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Research Article

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Trends in Serum Insulin, C-Peptide and IR Levels in Non-Diabetic Young Adults – A Case Control Study

Nadeeka Dimuthu Kumari Ranadeva*[1], Usha Pushkala Kumari Hettiaratchi[2] and Lohini Vijayendran Athiththan[2]

[1]*Lecturer, Department of Biomedical Science, Faculty of Health Sciences, KIU, Sri Lanka (nadeeka@kiu.ac.lk)*

[2]*Professor, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura (usha@sjp.ac.lk)*

[2]*Professor, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura (lohinia@sjp.ac.lk)*

***Corresponding author**

Nadeeka Dimuthu Kumari Ranadeva, Lecturer, Department of Biomedical Science, Faculty of Health Sciences, KIU, Sri Lanka, E-mail: nadeeka@kiu.ac.lk

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Abstract

Background: *Insulin resistance (IR), Body Mass Index (BMI), Waist Circumference (WC), Waist to Hip ratio (WHR) at risk levels have been identified as major predisposary factors for the development of Metabolic Syndrome (MS).*

Objectives**:** *To compare the trends in serum insulin, C-peptide and IR levels in non-diabetic obese and non-obese young adults (20-40 years).*

Methods**:** *The study was designed as a case control study and was conducted to compare serum parameters and selected anthropometric parameters in obese subjects (n=50) and non-obese subjects (n=50). After obtaining written consent, 100 non – diabetic (fasting blood sugar < 100 mg/ dL) aged between 20-40 years were recruited for the study. The study was conducted at the clinic of Family Medicine, University of Sri Jayewardenepura after obtaining ethical clearance from ethics review committee of University of Sri Jayewardenepura. According to BMI subjects were categorized as obese (BMI ≥ 25 kgm^{-2}) and non-obese (BMI ≤ 25 kgm^{-2}). WC, Hip Circumferences were measured and WHR was calculated. Fasting serum glucose (Glucose oxidase kit), serum insulin and serum C-peptide (ELISA method) levels were measured and IR was calculated according to HOMA-IR equation.*

Results**:** *Among the parameters assessed; anthropometric parameters WC (p= 0.000) and WHR (P= 0.03) and biochemical parameters IR (P=0.003), fasting serum insulin (P = 0.006), glucose (P = 0.022) and C peptide (P= 0.012) showed a significant difference in the obese group when compared to non-obese group. Higher number of hyperinsulinaemic (40%) and IR (38%) subjects were in the obese category compared to non-obese category (Hyperinsulinaemia: 6% and IR: 8%).*

Conclusions**:** *In healthy non-diabetics**,** the obese group had significantly higher WC, WHR, IR, serum insulin and C-peptide levels indicating the risk of developing non-communicable diseases. In addition to the known risk factor (WC, WHR, Insulin, and IR) significant elevation of c-peptide in obese individuals might have the potential of being a promising screening tool in obese subjects.*

Keywords: Insulin, IR, C-peptide, BMI

Introduction

WHO epidemiology reports indicate that, the number of adults with type 2 diabetes mellitus (T2DM) will increase from 135 million in 1995 up-to 300 million in 2025 [1]. The apparent rapid increase in the prevalence of T2DM have a direct link with obesity [2].

Individuals worldwide are at an increasing threat regard to the increasing prevalence rates of metabolic syndrome (MS), that leads to the development of non-communicable diseases (NCDs). With the evidential data from numerous research studies, it is apparent that obesity is a key component that contributes to the increased rates of NCDs [3, 4]. Obesity has become an epidemic not only in developed countries, but also in developing countries. Over the past two decades, the prevalence rates of obesity and NCDs such as T2DM has increased at an alarming rate, especially in South Asian countries including Sri Lanka and India [5].

Insulin resistance (IR) is defined as peripheral tissues becoming insensitive to action of insulin. In this phenomenon the insulin level is higher than the normal serum insulin levels. In obese

individuals, one of the striking features is the rise in insulin levels, which in turn increases IR [6]. The relationship between obesity and insulin resistance is studied in many ethnic groups. A wide variety of research studies have indicated that the risk of developing diabetes following insulin resistance rises with increasing BMI, which is a good measure of general adiposity [7]. Central adiposity which can be measured by waist circumference (WC), is believed to have better correlation with IR than general adiposity [8]. The factors that links obesity and insulin resistance has not been adequately explained [9]. Therefore, further studies are necessary to establish the relationships.

