ORIGINAL ARTICLE

INSULIN DEFICIENCY AMONG NEWLY DIAGNOSED TYPE 2 DIABETICS

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Background: Any patient above the age of 40 years, coming with the symptoms of diabetes is labelled as type 2 diabetic. If insulin levels are included in the protocol for initial investigations of diabetic patients, they can be differentiated as having insulin deficiency or insulin resistance. They can thus be treated accordingly. This study was conducted to see the prevalence of insulin resistance and insulin deficiency in newly diagnosed type 2 diabetics. **Methods:** This study was conducted on 75 newly diagnosed diabetic subjects, and 75 control subjects for comparison. Fasting serum insulin was assayed by ELISA and HOMA-IR index was calculated. The diabetic subjects with fasting hyperglycaemia and serum insulin level below 20 µIU/ml and HOMA-IR index below 3.5 were grouped as insulin deficient (Group-A), and the diabetic subjects with fasting insulin level above 20 µIU/ml and HOMA-IR index above 3.5 were grouped as insulin resistant (Group-B). **Results:** Twenty-eight percent subjects were found to have insulin level below 20 µIU/ml while 72% subjects had insulin resistance. When gender was taken into consideration, it was seen that 18.7% males had fasting insulin level of 6.98±0.737 µIU/ml and 9.3% females had fasting insulin level of 5.21±0.885 µIU/ml while 32% males and 40% females had insulin resistance. The mean age of male subjects with insulin resistance was significantly higher compared to the male subjects with insulin deficiency. Mean weight and body mass index of the male and female subjects having insulin resistance was significantly higher than their respective control groups and also higher than the subjects with insulin deficiency. Pearson coefficient of correlation was calculated for fasting serum insulin level with age and BMI. A significant positive correlation was observed between fasting serum insulin and age of females with insulin resistance. **Conclusion:** A considerable number of persons who develop diabetes after 40 years of age but are not insulin resistant. Twenty-eight percent subjects have relative insulin deficiency, and 72% subjects have insulin resistance.

Keywords: Type 2 diabetics, insulin deficiency, BMI

INTRODUCTION

Type 1 diabetes, previously called insulin-dependent diabetes, results from a cellular mediated autoimmune destruction of β cells of pancreas.[1] Baekkeskov *et al* in 1998 proved that markers of the immune destruction of the β cell include islet cell auto-antibodies (ICAs), auto-antibodies to insulin (IAAS), auto-antibodies to glutaminc acid decarboxylase (GAD65) and auto-antibodies to tyrosine phosphates IA-2.[2]

Zimmet *et al* showed that in diabetes, the rate of β cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults).[3] Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and or ketoacidosis in presence of infection or stress. Banerji and Lebovitz postulated that some adults may retain sufficient β cell function to prevent ketoacidosis for years. They eventually become dependent on insulin for survival and at this later stage of disease, they have very little or no insulin secretion. Immune mediated diabetes occurs in childhood and adolescence, but it can occur at any age even in the 8th and 9th decades of life.[4]

Shortly after the original description of islet cell antibodies (ICAs) as a marker of childhood type 1 diabetes, it was realised that some adult onset patients are also ICA positive. With the discovery of GAD antibodies as another marker of type 1diabetes, Tuomi *et al* used the term 'latent autoimmune diabetes of adults' (LADA). Typical patients are positive for GAD antibodies, 35 years of age or older, non-obese and present without ketoacidosis and weight loss.[5] Palmer and Hirsch suggested that besides LADA, these patients can also be named as 'type 1.5 diabetes', 'slowly progressive type 1 diabetes', 'latent type 1 diabetes' 'youth onset diabetes of maturity' and even 'LADA type 1' and 'LADA type 2' diabetes.[6] Nabhan *et al* showed that patients with LADA have several features of both type 1 and type 2 diabetes. Like type 1 diabetes patients, they have pancreatic auto antibodies and low c-peptide and insulin levels.[7] About 20% of persons diagnosed as type 2 diabetics may have LADA. This

accounts for 5–10% of the total diabetic population in the US, the same number as type 1 diabetes.[8]

Although the specific aetiology of type 2 diabetes is not known, autoimmune destruction of β cells does not occur and patients do not have any of the other causes of diabetes as in other specific types. However many environmental factors may contribute towards its expression. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance.[9] Keto-acidosis seldom occurs and when seen, it is in association with the stress of another illness such as infection.[10]

MATERIAL AND METHODS

Seventy-five adults above the age of 40 years, comprising equal number of males and females, who were diagnosed to have diabetes mellitus for the first time and had not started any medication for diabetes yet, were included in the study. Seventy-five healthy subjects, same age group, equal number of males and females as the diabetic study group, who had normal glucose levels, were used as control group. The diabetic subjects were taken from the Diabetic Clinic of Shaikh Zayed Hospital, Lahore, and the medical outpatient department. The control group was selected from general population. Both patients and controls participated willingly with prior consent to undergo tests and examination. History was taken on prescribed Performa. It included questions on medical history, family history, smoking, occupation, physical activity etc.

