Full Length Research Paper

Three physical markers of insulin resistance (body mass index, waist circumference and acanthosis nigricans): A cross-sectional study among children in south India

Vijayan A. P.*, Varma K. K., Meera Bhagyanathan, Dinesh K. B., Divia Nath K. R. and Bijayraj R.

Department of Pediatrics, Dermatology and Family Medicine, Malabar Institute of Medical Sciences, Calicut, Kerala, India.

To study the sensitivity of body mass index (BMI), waist circumference (WC) and Acanthosis Nigricans (AN) to recognize insulin resistance in children, A cross-sectional school-based study was conducted in a semi-rural environment in the Kerala state of India. A total of 283 children between the ages of 10 to 17 were evaluated. The selected children underwent clinical evaluation which included measurement of height, weight, waist circumference and blood pressure. Acanthosis nigricans was recorded as present or absent on close visual inspection of neck area. Fasting blood samples were collected to determine blood sugar, Insulin and lipids. Associations were summarized using cross-tabulations and analyzed using SPSS package, version 10.1. The prevalence of insulin resistance was 35%, which was estimated using homeostasis model assessment of insulin resistance (HOMA-IR). Among the children studied, 30% had waist circumference above 75th percentile and 18.7% had BMI above 85th percentile. Acanthosis nigricans was diagnosed in 39.6% of the study population. Significantly high prevalence of insulin resistance was noticed among the children either with waist circumference exceeding 75th percentile or with BMI more than 85th percentile or the ones with Acanthosis Nigricans. The most sensitive physical marker of insulin resistance was acanthosis nigricans (90%) and the most specific was BMI (91%). The sensitivity of these parameters can be increased to 94% and negative predictive value to 96% by combining them together. Easily identifiable physical markers can effectively predict insulin resistance among children. This would be of relevance to many South Asian countries where resources are scarce, but the insulin resistance and the associated diabetes mellitus and cardiovascular morbidity are highly prevalent and is increasing exponentially. We should look into the possibilities of designing simple screening programs for early identification of at-risk children, which would be helpful in implementing strategies to reverse these metabolic changes at an early stage.

Key words: Risk factors, diabetes, insulin resistance, acanthosis nigricans, body mass index (BMI), waist circumference.

INTRODUCTION

Background

South Asians are inherently at high risk of type 2 diabetes and cardiovascular disease and have an insulin-resistant phenotype, characterized by low muscle mass, upper-body adiposity, and high percentage of body fat (McKeigue et al., 1993; Ramachandran et al., 2004). Insulin resistance is defined as the decreased ability of insulin to perform its biological functions, hypothetically represent the primary physiologic defect underlying the metabolic syndrome, which includes hyperinsulinemia,

*Corresponding author. E-mail: drvijayan.ap@gmail.com Tel: 09895991502. Fax: 04952741329

glucose intolerance and/or type 2 diabetes mellitus, visceral obesity, hypertension, and dyslipidemia (Mercado et al., 2002; Steinberger and Daniels, 2003; Ramachandran et al., 2004). While insulin resistance has some proven genetic basis, it is often lifestyle factors that trigger the cardio metabolic disease processes (Steinberger and Daniels, 2003). Insulin resistance is not only associated with type 2 diabetes and cardiovascular disease, but also with their risk factors, even though such associations are incredibly complex and not yet fully understood (Ramachandran et al., 2004; Ferrannini et al., 1996). Insulin resistance and associated metabolic syndrome has been studied in detail in adults, but much less is known about its physiology and associations in children, especially in South Asia. Further, because childhood metabolic syndrome likely tracks into adulthood, early identification may help to target interventions to improve future cardiovascular morbidity in susceptible population (Duncan et al., 2004).

The relationship between body weight and insulin resistance is an interesting area for scientists and has been extensively studied. Even though the relationship between these two factors are very sophisticated, there is enough evidence to say that body fatness causes insulin resistance, and insulin resistance seemingly exacerbates the adverse effects of obesity (Scott, 2004). A surrogate marker for body fat content is the body mass index (BMI), which is determined by weight (kilograms) divided by height squared (square meters) (Clinical guidelines, 1998). The more accurate way to estimate obesity in daily clinical practice is to measure waist circumference. This is because an excess of abdominal fat is most tightly associated with the metabolic risk factors (Bosello and Zamboni, 2000; Moreno et al., 2002). There is also good evidence to suggest that waist circumference is associated with risk factors for cardiovascular disease and insulin resistance in children and adolescents (Moreno et al., 2002; Maffeis et al., 2003; Savva et al., 2000).

