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STUDY OF VITAMIN B12, FOLATE AND ADIPOKINES WITH RESPECT TO INSULIN RESISTANCE (IR) AND ANTHROPOMETRY (BIRTH WEIGHT AND BMI) IN CHILDREN (AGE 10 – 20 YEARS)

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ABSTRACT

Background: Childhood obesity is set to increase phenomenally in India in near future. Birth weight a reflection of maternal nutrition and fetal programming, which may predict the cause of obesity. Micronutrients (Vitamin B12, Folate) and Adipokines (adiponectin and leptin) also play an important role in development of insulin resistance and obesity. The aim of the study is to evaluate the combined effect of micronutrients and adipokines on insulin resistance in Indian school-going children in the age group of 10–20 years. This study also describes distribution of micronutrient concentrations and it's interrelationship with birth weight, body mass index (BMI), and insulin resistance. **Methods**: A cross-sectional study involves a total of 200 school going children between the age group of 10-20 yrs. Fasting plasma glucose, insulin, adiponectin, leptin, Vitamin B12, and folate were measured for the school going children and birth weight was recorded. BMI body mass index was defined as weight (kg) divided by height (m) squared. Insulin resistance was assessed using the homeostasis model (HOMA-IR). **Results**: Birth weight was not related to folate concentration but inversely to leptin and insulin resistance. Obese Children had higher leptin, birth weight, insulin with lower folate, and adiponectin levels. **Conclusion**: Adipokines partially protect fat induced obesity. Leptin induces insulin resistance and inflammation. Insulin resistance, leptin, and adiponectin were associated with fat mass. Folate consumption protects from developing obesity and adiposity. However the relationship of birth weight and BMI with micronutrients and adipokines needs further evaluation.

KEYWORDS: Adiponectin and Leptin, Vitamin B12, Folate, Birth weight, BMI, and Insulin resistance.

INTRODUCTION

Obesity is the major concerned with respect to metabolic syndrome worldwide. Epidemiologic researches showed that increased body weight and abdominal body fat accumulation cause insulin resistance (IR) and its complications.[1] In 2015 World Health Organization (WHO) reported that 39.0% of adults are overweight and 13.0% are obese.[2] Obesity, insulin resistance and type 2 diabetes have become unprecedented public health challenges around the world.[3,4] In developing countries, the prevalence of overweight and obesity has also increased from 8.1% in 1980, to 12.9% in 2013 for boys and 8.4 to 13.4% for girls.[5] This modern epidemic is expanding rapidly into less developed countries such as India and China.[6,7] There is increasing prevalence of childhood obesity in their adolescent age. Childhood obesity in the United States has more than doubled in the past 30 years: from 7% in 1980 to nearly18% in 2012[.8, 9] Childhood obesity is closely associated with adult obesity[10,11] and increased risk for insulin resistance, type 2 diabetes and cardiovascular diseases.[12–15] Adipokines and micronutrients may contribute to the development of obesity and insulin resistance.

Lots of research work has been carried out to study the root cause of obesity and its relation to various factors like, environmental, genetics, maternal nutrition, birth weight, inflammatory markers, adipokines and micronutrients. Indians are also reported to have low body mass index but higher adiposity.[16] Rising prevalence of obesity in developing countries is attributed to rapid sedentary life style and reduced physical activity. On the other hand, cytokines, such as tumor necrosis factor (TNF-α) and resistin, play an important role in cardiovascular risk and insulin resistance. Adipokines such as leptin, resistin, TNF-α, adipsin, visfatin, interlukines-6 and adiponectin are biological active molecules produced by adipose tissue. Reduction in adiponectin plays a primary role in the development of insulin resistance. Obesity is associated with low insulin sensitivity and high plasma leptin concentrations,[17] Limited data suggests that adiponectin

is 33% lower in obese children with diabetes than non diabetic.[18]

Birth weight is the reflection of maternal nutrition and fetal programming. Data analysis suggests that birth weight and obesity in adolescent age are interrelated. Studies show that there is a positive relationship is reported between birth weight and BMI in childhood or adulthood. This focused attention on the importance of intrauterine life as a determinant of later health. The link between intrauterine exposure and obesity and type 2 diabetes has been explained by the concept of fetal programming in relation to nutrition.[19,20] In 1991 Hales & Barker reported that low birth weight and thinness at birth are risk factor for type 2 diabetes,.[21,22] Adults with low birth weight were found to have an increased risk of developing diabetes, hypertension, coronary heart disease and stroke in adulthood.[23]