Collectively, numerous research studies have found that IR is one of the major factors that contribute to the development of MS. IR has been recognized as a fundamental factor for the development of T2DM, and International Diabetes Federation (IDF) has introduced IR as one of the significant criteria in predicting MS. The level of fasting serum insulin is also considered a valid measure mainly in predicting the risk of developing metabolic syndrome [10].

C-peptide is secreted into the blood stream in equimolar amounts with insulin and has a longer half-life than insulin with a higher stability. It's potency to be used as a biomarker for determining insulin secretion is widely studied, and many studies have indicated the possibility of utilizing C-peptide as a marker for detecting disorders in glucose metabolism [11, 12]. Research evidence also suggests C-peptide as an indicator of insulin secretion [13].

It is evident that people in South Asian countries are more prone to acquire metabolic complications including T2DM, at an early stage of life [14]. In addition, use of C-peptide, IR and anthropometric measures are not fully utilized in Asian countries.

NCDs are not curable but could be controlled through medication and lifestyle modifications. Since the prevalence rates of NCDs such as T2DM has increased among the younger community during the last few decades, special attention must be given to early prediction of the risk factors.

Hence the current study was targeted at the age group 20-40 years in order to observe the trends of insulin, C-peptide and IR levels in both obese and non-obese subjects who were not diagnosed as having diabetes to predict the risk of developing metabolic complications. This study was the first study done in Sri Lanka as well as in South Asia to assess the c-peptide in nondiabetic and also to compare the levels of C-peptide between nondiabetic obese and non-obese subjects.

Materials and Methods

Study participants

A total number of 100 apparently healthy non-diabetic subjects aged between 20 – 40 years were enrolled in this study. Subjects having normal fasting serum glucose levels (70 – 100 mg/dL, 4.0 – 5.6 mmol/L) were only enrolled for this study. Subjects with diabetes, malignancies, other severe illnesses, physical or cognitive impairments and pregnancy were excluded. The study subjects were enrolled into two subgroups as obese (BMI $\geq$ 25 kgm^{-2}) and non-obese (BMI $\leq$ 25 kgm^{-2}) with age sex matched equal numbers (n = 50) in each group [15]. Informed written consent was obtained from all the participants prior to the study.

Study Design & Setting

The study was a case control study and the study setting was at the clinic of Department of Family Medicine, University of Sri Jayewardenepura, which is located in the suburbs of Colombo, Sri Lanka. Ethical approval was obtained from the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (Ref no HB2/14) After educating all the study subjects about the study protocol informed written consent was obtained from all study subjects. Fasting blood samples were collected adhering to standard WHO protocol.

Investigations

Socio-demographic data were obtained using a standardized interviewer administered questionnaire. Selected anthropometric parameters (weight, height, waist circumference, hip circumference) were measured and BMI and WHR were calculated. WC $\geq$ 80 cm for females and $\geq$ 90 cm for males were considered as the risk cut off values. Waist/ Hip ratio $\geq$ 0.8 for females and $\geq$ 0.9 for males were considered as high risk of developing metabolic complications [16].

An overnight fasting of 10 hour blood samples (3 mL) were drawn from the study participants and the laboratory tests were done according to the standard protocols. Biochemical parameters (Fasting serum glucose, serum insulin, and c-peptide) were measured and insulin resistance was calculated according to Homeostasis Model of Assessment – IR (HOMA - IR) equation [17]. Quantitative *in vitro* determinations of serum glucose were done using the enzymatic Glucose Oxidase /Peroxidase kit method (GD/GLUL/0513/00kit).

ELISA method was used to quantify the serum insulin (Sandwich ELISA) and serum C-peptide (Competitive ELISA) levels. Standard curve was used to extrapolate the concentrations (Prism Graph pad 2010 version). A fasting serum insulin level above 13.45 ± 7.8 µU/mL was considered as Hyperinsulinaemia [18].

