Original Article

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Biochemistry Section

The Clinical and Biochemical Parameters in Relation to the Serum Neopterin Levels in Indian Children and Adolescents

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ABSTRACT

Introduction: An adverse pattern of blood lipids and cardiovascular abnormalities starts in obese children during childhood and neopterin serves as a marker for cardiovascular disease. Unfortunately, the data for children and adolescents, particularly, in the Indian population, are scarce. The present study aimed at evaluating the levels of serum neopterin in obese and overweight children and adolescents of the Indian population.

Methods: The study groups included 296 school going children and adolescents. (96 obese and 97 overweight subjects were compared with 103 normal controls who were aged between 10-17 years). The anthropometric variables, the lipid profile, the fasting serum glucose which was analyzed by using an autoanalyzer and the serum neopterin levels were assayed by HPLC (Shimazdu) by using the method of Palfrey *et al*., 1993. The serum insulin levels were measured by using ELISA kits.

Results: The serum neopterin levels (nmol/l) were elevated significantly in the obese (7.4±1.4) and overweight (6.4±0.8) ($p<0.001$) children and adolescents than in the controls (4.9±0.9). The serum neopterin levels showed a positive correlation with the BMI (r=0.79), WHR (r=0.5), systolic (r=0.44) and diastolic blood pressures (r= 0.25), insulin (r=0.57), HOMAIR (r=0.55), total cholesterol (r=0.35), triglycerides (r=0.20) and LDL-C (r=0.27) and they showed a negative correlation with HDL-C (r=-0.15) and fasting glucose (r= -0.3).

Conclusion: This study revealed a good relationship between serum neopterin and the anthropometric and biochemical parameters. We, therefore, aim to conduct regular camps at schools to counsel and advise the identified overweight and obese children to go for physical exercise and a balanced diet. The implementation of preventive measures from early childhood will have far reaching benefits, as even the prevalence of other obesity related disorders could decline.

Key Words: Children, Obesity, Neopterin, HPLC

INTRODUCTION

The occurrence of overweight and obesity among children and adolescents is rapidly growing in India. It has been well established that overweight children and adolescents have an increased risk of adult obesity [1]. Compelling evidence has emerged in recent years, regarding the relationship between obesity, inflammation and cardiovascular diseases [2]. Inflammation plays an essential role in the natural history of atherosclerosis, which includes the initiation, progression and the rupture of the vascular plaque [3]. Several lines of evidence have supported a link between the adipose tissue and the impaired immune function in both humans and genetically obese rodents. There is a great deal of evidence that the obese adipose tissue is infiltrated by macrophages. Weight loss is associated with a reduction in the macrophage infiltration of the adipose tissue and an improvement in the inflammatory profile of the gene expression [4]. The growing appreciation of the role of inflammation in atherogenesis has triggered interest, as to whether the circulating inflammatory biomarkers may help in identifying the subjects who are at a risk of future cardiovascular events. Therefore, finding the markers of inflammation would be of great importance for the screening and early treatment of children and adolescents. The scientific literature has revealed the presence of increased circulating levels of serum neopterin in obesity, diabetes and cardiovascular diseases [5-7]. Neopterin is a pteridine compound which is produced by human and primary monocyte/macrophages upon the activation by proinflammatory stimuli Th-1 type cytokine interferon gamma. Neopterin enhances the macrophage cytotoxicity through its interaction with reactive oxygen, nitrogen and chloride species, which results in the production of cytotoxic NO radicals, which promotes oxidative stress-triggered apoptosis of the vascular smooth muscle cells and also plaque growth [8,9]. It was of particular interest for us to study the possible role of serum neopterin in Indian children and adolescents and its correlation with the anthropometric and biochemical parameters. To date, no published data are available on the serum neopterin levels in Indian children and adolescents.

METHODS

A total of 296 school children of Chennai, India, whose ages ranged from 10 to 17 years, were enrolled as the study participants. Informed written consents were obtained from their parents before the commencement of the study. The children with overweight and obesity were included in the study. The study was carried out at the International Centre for Cardio-Thoracic and Vascular Diseases, a unit of Frontier Lifeline Hospitals, Chennai, India. The children with the secondary causes of obesity and insulin-dependent and insulin-independent diabetes mellitus and the children who underwent treatment for any other disorder, were excluded from the study. The anthropometric measurements such as height, weight, BMI and the waist-to-Hip Ratio (WHR)