A Micronutrient deficiency continues to be major public health issue concerned in number of regions of the world, not only in the developed countries but also in developing countries like India. Deficiency in maternal folate during pregnancy may lead to adverse health outcomes and low infant birth weight, resulting in risk of long term. However some studies have found that overweight and obese adults had low blood concentration of thiamine, vitamins B6, B12, and folic acid.[24,25] There has been a study available showing the impact of micronutrients supplementation in pregnancy and its out comes in relation to birth size.[26] In recent studies, especially vitamin D, vitamin B12 and folic acid deficiency have been detected in obese individuals.[27-29]

Studies in India have shown a low dietary consumption of vitamin B12 due to dietary pattern of vegetarianism and poor consumption of milk and milk products. In vegetarian Indians, deficiency of vitamin B12 is commonly seen while concentrations of folate are adequate.[30,31] Plasma vitamin B12 and folate are required for the synthesis of methionine from homocysteine. Methionine is the precursor to S-adenosyl-L-methionine (SAM), a universal methyl donor active in metabolic pathways related to the synthesis of hormones, neurotransmitters, nucleic acids, and proteins, particularly in the brain. Elevated plasma homocysteine[32,33] has been associated with low concentrations of SAM in plasma and cerebrospinal fluid[34], and with depressive symptoms.

Obesity is one of the factors for insulin resistance, which is better understood by studying the supplementary factors influencing insulin resistance. There are several factors responsible for obesity leading to metabolic syndrome. Therefore, in this study we aimed to examine the associations between serum vitamin B12, folate, cytokines, and birth weight in the general school going urban population of Pune.

MATERIALS AND METHODS

Subjects

This study involves a total of 200 school going children between the age group of 10-20 yrs. The children participating in this study mostly came from educated families who lived in an urban setting with relatively higher socio-economic background.

Design

A cross-sectional study and total students were categorized into two groups, based on their BMI: group I (120 subjects) was categorized as non obese with a BMI <25 kg/m^2, group II (80 subjects) was categorized as obese subjects with BMI ≥ 25 kg/m^2 respectively. Obesity was defined as BMI ≥ 25 kg/m^2.[36-38] All the participants have given their informed consent and the study was approved by the ethics committee of B. J. Medical College & Sassoon General Hospital, Pune (Ref letter no. BJMC/IEC/Pharmac/D1210156-58).

Inclusion criteria

All the willing school going subjects in the age group of 10-20 years children not suffering from any major illness.

Exclusion criteria

It consists of subjects not knowing the birth weight, those with diabetes mellitus or other chronic illnesses, and those on medications at the time of the study.

Participants

The participants were boys and girls who represent the middle socio-economic population of Pune School.

Indexes

The BMI was calculated as body weight (kg) / height (m)^2. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR= fasting glucose (mg/dL) X fasting insulin (uU/mL) /405.[39]

Biochemical methods

Blood samples of 10 ml were collected in the morning by venous puncture after overnight fasting (at least 08 – 12 hours of fasting) to measure the following biochemical parameters: serum insulin, adiponectin, leptin, concentration were measured by enzyme linked immunoassay respectively (ELISA; Calbiotek, Biovendor, and Diagnostics Biochem). Vitamin B12 and folate were measured by MEIA and ion capture assay. Glucose was measured by GOD-POD method. Intra and inter kit deviation was less than 8%.

Statistical Method

Statistical Analysis was performed using Statistical Package of Social Sciences (SPSS, version 12). Means, Medians and Coefficient of Variation (CV) were calculated for all parameters. Independent Student's t test was used to perform gender comparisons as well as between obese and non-obese children. Pearson

correlation coefficient (*r*) was used for correlation analysis. A p value ≤ 0.05 was considered significant and ($p<0.001$) highly significant.

RESULTS

Mean age of children studied was 13.6 years with 22.19 kg/m^2 BMI. Folate levels were lower in male as compared to females, but not significant. Vitamin b12 levels were almost similar in male and female children. Insulin resistance was not associated with fat mass (Table1). Females had higher BMI ($p<0.05$), lower adiponectin and higher fat mass as compared to males. 50% of children had low birth-weight. Obese children had higher leptin ($p<0.0001$) and lower adiponectin ($p<0.001$) concentration independent of folate and vitamin B12. 40% of the children were obese but none was diabetic (less than 110 mg/dL). Low birth weight children remained thin at adolescence. There was significant inverse correlation between leptin and adiponectin. Glycemic status was directly related to adiponectin and inversely related to fat mass with no association with folate and vitamin B12.