Acanthosis nigricans (AN) is a disorder characterized clinically by thickened, dark brown skin with greater evidence of the groove and relief pattern and histologically by hyperkeratosis and hyperplasia of the dermal papillae. A number of studies have shown a significant association between acanthosis nigricans (AN), insulin resistance and the consequent hyperinsulinemia (Ponciano et al., 1992; Garofalo et al., 1988; Rave, 1988; Yamazaki and Yoshida, 2003). The factor stimulating the keratinocyte and fibroblast growth is probably the excess insulin in subjects with AN (Garofalo et al., 1988). Patients with AN are at risk for all components of the metabolic syndrome, such as, obesity, hypertension, elevated triglycerides, low/high-density lipoprotein cholesterol (HDL), and impaired glucose tolerance (Rave, 1988; Yamazaki and Yoshida, 2003; http://www.win.niddk.nih.gov/statistics/index.htm).

The prevalence of AN varies in different racial groups.

A recent study from the USA reports the prevalence of AN as 3% among Caucasians, 19% in Hispanics and 28% in American Indians (Kong et al., 2007). More recently, the studies from Sri Lanka and south India shows the prevalence of AN as high as 17.4% and 16.1% respectively, in the general adult population (Anuradha et insulin resistance (IR) in different parts of the world (Yamazaki and Yoshida, 2003; Guran et al., 2008; Murphy et al., 2009). But there is hardly any evidence that exist about the interaction of these factors among the children and adolescents of south Asia.

There are multiple published guidelines to define overweight (Himes and Dietz, 1994), hypercholesterolemia (National High Blood Pressure Education Program, 1996) and hyperglycemia (National High Blood Pressure Education Program, 1987) among children and adolescents. Even though there is no universally accepted guideline for the grading of acanthosis nigricans, it can be easily diagnosed by the presence of dark, thick, velvety, pigmented skin in the neck, axilla, groin, and other flexural areas. The neck area is found to be involved in 93 to 99% of the cases as evident from the previous studies (Stuart, 1998; Burke et al., 1999). We used the neck area to identify AN in our study as it is easy to expose the neck area in practical settings and is more acceptable for the students.

By using recommended standards, the current study examines the relationship of these easily recordable physical factors with insulin resistance among school students in the South Indian state of Kerala.

METHODS

The study was conducted in a semi-rural school in the northern part of Kerala state in India. It was done as a part of MIMSSSI (Malabar Institute of Medical Sciences Safe School Initiative) programme, which was established to provide guidance and clinical support in health related activities in the schools of the region. This study was done as a pilot project of the clinical research wing of MIMSSSI, which aims to do more quality research in the field of cardio-metabolic abnormalities among school children.

The children involved were aged between 10 to 17 years and included both boys and girls. Before starting the study, the research team visited the school and conducted multiple sessions to explain the purpose of the study and the procedures to be carried out with potential hazards and benefits. All the students who were willing to participate in the study were selected for further evaluation.

The final sample size was 283, including 208 boys and 75 girls. The number of girls in our study was relatively low partially because of the lower number of girls studying in the school (as many parents in this region prefer 'girls only schools' than mixed schools) and also because a relatively higher percentage of girls chose not to participate in the study. Informed, written consents were obtained from the students or their legal guardian. Ethical approval for the study was obtained from the Ethics Review Committee of the MIMS Research Foundation.

Details of medical history and family history of diabetes, hypertension and cardiovascular disease were obtained using a questionnaire, with parental help when necessary.

Height was measured to the nearest 0.1 cm using a standard height board attached against the wall. The subject was asked

Table 1. High insulin resistance (HOMA.IR >2.5) in 10 to 17 years old children with family history of diabetes, hypertension and coronary heart disease.

Family history	HOMA-IR >2.5	HOMA-IR<2.5	Total	P value
Diabetes	43	49	92	<0.05
Hypertension	25	29	54	<0.05
Coronary heart Disease	03	09	12	>0.05

Table 2. High Insulin Resistance (HOMA.IR >2.5) in 10 to17years old children with Acanthosis, Waist Circumference above 75th percentile and BMI above 85th percentile.