were recorded. Weight was measured by using a beam balance to the nearest 0.1 kg and height was measured to the nearest centimeter by using a tape which was stuck to the wall. The abdominal girth was measured at the level of the umbilicus, with the subject in a relaxed mood and in a standing posture. The hip girth was measured at the widest point of the hips, at the level of the greater trochanter, with the patient standing with both feet together. The Waist-to-Hip Ratio (WHR) was calculated from these measurements. The children with a BMI of > the 85th percentile for age and gender were considered as overweight and the children with a BMI of > the 95th percentile for age and gender were considered as obese by using CDC growth charts. The blood pressure levels of all the children were also recorded by using a mercury sphygmomanometer. A detailed questionnaire regarding the medical history of the parents and the children were recorded. This study was approved by the Institutional Ethics Committee.

Twelve hours fasting blood samples were collected from all the children, the sera were separated and the samples were stored at -20^{0}C till the time of their analysis. The lipid profile, which included total cholesterol, LDL-C and HDL-C were analyzed by enzymatic methods, triglycerides were analyzed by the GPO-PAP method and the fasting blood glucose levels were analyzed by the GOD-POD method by using an autoanalyzer (Randox Daytona). The serum Neopterin levels were assayed by HPLC (Shimazdu) by using the method of Palfrey *et al*., [10]. The serum insulin levels were measured by using ELISA kits (Monobind Inc USA). The homeostasis model assessment (HOMA-IR) was calculated by using the following formula.

HOMA-IR = fasting insulin (μiu/ml) × fasting glucose (mg/dl)/22.5.

STATISTICAL ANALYSIS

The statistical analysis of the data was carried out by using the SPSS package, version 9.0. The results were expressed as Mean ± SD and P value of < 0.05 was considered to be statistically significant. The data of significance among the groups were analyzed by one way ANOVA and Bonferroni comparisons. A correlation analysis was done by using Pearson's correlation at a 5% level of significance. Since some of the parameters were slightly skewed, we applied logarithmic transformations for all the statistical analyses.

	Control (n=103)	Overweight (n=97)	Obese (n=96)
Age	14.45±1.4	13.93±1.5	14.07±1.6
BMI(Kg/m^2)	18.0±2.1	24.1±1.4**	28.4±2.6**
WHR	0.85±0.09	0.99±0.13**	1.02±0.13**
Systolic B.P(mmHg)	117.6±5.5	120.5±7.1†	124.5±8.8**
Diastolic B.P(mmHg)	75.4±6.7	77.9±9.0 NS	76.6±9.0 NS
Neopterin (nmol/L)	4.9±0.9	6.4±0.8**	7.4±1.4**
Insulin (μU/mL)	3.4±2.8	13.9±5.9**	20.1±8.4**
HOMA-IR	0.7±0.6	2.9±1.3**	4.0±1.7**
TC (mg/dl)	140.9±18.2	151.9±21.8**	164.2±22.1**
TG (mg/dl)	75.7±26.9	90.0±32.3†	103.3±36.2**
LDL-C (mg/dl)	87.9±12.3	90.7±12.3 NS	92.6±12.3 NS
HDL-C (mg/dl)	39.5±4.8	39.0±4.0 NS	38.4±3.5 NS
Fasting glucose (mg/dl)	87.8±6.6	81.9±6.2**	81.0±6.5**
Non Veg (%)	64.0	74.2	86.4

[Table/Fig-1]: Comparison of various parameters between controls and overweight, obese, children.
Results are expressed in Mean ± SD.
**P<0.001; *P<0.005; ††P<0.01; †P<0.05; NS-non - significant.
BMI- Body Mass Index, WHR-Waist-to-hip ratio, TC- Total Cholesterol, TG- Triglycerides.

RESULTS

The mean levels of the biochemical and the anthropometric parameters have been summarized in [Table/Fig-1]. Among the 296 subjects (103 control, 97 overweight, 96 obese) 156 were boys and 140 were girls, with an age range of 10-17 years. The mean serum neopterin levels (nmol/l) were elevated significantly in the obese (7.4±1.4) and overweight (6.4±0.8) (p<0.001) subjects than in the controls (4.9±0.9). The anthropometric measurements were found to be significantly higher in the overweight and obese children as compared to those in the controls. This was natural as per the diagnostic criteria. With respect to the comparison between boys and girls, the serum neopterin levels did not vary significantly between the boys and girls in all the groups. The descriptive statistics have been shown in [Table/Fig-2].