Insulin resistance (IR) was calculated using the equation for HOMA-IR).

$$HOMA - IR = \frac{Fasting\ Insulin\ \left(\mu\frac{U}{mL}\right) X\ \text{fasting glucose}\ \left(\frac{mmol}{L}\right)}{22.5}$$

HOMA-IR $\geq$ 2.6 was considered as elevated insulin resistance [17, 19].

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) software version 16.0. Data were expressed as mean ±

standard deviation. The two groups were compared by independent sample t-Test and P < 0.05 was considered to be significantly different.

Results

Majority of the study population were residents from a sub urban area in Colombo district, Sri Lanka. Mean values of selected anthropometric parameters in obese and non-obese subgroups are presented in Table 1.

Among the anthropometric parameters assessed, the mean WC and waist/hip ratio showed significant difference (p = 0.000 and p = 0.030) respectively between obese and non-obese group (Table 1).

Table 1: Mean values of selected anthropometric parameters in obese and non-obese subgroups.

Parameter	Obese (n=50) / Non-obese (n=50)	Mean ± SD	P value	Percentage at risk
WC (cm)	Obese	91.15 ± 8.35**	0.000	82%
	Non-obese	77.08 ± 7.09		16%
BMI (kgm-2)	Obese	27.73 ± 2.67**	0.000	100%
	Non- obese	21.17 ± 1.93		0%
WHR	Obese	0.8919 ± 0.06*	0.03	82%
	Non-obese	0.8374 ± 0.07		34%

*Difference is significant at 0.05 level, **Difference is significant at 0.001 level
WC - Waist Circumference, BMI - Body Mass Index, WHR - Waist to Hip Ratio

Among the obese subjects 78% or more of the subjects were at risk of developing metabolic complications based on more than two or three parameters (Table 2).

Table 2: Number of subjects who are at risk of developing metabolic complications, with different anthropometric parameters among obese and non-obese group

Anthropometric parameter and the risk numbers	Obese (n = 50)	Risk percentage in obese category	Non obese (n = 50)	Risk percentage in non-obese category
Risk WC	41	82%	8	16%
Risk WHR	41	82%	17	34%
Risk BMI	50	100%	9	18%
ALL three anthropometry	39	78%	0	0%
BMI and WC	41	82%	3	6%
BMI and WHR	45	90%	6	12%
WC and WHR	39	78%	8	16%

WC - Waist Circumference, WHR - Waist to Hip Ratio, BMI - Body Mass Index

The mean values for all the biochemical parameters (IR, fasting serum insulin, c-peptide and glucose), showed significant mean differences between the obese and non-obese groups (Table 3).

Table 3: Comparison of mean biochemical parameters and IR between non-diabetic obese and non-obese subgroups

Biochemical parameter	Subgroup	Mean	P value
Fasting serum glucose (mg/dL)	Obese	79.9 ± 7.7 *	0.022
	Non-obese	76.9 ± 4.9	
Fasting serum insulin (µIU/mL)	Obese	14. 3 ± 8.8 *	0.006
	Non-obese	10.2 ± 5.3	
Fasting serum C-peptide levels (ng/mL)	Obese	4.7 ± 1.6 *	0.012
	Non-obese	4.0 ± 1.0	
IR	Obese	2.8 ± 1.8 *	0.003
	Non-obese	1.9 ± 0.9	

* Difference is significant at 0.05 level

Apart from these main observations, the obese group not only had a higher mean fasting serum insulin and IR values but also had considerably higher number of hyperinsulinaemic and insulin resistant subjects compared to non-obese group. There were 20 hyperinsulinaemic obese subjects accounting 40 % of the total obese group and only 3 (6%) non-obese hyperinsulinaemic subjects were present in the non-obese group. Among the obese, 38% of the subjects were insulin resistant; while among the non-obese there were only 8%.

There were 23 hyperinsulinaemic subjects in the total subject population where 21 had HOMA IR ≥ 2.6. Further; fasting serum C-peptide was also significantly higher in obese group compared to the non-obese group (Table 3).