Bio-physical markers	HOMA-IR>2.5	HOMA-IR<2.5	Total	P Value
Acanthosis	89	23	112	<0.0001
Waist C >75	51	34	85	<0.0001
BMI > 85	37	16	53	<0.0001
BMI >95	17	04	21	<0.0001

to stand erect with shoulders and heels flat against the wall; looking straight ahead. The weight was measured using a balance-beam metric scale to the nearest 0.1 kg, which was calibrated every day with a standard weight. The height and weight were measured after removing the footwear but no adjustments were made for the weight of shirt, pant, underpants, or socks worn during the examination. BMI was calculated by dividing body weight (kilograms) by the square of the height (meters). Waist circumference was measured at the smallest girth between the coastal margin and the iliac crest using a standard measuring tape. Blood pressure was measured by a standard mercury sphygmomanometer (Erkameter 3000, Germany), after the subject had rested for 5 min in the sitting position, using the appropriate cuff sizes. Two readings were taken in sitting position at an interval of 5 min and the mean of the two values was taken as the final reading.

Venous blood samples were drawn after a minimum of 8 h fasting and were transported to the central laboratory immediately on cold chain conditions. Blood sugar was measured using the glucose oxidase-peroxidase method (Srinivasan et al., 1976). Serum concentrations of total cholesterol (TC) and triglycerides (TG) were determined by using enzymatic procedures in the laboratory. Determinations of low-density lipoprotein cholesterol (LDLC) and high-density lipoprotein cholesterol (HDLC) were done by direct assay method (De Ferranti et al., 2004). Biochemical assays were done on a Hitachi 912 auto analyzer using regents from Roche Diagnostics, Mannheim, Germany with appropriate quality control methods. Plasma insulin was measured using a radioimmunoassay kit (Elecsys2010 Roche Hitachi).

In the absence of a national consensus on the normal cut-off values for anthropometric and biochemical parameters of Indian children, we used the guidance from America to interpret our data (De Ferranti et al., 2004; http://www.win.niddk.nih.gov/statistics/index.htm; American Academy of Pediatrics, 1998; National Cholesterol Education Program, 1991). Overweight was defined as BMI above 85th percentile and the cut-off value for waist circumference was 75th percentile for age and sex (American Academy of Pediatrics, 1998). High levels of TC and LDLC were defined as >200 mg/dL and >130 mg/dL, respectively (National Cholesterol Education Program, 1991). A Triglyceride level of >130 mg/dL was considered high, and HDLC level of <35 mg/dL was considered low (National Cholesterol Education Program, 1991; Matthews et al., 1985). Insulin

Resistance (IR) was calculated by the formula FPG (mmol/l)×FPI (mU/l)/22.5, as per the homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985). As the reference intervals for HOMA-IR for Indian population has not yet been clearly established, in our study, Insulin resistance was defined as HOMA-IR >2.5, which was adapted from previous research evidence (Regina et al., 2005; Ah Changa et al., 2004; CMadeira et al., 2008; Chizumi et al., 2011). BMI, waist circumference, blood pressure and biochemical markers were categorized into two groups (high vs. not high) for analysis. Associations were summarized using cross-tabulations and analyzed using Chi-square test. Sensitivity, specificity, positive and negative predictive values for risk factors were calculated. Group comparisons were made using median test or χ^2 test as relevant. The risk variables included were values of fasting insulin, insulin resistance, fasting plasma glucose, HDL and LDL and triglycerides. SPSS package, version 10.1, was used for the analyses.

RESULTS

The difference in the prevalence of high insulin resistance among 32 girls (n=75) and 67 boys (n=208) found in the current study, with cutoff for normal HOMA-IR as 2.5, has no statistical significance (P > 0.05).

Family history of diabetes was reported in 32.1%, hypertension in 19% and coronary heart disease in 4% of the children studied. Offspring of parents with diabetes and hypertension were found to have increased risk for high insulin resistance than healthy parents. This relationship is not evident in the case of coronary heart disease (Table 1).

Elevated waist circumference >75th percentile for age and sex, was present in a total of 85 children (30%) and >90th percentile in 24 children (8.5%). The typical skin changes described in early diabetes, that is acanthosis nigricans (AN), was present in 39.6% of the children examined. Overweight children showed higher rates of insulin resistance. Prevalence of AN, increased waist

Table 3. HOMA.IR >2.5 in children (10 to 17 years) with high fasting blood sugar (>100 mg), cholesterol (>200 mg), triglycerides (>130 mg), LDLC (>130 mg) and low HDL (<35).