The relationship between the serum neopterin levels and the biochemical and the anthropometric parameters for all the groups has been shown in [Table/Fig-3]. The serum neopterin levels

	Control		Overweight		Obese	
	Boys(55)	Girls(48)	Boys(47)	Girls(50)	Boys(54)	Girls(42)
Age	14 ± 1.4	14.4±1.3	14.0±1.4 NS	13.8±1.5 NS	14.2±1.5 NS	13.9±1.6 NS
BMI (Kg/m^2)	18.1±2.2	18.0±2.1	24.0±1.4**	24.2±1.4**	28.1±2.6**	28.7±2.4**
WHR	0.86±0.08	0.84±0.10	0.97±0.10**	1.01±0.15**	0.99±0.11**	1.05±0.15**
Systolic B.P (mmHg)	116.7±5.8	118.5±5.1	120.9±6.9†	120.2±7.4 NS	124.3±8.8**	124.8±8.9**
Diastolic B.P (mmHg)	74.4±7.6	76.7±5.2	77.0±9.5 NS	78.8±8.5 NS	73.9±8.1 NS	80.0±9.1 NS
Neopterin (nmol/L)	4.9±0.9	5.0±0.8	6.5±0.8*	6.3±0.9*	7.6±1.5*	7.2±1.2*
Insulin (μU/mL)	3.0±2.6	3.9±2.9	13.6±6.4**	14.2±5.5**	18±7.2**	22.9±9.1**
HOMA-IR	0.6 ±0.5	0.8±0.6	2.8 ±1.4**	2.9 ±1.1**	3.6 ±1.4**	4.6 ±1.8**
TC (mg/dl)	139.2±19.7	142.9±16.4	148.6±18.5 NS	155.1±24.2†	159.4±20.8**	170.3±22.4**
TG (mg/dl)	77.5±28.6	73.7±24.9	85.6±32.0 NS	94.1±32.4†	97.9±32.2††	110.1±40.1**
LDL-c (mg/dl)	86.6±13.6	89.4±10.5	89.2±12.9 NS	92.2±11.6 NS	90.7±12.1 NS	94.9±12.4 NS
HDL-c (mg/dl)	39.1±4.9	39.9±4.7	39.3±4.5 NS	38.7±3.6 NS	38.1±3.5 NS	38.7±3.5 NS
Fasting Glucose (mg/dl)	86.4±6.2	89.5±6.7	80.5±6.0**	83.4±6.0**	80.2±6.3**	81.9±6.7**

[Table/Fig-2]: Comparison of various parameters between boys and girls of different groups.
Results are expressed in Mean ± SD.
**P<0.001; *P<0.005; ††P<0.01; †P<0.05; NS-non - significant.
BMI- Body Mass Index, WHR-Waist-to-hip ratio, TC- Total Cholesterol, TG- Triglycerides.

	Overall (296) rho	pvalue	Boys (156) rho	pvalue	Girls (140) rho	pvalue
BMI(Kg/m^2)	0.79	0.001	0.80	0.001	0.79	0.001
WHR	0.50	0.001	0.60	0.001	0.44	0.001
Systolic B.P (mmHg)	0.44	0.001	0.47	0.001	0.40	0.001
Diastolic B.P(mmHg)	0.25	0.001	0.22	0.003	0.33	0.001
Insulin (µU/mL)	0.57	0.001	0.56	0.001	0.59	0.001
HOMA-IR	0.55	0.001	0.55	0.001	0.59	0.001
TC(mg/dl)	0.35	0.001	0.35	0.001	0.38	0.001
TG(mg/dl)	0.20	0.001	0.39	0.06	0.37	0.001
LDL-C(mg/dl)	0.27	0.001	0.26	0.001	0.30	0.001
HDL-C(mg/dl)	-0.15	0.004	-0.18	0.01	-0.11	-0.10
Glucose(mg/dl)	-0.3	0.001	-0.36	0.001	-0.22	0.005

[Table/Fig-3]: Pearson's Correlation Analysis between Serum Neopterin and Anthropometric, Biochemical Variables of the Study Subjects.

	Control (%)	Overweight (%)	Obese (%)
Obesity	20.3	56.7	55.2
Diabetes	11.6	36	30.2
Hypertension	15.5	32	37.5
Heartdisease	4.0	9.2	7.3

[Table/Fig-4]: Comparison of family history between obese, overweight and controls.

showed a positive correlation with the BMI (r=0.79), WHR (r=0.5), the systolic (r=0.44) and diastolic blood pressures (r= 0.25), insulin (r=0.57), HOMAIR (r=0.55), total cholesterol (r=0.35), triglycerides (r=0.20) and LDL-C (r=0.27) and they showed a negative correlation with HDL-C (r=-0.15) and fasting glucose (r= -0.3).

