prawn both in-vitro and in-vivo.

In the present study thus an attempt has been made to use dried *Spirulina* (pelleted) alone and *Spirulina* mixed with conventional feed in the feed experiment of rearing of juvenile prawns in the laboratory (i.e. in -vitro studies).

MATERIALS AND METHODS :

Spirulina, a blue-green microalgae was cultured in glass bottles (5 litres capacity) using Zarrouk's medium under laboratory and field conditions. After 7-10 days of growth, algal mass was harvested by filtering through very fine linen and the cell mass was washed and dried. Air dried algal mass was then powdered and pelleted using Carboxy-methyl-cellulose (CMC) as binding agent.

In addition, *Spirulina* powder was mixed with mixture of soyabean meal and shrimp meal and maize meal (in the ratio 20:20:10) in equal quantity (i.e. *Spirulina* powder : mixture = 1:1) and then pellets were made using CMC as binder.

Another conventional pelleted feed was prepared using shrimp meal, soyabean meal, maize meal and wheat flour (in the ratio 30:30:30:10) fortified with vitamins and

trace elements.

The feed trials were carried out separately in aquaria (size 3.5ft x 1.5ft x 1.5ft). In each aquarium ten juvenile prawns were used for testing (average length 5.0 cm). The feed was applied at the rate of 10% of the body weight of the test animal per day to each aquarium. The aquaria were placed under constant aeration and 50% of the water of each aquarium was replaced at every 48 hours interval. Each experimental set was replicated thrice in the same environmental conditions.

The relative growth rates with respect to size and weight were examined at an interval of seven days up to twenty eight days. All these data were recorded separately. The final result is expressed in tabular and graphical ways after proper statistical analysis.

The physico-chemical properties viz. dissolved oxygen, temperature, pH total alkalinity and Ammonium nitrogen of water in each aquarium were measured at an interval of seven days using the conventional method of APHA (1985). The mean values of each parameter are tabulated in Table-3.

The weekly growth of the juvenile prawns were measured in terms of size and body weight increments. The data thus recorded is presented in Table-2 and Fig-1 and Fig-2.

RESULTS :

The temperature of the aquaria water was maintained at $29 \pm 1^\circ\text{C}$ and pH ranged 8.0 to 8.5. The dissolved oxygen content, total

Table-1. Physico-chemical parameters of water

Water parameter	7 th Day					14 th Day					21 st Day					28 th Day				
	Temp	pH	D.O.	T.A.	NH ₄ N	Temp	pH	D.O.	T.A.	NH ₄ N	Temp	pH	D.O.	T.A.	NH ₄ N	Temp	pH	D.O.	T.A.	NH ₄ N
Aquaria with <i>Spirulina</i> feed	29	8.1	6.2	35.6	1.68	29.5	8.2	6.4	80.5	1.46	30.2	8.3	5.8	120	1.50	28.5	8.2	6.12	110.20	1.58
Aquaria with conventional feed and <i>Spirulina</i> feed mixture	29.5	8.2	4.4	20.24	1.46	29.4	8.10	6.3	50.8	1.58	29.8	8.3	8.4	61.6	1.62	28.8	8.3	6.8	140.2	1.12
Aquaria with conventional feed	29.2	8.3	6.6	50.5	2.2	29.5	8.4	7.6	70.4	1.68	30.2	8.1	6.8	140.8	1.18	28.6	8.4	6.61	136.8	1.26

Temp – Temperature °C; D.O. – Dissolved Oxygen mg l⁻¹;

T.A. – Total Alkalinity mg l⁻¹; NH₄N – Ammonium Nitrogen mg l⁻¹

alkalinity and ammonium nitrogen content of water samples varied from 4.4 to 8.4; 20.24 to 140.8 and 1.12 to 2.2 mg l⁻¹ respectively.

The total size increment of the juvenile prawn was higher in *Spirulina* feed than *Spirulina*-conventional feed or conventional mixture (Table. 2 & Fig. 1 & 2). The body weight increment (i.e. yield rate) was highest in *Spirulina* feed ((1.53 gms) which is followed by *Spirulina* conventional feed (1.20 gms) and the lowest was recorded in conventional feed (0.76 gm) the percentage increase of growth rate in case of *Spirulina* feed was 57.36% in contrast to *Spirulina*-conventional feed (50%). The food conversion ratio (FCR) in case of the

Spirulina feed was calculated to be 3.18 and that of the *Spirulina*-conventional feed in 6.9. All these imply that there is a great potentiality of prawn rearing by *Spirulina* feed.

The natural quality of the feeds use in the experiments were also considered for growth rate interpretation. Table-3 represent the data relating to the major nutritional values of the feed types used.