Parameters	HOMA-IR >2.5	HOMA-IR<2.5	Total	Percentage	P Value
>100 mg FBS	25	10	35	12.00	<0.001
>200 mg cholesterol	06	06	12	04.00	>0.05
>130 mg triglycerides	10	02	12	04.00	<0.001
<35 mg HDLC	40	68	108	38.00	>0.05
>130 mg LDLC	01	03	04	1.30	>0.05

Table 4. Sensitivity, specificity, positive predictive values and negative predictive values of high fasting blood sugar (>100mg), Cholesterol (>200 mg), triglycerides (>130 mg) LDLC(>130 mg) and low HDL (<35) in children with HOMA IR >2.5.

Bio-chemical markers	HOMA-IR >2.5	HOMA-IR <2.5	Total	Sensitivity As %	Specificity As %	Predictive value Positive test As %	Predictive value Negative test As%
>100 mg FBS	25	10	35	25.30	94.50	71	70.00
>200 mg cholesterol	06	06	12	06.00	96.70	50	65.60
>130 mg triglycerides	10	02	12	10.10	98.90	83	67.00
<35 mg HDLC	40	74	114	40.40	59.70	35	65.00
>13 0mg LDLC	01	03	04	01.00	99.40	25	65.60

circumference and overweight are found to be disproportionately high among children with high insulin resistance (Table 2). Statistical analysis showed that HOMA-IR is independently associated with > 85th percentile BMI (P < 0.0001), >75th percentile Waist circumference (0.0001) and AN (P < 0.0001).

Out of the evaluated biochemical parameters only fasting blood sugar more than 100 mg (P<0.001) and triglycerides more than 130 mg (P<0.001) had strong association with high HOMA IR in children. Though well known as risk factors for diabetes, statistical analysis failed to establish this relationship with high cholesterol (>200 mg), low HDL cholesterol (<35 mg) or high LDL cholesterol (>130 mg) values in the children

studied. A total of 183 children (64.8%) had abnormal values for one or more biochemical diabetic risk factors. The prevalence of each abnormality is shown in the Table 3.

Overall, the proportion of persons with adverse lipid levels ranged from 1.3% LDL cholesterol to 38% low HDL cholesterol. Compared to the very high (35%) prevalence of abnormal IR the incidence of the studied biochemical parameters were very low (except low HDL Cholesterol) a fact which make them less useful diagnostic tools in predicting the presence of IR accurately. Though prevalence is high, unlike other parameters, HDL cholesterol has no statistical association with abnormal insulin resistance.

Analysis of the estimated biochemical markers

to predict presence of high insulin resistance showed that none of them have acceptable sensitivity (ability of the test to identify correctly all those who have high IR).(Table 4) The highest sensitivity was recorded for low HDL Cholesterol (40.4%) followed by fasting blood sugar (25.3%), high triglycerides (10.1%), total cholesterol (6%) and high LDL (01%).

All biochemical markers except HDLC have specificity above 90%. HDLC, which has the best sensitivity, was found to have least specificity (59.7%). The predictive value of a biochemical marker to indicate the probability that the children with abnormal result have, in fact, high IR is maximum for triglycerides (83%) and minimum for low LDLC (25%). The predictive value of a

Table 5. Sensitivity, specificity, positive predictive values and negative predictive values of Acanthosis, waist circumference above75th percentile and BMI above 85th percentile and their combination in 10 to 17 years old children with HOMA IR >2.5.

Biophysical markers	HOMA IR >2.5	HOMA IR <2.5	Total	Sensitivity As %	Specificity As %	Preditive value Positive test %	Predictive value Negative test %
Acanthosis (ACN)	89	23	112	90.00	87.00	79.50	94.00
Waist C >75	51	34	85	52.00	83.00	60.50	75.20
BMI > 85	37	16	53	38.00	91.00	69.80	73.00
ACN+WC+ BMI	93	53	146	94.00	72.00	64.00	96.00

normal test to indicate the probability that the children have normal IR is maximum with fasting blood sugar (70%) and minimum with HDLC (65%). Sensitivity of acanthosis nigricans (AN) to identify high HOMA-IR correctly was found to be 90%.