The family histories of obesity, diabetes, hypertension and heart diseases for all the children who were enrolled in the study have been shown in [Table/Fig-4]. The socioeconomic statuses and the physical training programmes of all the children who were enrolled in the study were found to be similar.

DISCUSSION

The medical literature has pointed out the vital role of serum neopterin in obesity and its correlation with inflammation [3,5]. However, no evidence is available so far, to describe the role of serum neopterin in children and adolescents of the Indian population. We, therefore, aimed to dissect the role of the serum neopterin levels and their correlation with the anthropometric, clinical and the biochemical parameters in Indian children and adolescents. We observed for the first time, to our knowledge, an increase in the serum neopterin levels in both obese and overweight children. Our results were identical to the reports of earlier studies which were done on adults [7-9,11,12] Our findings reinforced the notion, that neopterin stimulates redox sensitive intracellular signal transduction cascades, thereby triggering an inducible NOS gene expression at the messenger ribonucleic acid levels, with a subsequent increase in the nitric oxide production, which has been shown in in vitro rat heart models to induce contractile failure [13] Accordingly, it is possible that the high levels of neopterin in adiposity would indicate the degree of activation of the cellular immune system and the role of inflammation in cardiovascular diseases. However, recently, one elegant study found no marginal differences in the serum neopterin levels between obese adolescents and non obese controls [14].

The results of this study indicate a close positive association between the serum neopterin levels and the anthropometric parameters. The available information suggests that BMI is the main determinant for the variations of the serum neopterin levels and our data agree with those which have been reported by others [5,15-16]. In the present work, we found elevated systolic and diastolic blood pressures in both obese and overweight children and adolescents as compared to those in the controls. Thus, it is generally believed that elevated neopterin levels in overweight and obese subjects indicate the pathophysiological role of neopterin in obesity related hypertension. Our results were also at par with those of other investigators [17,18].

Like in other studies, we also observed higher serum insulin levels and HOMA-IR levels in both obese and overweight children and in adolescents than in the controls [18-20]. Nevertheless, a good correlation was seen between the neopterin levels and insulin and HOMA-IR. At this juncture, it is appropriate to mention that the infiltrated macrophages probably contribute to the pathogenesis of the insulin resistance [5,7]. We observed a strong positive correlation between the serum neopterin levels and TC, TG and LDL-C and a negative correlation with HDL-C. Taken together, our findings suggested that higher neopterin levels in childhood obesity were associated with dyslipidaemia. One of the most fascinating aspects in our data was that the fasting glucose levels were found to be decreased in both obese and overweight children and in adolescents and this implied a negative correlation between neopterin and fasting glucose. The possible explanatory mechanism for this could be that in obesity, insulin probably decreases the blood glucose concentration by reducing the hepatic gluconeogenesis and the glycogenolysis and by enhancing the glucose uptake into striated muscles and adipocytes [20]. In contrast to our report, other authors found a positive association between the neopterin levels and blood glucose [5].

On further comparison of the serum neopterin levels between boys and girls, we found no marginal differences between them in all the groups. This indicates that probably the sex hormones do not influence the serum neopterin levels in children and adolescents. The present study confirmed the results of previous studies [21,22]. The strengths and limitations of our study deserve comments. The potential limitation of our study was a lack of the puberty assessment and the lack of a detailed measurement of the dietary habits of children and adolescents. Also, the sample size was too small, to draw valid conclusions.

Among the overweight and obese children and adolescents the parental history of obesity, hypertension, diabetes and heart diseases was 56%, 35%,33% and 8.5% respectively. This confirms the strong genetic influence on the childhood obesity, diabetes and the cardiovascular diseases [23].

In conclusion, our findings indicate that higher neopterin levels enhance the oxidative stress and the immune stimulation and that they act as proinflammatory biomarkers of the cardiovascular

diseases. We, therefore, aim to conduct regular camps at schools to counsel the identified overweight and obese children and to encourage physical exercise, which would help in reducing the risk of these children being prone to the major cardiovascular anomalies in adulthood. Furthermore, the future research should endeavour to build upon the role of serum neopterin in childhood obesity and its complications.

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