From the study of nutritional quality of different feeds it appears that *Spirulina*-conventional feed mixture and *Spirulina* powder were balanced feed for shrimp growth. This observation was reflected in the fish yield assay experiment in-vitro.

Table-2. Feed trial experiments: Growth rate analysis (\pm SE)

Feed type	Size increment (cm) at different period (days)				Body weight increment (gms) at different period (days)				Total body weight increment (gms)	% of growth	FCR
	7	14	21	28	7	14	21	28			
<i>Spirulina</i> feed (pelleted)	* 0.62 \pm 0.15	* 0.33 \pm 0.10	0.28 \pm 0.05	0.25 \pm 0.10	* 0.65 \pm 0.15	0.35 \pm 0.05	0.28 \pm 0.10	* 0.25 \pm 0.50	1.53 \pm 0.15	57.36	3.18
<i>Spirulina</i> powder mixed with conventional feed (1:1)	0.33 \pm 0.20	0.25 \pm 0.10	0.15 \pm 0.05	0.11 \pm 0.10	0.22 \pm 0.10	0.15 \pm 0.10	0.20 \pm 0.05	0.16 \pm 0.10	1.20 \pm 0.10	50.0	6.19
Conventional feed	* 0.58 \pm 0.10	* 0.30 \pm 0.05	* 0.19 \pm 0.10	* 0.15 \pm 0.05	* 0.31 \pm 0.05	0.20 \pm 0.50	0.15 \pm 0.01	0.10 \pm 0.05	0.76 \pm 0.10	42.4	6.88

* significant at 5% level

Fig-1. Effect of Feed Trial on Size Increment

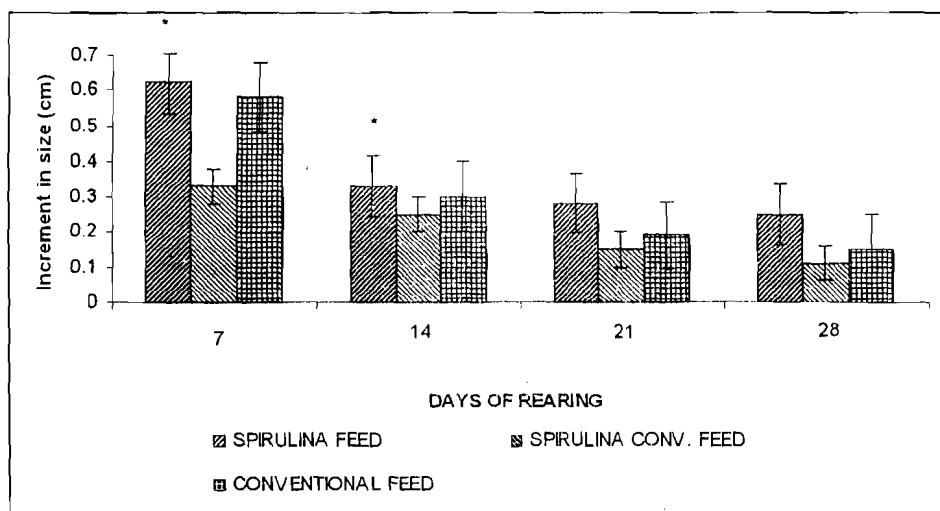
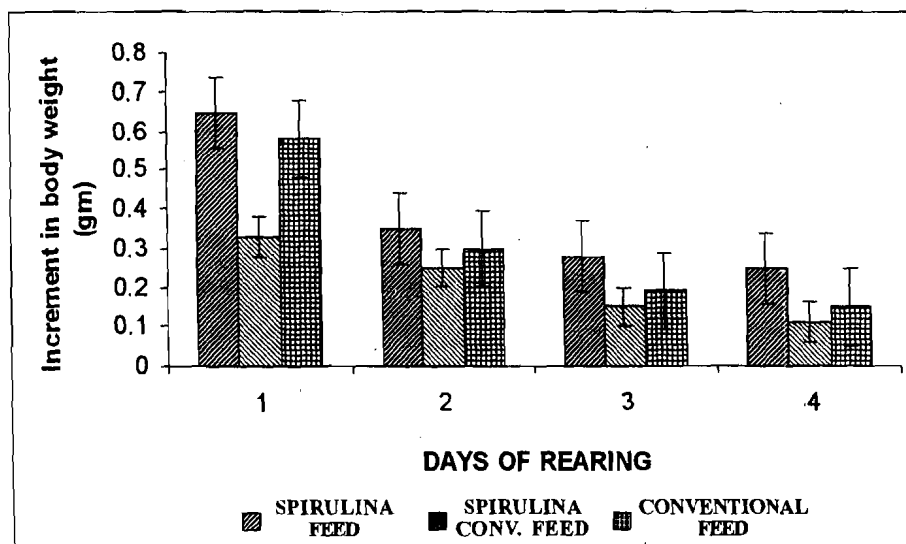