Discussion

In the present study, selected anthropometric parameters and biochemical parameters were assessed in 100 individuals and were compared between the obese (n=50; BMI ≥ 25kgm^{-2}) and non-obese (n=50; BMI ≤ 25kgm^{-2}) groups. It was observed that among the assessed anthropometric parameters mean WC and WHR showed significant mean difference between the obese and non-obese ($p = 0.000$ and $p = 0.03$). Shirur et al also had a similar result with WC in young Indian adults (age range 18-23 years). Further when considering the biochemical parameters, it was clearly observed that insulin resistance was significantly higher in the obese group compared to non-obese group [4]. A study conducted in an Indian non-diabetic population by Premanath et al in 2014 also showed the obese non-diabetics had a significantly higher IR level (3.88 ± 0.49) in comparison with the non-obese individuals (mean IR = 1.60 ± 2.25) [20]. These values were closely related to the present study. Hence these two studies strengthen the evidence for the relationship between increased IR levels in obese South Asian populations. Increase in both serum insulin and IR levels indicate that subjects are at a high risk of developing metabolic complications. According to IDF criteria hyperinsulinaemia and insulin resistance are considered to be the main determinants of MS [20, 21].

Obesity and T2DM is thought to be the result of reduced insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle. Impaired suppression of hepatic glucose output is also thought to contribute to IR in obese individual [22]. IDF defines, central obesity indicated by increased WC as the primary criteria of metabolic syndrome. A high number of subjects in obese group (n = 41) were having increased WC in the present study, which is an alarming situation as this study had young adult population. Increased IR, impaired fasting serum glucose, hypertension and dyslipidemia are the other criteria needed to assess the risk of development of metabolic syndrome [23].

Although this population is apparently healthy non-diabetics, the findings of the current study suggest that the subjects in the risk category have higher tendency of developing metabolic complications at an early stage of their life. Studies have reported that the risk of developing metabolic diseases increases with age [24]. Hence even though there was a significantly higher IR in the study population the presence of normal FBS could be attributed to the younger age of the population with high active lifestyle.

The present study further observed that more than 40 subjects (> 80%) in obese category had at least one of the anthropometric parameters (increased WC or WHR) at risk level. More than 39 individuals (≥ 78%) in the obese category were at risk of developing metabolic complications according to 2 anthropometric parameters and 39 individuals (78%) were at risk according to all three parameters that were assessed. These findings further emphasise the fact that these young obese adults are at a higher risk of developing metabolic complications if necessary, precautions are not taken.

The present study also showed a significantly higher value for fasting serum C-peptide in obese subjects compared to the non-obese subjects ($p \leq 0.05$). Therefore, this study also suggests that serum C-peptide could be used as a novel marker of metabolic complications but further studies are necessary to define normal cut – off values for c-peptide level, and to confirm the findings of the current study as there is little data available of the fasting serum c-peptide levels in non-diabetics in South Asian populations as well as in other communities. The optimization of C-peptide analysis can be clinically important as monitoring the variations of insulin secretion is critical to the treatment of Type 1 and Type 2 diabetes. Maintaining the temperature, minimizing pipetting delays can be practiced in the optimizing process. C-peptide is considered as a good marker for insulin secretion, since it is released into the blood stream in equimolar concentrations with insulin [25]. The half-life of C-peptide is 30 minutes and this is 8 times longer than that of insulin and it also has other important physiological characters such as, negligible amount of liver extraction and constant peripheral clearance [26, 27]. Although there are limitations in C-peptide assay, many studies have pointed out that measuring insulin secretion using C-peptide is a more reliable method than measuring insulin itself with the advancement of assay technologies [28]. This was the first study to compare C-peptide in non-diabetic subjects.

Conclusion

Higher number of hyperinsulinaemic and IR individuals were present in obese group compared to non- obese individuals. As such these individuals are at a higher risk of developing metabolic disorders in the future. Therefore, it will be greatly beneficial if individuals with risk anthropometric parameters such as increased WC and BMI to have a routine check not only for the FSG but also the insulin levels and to calculate the IR, in-order to assess their risk level regularly.

IR value must be included in routine checkup reports in addition to serum glucose. This can pave the way. for them to control their diet pattern and take other necessary precautions to prevent future health risks.

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