Obese children had significantly lower folate levels as compared to non obese. However no significant changes were observed in vitamin B12 levels. Obese children were older than the non-obese children with higher birth weight having no association with circulating vitamin B12. Children belonged to medium Socio economic status and physical activities were similar in all children. There was no relation between adiponectin and plasma B12 in obese groups as compared to the non- obese group. Birth weight was inversely associated with plasma adiponectin and glucose and directly with BMI at the time of study age with no association with vitamin B12.

Birth weight had positive co-relation with BMI & negative co-relation with plasma glucose (0.05), folate (0.009) & adiponectin (0.01) but not with B12. **BMI** showed negative correlation with plasma glucose (0.01), Adiponectin (0.01) and positive with plasma insulin (0.05), leptin (0.01), and birth weight (0.01). **Adiponectin** showed negative co-relation with plasma insulin (0.05), HOMA-IR (0.05), leptin (0.01) birth weight (0.01) and positively correlated with glucose (0.05). **Leptin** showed negative correlation with plasma glucose (0.05). **HOMA-IR** showed positive co-relation to plasma glucose (0.01), insulin (0.01) (Table 3).

Table 1: Gender wise distribution of Group statistics.

Variables	Male Mean	Female Mean	Significance (p) value
Age (years)	13.29 ± 2.97	13.93 ± 2.898	0.147
Weight (Kg)	49.83 ± 22.89	50.62 ± 17.99	0.004*
Height (cm)	147.65 ± 16.89	146.97 ± 12.9	0.005*
Birth Weight (Kg)	2.70 ± 0.712	2.63 ± 0.67	0.423
BMI (Kg/m^2)	21.66 ± 6.172	22.67 ± 5.95	0.045*
Glucose (mg/dL)	90.97 ± 18.93	92.38 ± 22.69	0.652
Insulin (mIU/L)	12.79 ± 19.32	15.25 ± 19.77	0.404
HOMA-IR	2.99 ± 4.49	3.74 ± 6.28	0.361
Vitamin B12 (pg/mL)	242.42 ± 131.26	263.54 ± 152.53	0.326
Folate (ng/ml)	8.11 ± 4.95	8.98 ± 4.18	0.060
Adiponectin (µg/mL)	48.44 ± 16.58	41.56 ± 17.10	0.007*
Leptin (ng/mL)	10.92 ± 13.04	16.67 ± 14.93	0.007*

Table 2: Characteristics of obese and non-obese children (kg/m^2).

	Group (I)*	Group (II) **			
	Mean	**Mean**	**Mean**	**Range**	**Significance**
Age (Yrs)	12.32	15.4	13.6	10 – 20	0.026
Birth weight (Kg)	2.58	2.80	2.67	1.0 – 4.6	0.607
Mean BMI (Kg/m^2)	18.04	27.81	22.1	11.9 - 39.6	0.0001
Glucose (mg/dL)	93.61	86.08	91.6	42 – 191	0.032
Insulin (mIU/L)	13.18	15.15	14.0	0.20 - 148.1	0.975
HOMA-IR	3.34	3.42	3.36	0.0 – 51.9	0.577
Vitamin B12 (pg/mL)	257.01	248.01	253.0	60.0 - 1055.0	0.427
Folate (ng/mL)	8.81	8.24	8.60	1.05 - 20.0	0.0001
Adiponectin (µg/mL)	53.8	32.9	44.89	13.87- 85.46	0.003
Leptin (ng/mL)	8.73	20.77	13.84	0.0 - 61.6	0.0001

*BMI <25Kg/m^2, N=120, Male=55, Female=65
** BMI >25Kg/m^2, N=80, Male=39, Female=41

Table 3: Correlations of different parameters of the study children.

	BIRTH-WEIGHT	BMI	GLUCOSE	INSULIN	HOMA-IR	Vit B12	Folate	ADIPONECTIN	LEPTIN
BMI									
(r)	0.243**	1.000	-.184**	0.163*	.100	-.056	-.008	-.726**	0.468**
(p)	.001	------	0.010	.019	.102	.479	.926	.000	.000
GLUCOSE									
(r)	-.171*	-0.184**	1.000	.195**	.288**	-.066	-.068	.161*	-.150*
(p)	.032	.010	-----	.005	.000	.387	.428	.017	.024
INSULIN									
(r)	-.087	.163*	.195**	1.0000	.970**	.076	.111	-.157*	.092
(p)	.173	.019	.005	-------	.000	.317	.197	.019	.227
HOMA-IR									
(r)	-.128	.100	.288**	.970**	1.000	.098	.092	-.132*	.046
(p)	.082	.102	.000	.000	------	.193	.283	.040	.547
VIT- B12									
(r)	-.016	-.056	-.066	.076	.098	1.00	.115	.078	.143
(p)	.862	.479	.387	.317	.193	----	.180	.307	.059
FOL ATE									
(r)	-.247**	-.008	-.068	.111	.092	.115	1.00	.147	-.188*
(p)	.009	.926	.428	.197	.283	.180	-----	.088	.028
ADIPONECTIN									
(r)	-.246**	-.726**	.161*	-.157*	-.132*	.078	.147	1.000	-.392**
(p)	.004	.000	.017	.019	.040	.307	.088	-------	.000
LEPTIN									
(r)	.102	.468**	-.150*	.092	.046	.143	-.188	-.392**	1.000
(p)	.135	.000	.024	.113	.273	.059	.028	.000	------