Table-3
Nutritional quality of the feed types (gms/100 gms)

Nutritional	<i>Spirulina</i> feed	Conventional feed and <i>Spirulina</i> mix.	Conventional feed
Carbohydrate	16.00	20.00	18.00
Protein	65.00	25.00	52.00
Fats	6.70	9.50	7.50
Minerals	2.50	1.50	1.75
Vitamins	0.05	0.02	0.03

Fig-2
Effect of Feed Trial on Body Weight Increment



DISCUSSION :

In most of the fish and shrimp culture ponds there are dense algal blooms due to the application of manures (Schroeder et al 1990; Zhu et al 1990) or simply as a result of nutrient release from feed applications. The large algal blooms contribute directly or indirectly (through a zooplankton or benthic micro fauna food- chain) to the nutrition of the animals. However, in such systems there is essentially no control of the algal or zooplankton populations. Indeed blue- green algal blooms often create problems (Severin - Reyssac and Pletikovic, 1991). But this system can not be considered as algal cultures, but may become such if management techniques could be perfected.

However, at present micro algae feeds have limited application in aquaculture because of its technical and economic reasons. In general, the cost of production is comparatively higher and skilled technical personnel are needed for commercial scale production of the algal feeds.

During the current years, among the algal feeds, *Spirulina* is being used as a feed in Salmon culture, Koi culture and also for shrimp culture (Mori et al 1987; Siegel, 1989). Shrimps are detritivores, they graze the ponds for food particles, which includes dead or living algal, bacterial and fungal particles and small benthic animals, in addition to the feeds supplied to the ponds by the aquaculturists, which again consists of a large variety of different food materials as may be locally available (New, 1987). The present study thus indicates the scope of the use of *Spirulina* feed alone for rearing

of prawn in a positive direction in future by assessing the cost-effectiveness in more rigorous ways.

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Crichton, Michael. 2006. *Next*. London: Harper (Paperback).

PP.X+431. Price: Rs.195/-

Michael's talent outscaled even his own dinosaurs of 'Jurassic Park.'

----- Steven Spielberg on Michael Crichton's death.

Before reviewing this excellent book for an academic journal, I asked about a dozen academic personalities (many of them belonged to the discipline of Anthropology) a common question: 'Have you heard the name Michael Crichton?' The answer which came from more than 95 per cent of my sample was in the negative. My immediately next question was: 'Have you heard the name of the novel Jurassic Park?' One hundred per cent of my respondents confirmed that they know the name of the novel which was filmed by Steven Spielberg.

Very few of us in the academic world in India know that Michael Crichton received his undergraduate degree in Biological Anthropology from Harvard University and later took his Masters' in Medicine from Harvard Medical School and was visiting lecturer in Anthropology at Cambridge University. He finally gave up academic profession and took up science fiction writing and became famous for his novels based on most advanced areas of scientific research and received a number of literary awards. Crichton was born on October 23, 1942 and died with throat cancer on November 4, 2008.

The novel *Next* is not simply a fiction. The first page of the book contains a sentence which reads as follows: 'This novel is a fiction, except for the parts that aren't.' It is a book

which must be read by the biological scientists who are pursuing their research careers in stem cell, transgenic animals and gene patenting. Because under the cover of a techno-thriller this book raised basic questions regarding the nature of our genes and the right of the private corporations to patent certain genes which may act to cure deadly diseases, like, leukemia.

The central characters of the book are not scientists but are affected and/or influenced by genetic research. The novel starts with one Frank Burnet who contracted leukemia and during medical check-ups it was found that his body cells developed immunity against the disease and he was then made to sign documents by the scientists of a famous university in USA which allowed the scientists to collect Mr. Burnet's cells for the greater cause of medical research. After that the cell lines from Brunet's cells were developed as curative drugs, the university sold the patent to a big corporate, a drug company. When Brunet and his daughter (a lawyer) came to know that the company is making staggering profit by selling Brunet cell lines they filed a legal suit and lost in the local court under the existing patent laws of USA. The thrill of the novel then begins and Crichton proceeded with a super fast speed packing enormous amount of information

from leading journals and books of the frontier areas of genetic research. The bibliography of the novel cited 35 books, 10 journal and newspaper articles (including articles in *Science* and *New England Journal of Medicine*) and 12 scientific articles published in the websites.