Abnormal waist circumference (>75 percentile) and high BMI (>85 percentile) had 52 and 38% sensitivity respectively. All these three tests had reasonably high specificity with BMI having the maximum specificity with 91% followed by AN 87%. Predictive value of presence of AN to correctly identify abnormal HOMA-IR was 79.5% and absence of AN has identified 94% healthy children with normal HOMA-IR (Table 5).

Combining of all these three parameters can correctly identify 94% of the children with abnormal HOMA-IR. Out of 99 children with high HOMA-IR, 93 were identified correctly by this combination of physical tests. 96% children with normal HOMA-IR have normal values in all these three parameters.

DISCUSSION

This study shows that children and adolescents of the studied region have increased prevalence insulin resistance and other cardio metabolic risk factors. Among the 283 school children studied,

35% had high insulin resistance (IR). There was no significant difference in the prevalence of high IR among boys and girls. The prevalence of acanthosis nigricans was 39.6% in our study, which is comparable to the previous evidence from studies done on obese children in Turkey (Guran et al., 2008) and Japan (Yamazaki and Yoshida, 2003). This supports the previous evidence that many normal weight South-Asian children are 'metabolically obese' and have higher insulin resistance compared with their counterparts from other ethnic backgrounds (Whincup et al., 2005; Ehtisham et al., 1979).

Body weight and waist circumference were found to have a strong statistical association with insulin resistance, but their usefulness in identifying children with IR is limited by their relatively low prevalence. In other words, though they performed well as 'good quality litmus papers' (69.8 and 60.5%) indicating presence of high IR, many children with high IR does not possess abnormalities in these physical markers (38 and 52%). The third physical factor, acanthosis nigricans, was found to have high prevalence and strong statistical association with elevated HOMA–IR. Appearance of Acanthosis Nigricans was found to be a reliable 'sign board', for indicating the presence of high IR in children of South Asian origin (90%). Similar results were obtained in previous research among children,

but done in different geographical locations and children from other ethnic groups (Yamazaki and Yoshida, 2003; Regina et al., 2004). To our knowledge, this is the only study published to date, which examined the interrelation of these three factors among south Asian children.

One of the limitations of our study is the questions regarding the validity of HOMA-IR as an accurate estimate of insulin resistance in children and adolescents. Since glucose clamp-the gold standard-is time-consuming, costly, and complex (Matsuda and de Fronzo, 1997; de Fronzo et al., (1979), HOMA-IR is used extensively in clinical research and clinical guidelines as a surrogate marker of Insulin Resistance in different settings (Haffner et al., 1997; Misra et al., 2004; Alireza et al., 2009; Chizumi et al., 2011; Ambady et al., 2007;Sarafidis et al.,2007; www.idf.org/webdata/docs/MetSyndrome_FINAL. pdf - United States).

Although, elevated fasting blood sugar and high triglycerides were found to be more in children with high insulin resistance, their overall prevalence in the studied population was relatively low. The abnormal (low) HDLC has high prevalence among the studied children, but found to have no statistical association with HOMA-IR.

So, these biochemical parameters performed poorly as screening tools in the studied population. The ease of recognition of

acanthosis nigricans makes it a very useful clinical indicator to be used for mass screening. Both the BMI and waist circumference can be measured accurately with simple instruments. Combining all three will make it a useful tool for identifying the individuals with high insulin resistance Our findings substantiate the previous efforts to use BMI and acanthosis nigricans as a screening tool for insulin resistance among children (www.cdc.gov/diabetes/news /docs/an.htm.

(www.utpa.edu/dept/tmbhco/tmbhco/response.htm; www.cdc.gov/diabetes/news/docs/an.htm) in order to make lifestyle measures to prevent them from developing Diabetes Mellitus and cardiovascular disease.

Conclusion

There is high prevalence of risk factors for Diabetes Mellitus and cardiovascular disease even among the healthy young children we studied. The presence of Acanthosis Nigricans, overweight and increased waist circumference are three biophysical markers, in combination can strongly predict the presence of high insulin resistance in otherwise healthy children. Low sensitivity of the biochemical markers makes them less useful as simple and cost-effective tools for mass screening of healthy children in resource poor settings. The measurements of these three physical markers need no sophisticated equipments or extensive training and can be easily performed on children in any community setting. More quality research is needed for further evaluation of this clinical tool, which has got a high potential to identify at-risk children in communities with high prevalence of early onset diabetes and cardiovascular mortality and the disadvantage of limited resources for health care.

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