****.**Correlation is significant at the 0.01 level.
***.**Correlation is significant at the 0.05 level.

DISCUSSION

The children participating in this study were from educated families who lived in an urban setting with relatively middle socio-economic background. The main purpose of the present study was to assess the interrelation between micronutrients, adipokines, birth weight, obesity at childhood, and glucose tolerance of adolescent school children in Pune, India and to predict obesity in adulthood.

Children with high birth weight were more obese in their adolescence while, the thin children remained thin. Obese children had lower glucose, higher insulin resistance and fat mass. Girls were more insulin resistant than boys. Bavdekar.et.al, found that low birth weight children who became obese at the age of 8 years due to over nutrition were at higher risk of type II diabetes than those who continued to be remain thin[40] explained as catch-up growth. Yajnik et al, reported that low birth weight children had higher plasma glucose and insulin concentration at 30 min post glucose load independent of their current size at 8 years of age with no significant difference in fasting glucose[41] which they attributed to maternal folate and vitamin B 12 status. They also reported that mean glucose and insulin concentration were 8.1 mmol/L and 46.22 mIU/L in children whose birth weight had been 2.4 kg or less, compared with birth weight more than 3.0 kg with the glucose and insulin concentration 7.5 mmol/L and 41.61 mIU/L respectively. Whereas we observed mean glucose and insulin levels of non-obese were 90.97 mg/dL and 13.18 mIU/L. respectively with mean birth weighed 2.58 kg, compared with obese group whose mean glucose and insulin were 86.08 mg/dL 15.15 mIU/L respectively, whose mean birth weight was 2.8 kgs without any association with vitamin B12.

The role of catch-up growth in influencing the cardio vascular risk and its implications for populations in economic and nutritional transition which we face in Pune, India, provide opportunities for an imbalance between nutrition in early life and diet and physical activity in childhood.[42]

Folate shows negative association with birth weight and leptin. Obese children were having significantly low levels of folate as compared to non obese. The implication of folic acid in the pathogenesis of type 2 diabetes is associated to vitamin B12 deficiency and its consequent hyperhomocysteinemia, although its deficiency is not widespread in this study. A low level of folate and normal vitamin B12 concentrations seems to be contributing to obesity. In addition folate supplementation improves glycemic control by reducing glycosylated hemoglobin fasting blood glucose, serum insulin and insulin resistance as well as homocysteinemia in type 2 diabetes patients.[43]

Leptin is considered to be the missing link between obesity and diabetes, as it has been shown to regulate blood sugar via its control on appetite and fat storage.[44-46] In our study, there was no significant correlation between birth weight and leptin, while adiponectin was inversely related. Leptin showed a highly significant rise with increasing BMI ($p < 0.01$) independent of folate and vitamin B12 concentrations; Obese children with reduced adiponectin levels were closely associated with insulin resistance. In this study we observed that obese children had low levels of adiponectin ($p \leq 0.003$) which is in concordance with Arita Y et al. and M. K. Garg et al and Kuo et al who also found that adiponectin concentration was lower in obese subjects as compared to non-obese subjects[47-48] though there was significant association with vitamin b12 and folate concentrations. On the contrary Hung et al reported an association of circulating adiponectin levels with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity.[49]

The vitamin B12 status of the migrant group (those who were not supplement users) in New Zealand was low, especially when they were vegetarian, as has been seen in other countries. Although no relation between serum vitamin B12 and IR was seen, the high levels of adiposity in this group are likely to overshadow any other contributing factor to IR.[50] Observational studies are limited because of such confounding factors and randomized controlled trial would be needed to conclusively answer whether low serum vitamin B12 status and IR have a cause-and-effect relation. However, this study does add to the knowledge of a significant and possible urban population group in Pune. It also raises some further questions that need to be investigated, including how obesity in childhood needs to be controlled with nutritional sources, folate and vitamin B12.

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