At the end of the book an 'Author's note' (pp. 417- 423) is added in which Crichton summarised his views on gene patenting which reads almost like the recommendations of a research paper dealing with contemporary problems. Here we must give some information on the impact of this brilliant book on the policy and law of the USA. This novel raised public interest on the implications of gene patenting, which according to Crichton are 'Facts of nature', like gravity, sunlight, and leaves on trees and therefore, they cannot be owned. Let me quote from the author: 'You can own a test for a gene, or a drug that affects a gene, but

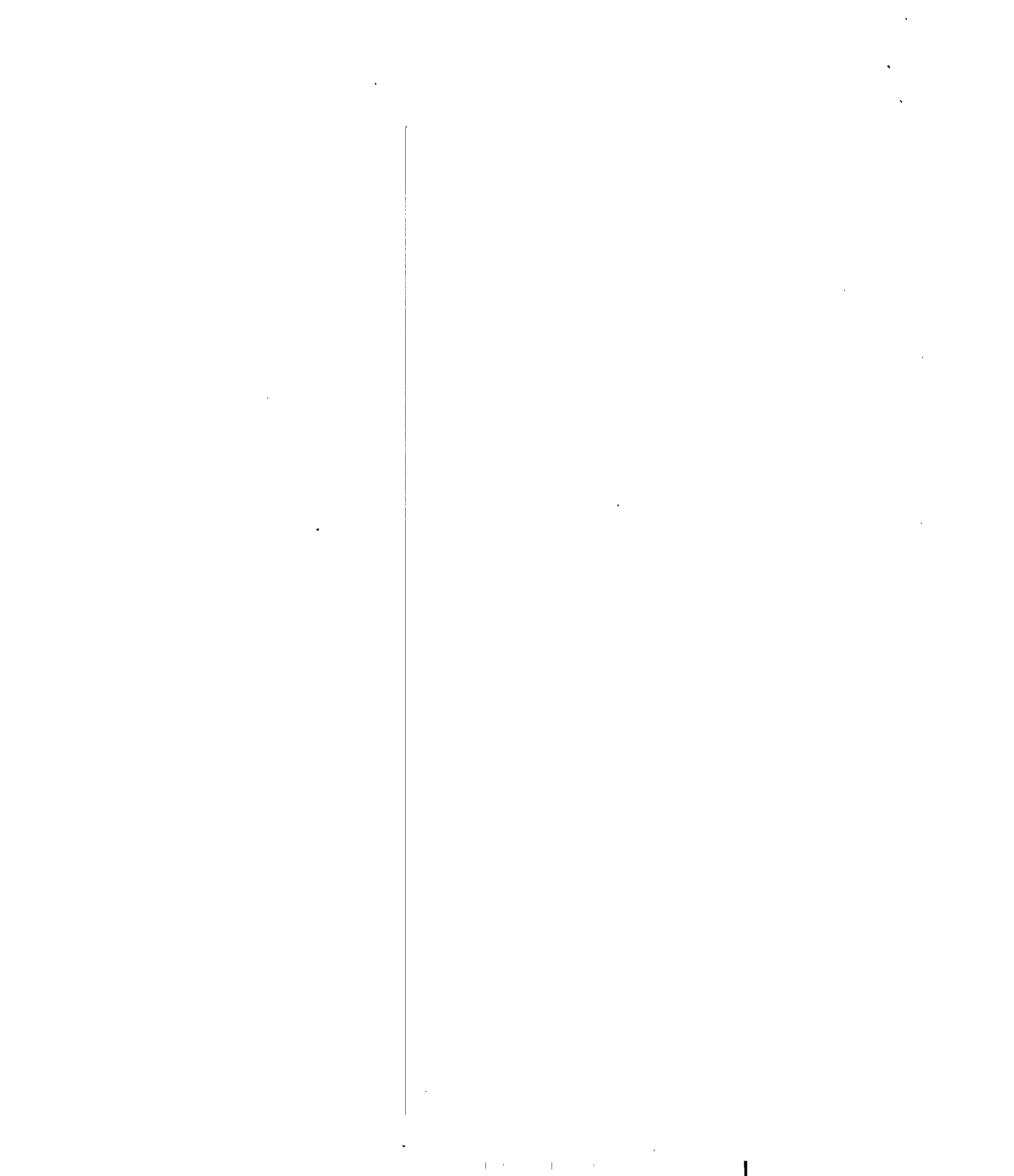
not the gene itself. You can own a treatment for a disease, but not the disease itself. Gene patent break that fundamental rule.'(p.417) This is the essential message of the novel *Next* which directs our attention to the future of research in biology and particularly biotechnology. It is no wonder that the biotechnology industry magazine *Genetic Engineering and Biotechnology News* vehemently criticized *Next* and hoped that the poor reviews of the book will reduce its impacts. But we learn from the Wikipedia that two Congressmen of the US Senate introduced a bill to ban future gene patents and abolish the existing ones. The biological scientists too much enthusiastic about gene patenting in India should read *Next* to enhance their ethical and social responsibility and this probably justifies the review of an unusual novel in a professional journal published by the academic departments of a non-traditional university.

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