The diagnosis of diabetes mellitus was confirmed using World Health Organization (WHO) diagnostic criteria:

- Fasting plasma glucose (FPG) ≥126 mg/dl
- Two hours postprandial glucose ≥200 mg/dl.

For anthropometric measurements, height was measured in centimetres on a standard height scale and weight was measured in Kg. The BMI Kg/m^2 was then calculated for each individual.

Venous blood sample (5 ml) was drawn from each subject and control on 12 hours fast was drawn. An aliquot of 1.5 ml was placed in fluoride EDTA tube (containing 3 mg sodium fluoride and 1 mg EDTA) for glucose estimation and 3.5 ml aliquot was placed in plain tube, allowed to clot for 1–2 hours and then centrifuged. Clear serum thus obtained was preserved in eppendroff tube and stored at -20 °C for later analysis for insulin.

Glucose estimation was done on fully automated instrument Dimension RXL from Dade Behring. Insulin assay was done by Enzyme Immunoassay (EIA).[11] A calibration curve was plotted and insulin concentration in samples was determined by interpolation from the calibration curve.

- HOMA-IR, i.e., homeostasis model assessment for insulin resistance was calculated as:

HOMA-IR= glucose (mg/dl) × insulin (μIU/ml) 405

where 405 is a constant[12]

- HOMA-IR greater than 3.5 was taken as an indicator of insulin resistane[13], and subjects who had fasting insulin level below 20 μIU/ml and HOMA-IR <3.5 were labelled as insulin deficient and placed in Group-A. Similarly the subjects who had fasting insulin level >20 μIU/ml and HOMA-IR >3.5 were labelled as insulin resistant and placed in Group-B.

RESULTS

In the male group 37 were control and 38 were diabetic. In the female group 38 control and 37 were diabetic.

Fasting plasma glucose in male and female diabetic subjects was found to be significantly higher (p<0.01) compared to the fasting plasma glucose of the respective controls (Table-1).

Table-1: Fasting plasma glucose and 2 hours postprandial glucose levels in subjects (Mean±SEM)

Group	Fasting glucose (mg/dl)	2 hours postprandial glucose mg/dl
Control		
Male (37)	84.78±2.09*	119.27±2.47
Female (38)	82.68±1.89	112.87±2.55
Diabetic		
Male (38)	193.68±6.03*	272.30±7.9*
Female (37)	188.70±7.56	274.59±8.97*

*p<0.01 compared to controls

The male control group had a mean fasting insulin level of 11.51±0.42 μIU/ml and HOMA-IR index of 2.36±0.06. The female control group had a mean fasting insulin level of 11.21±0.46 μIU/ml and their HOMA-IR index was 2.24±0.79 (Table-2). Depending on the fasting insulin level and HOMA-IR index of the male and female diabetic subjects, they were divided into two groups.

The number of subjects in Group-A was 21 (28%) and this included 14 males (18.7%) and 7 females (9.3%). The mean fasting insulin level of male subjects in Group-A was 6.98±0.73 μIU/ml and their mean HOMA-IR index was 3.35±0.41 μIU/ml. The mean fasting insulin level of female subjects in this group was 5.21±0.88 μIU/ml while their mean HOMA-IR index was 2.48±0.87 μIU/ml. The number of subjects in Group-B was 54 (72%) and included 24 males (32%) and 30 females (40%). The mean fasting insulin level of male subjects in this group was 48.06±3.33 μIU/ml and their HOMA-IR index was 23.18±1.79. The female subjects in Group-B had mean fasting insulin level of 40.46±0.79 μIU/ml and their HOMA-IR index was 18.89±0.79 (Table-2).

A comparison of fasting insulin level and HOMA-IR index was made between the control group and Group-A and B. The diabetic Group-A and B were also compared with each other. The fasting insulin

level of control groups was significantly higher (p<0.001) than the fasting insulin level of subjects in Group-A. The fasting insulin level of subjects in Group-B was significantly higher (p<0.001) than the control group as well as significantly higher (p<0.001) than the subjects in Group-A. The HOMA-IR index of control groups was not significantly different from the HOMA-IR of the diabetic groups. HOMA-IR index of subjects in Group-B was significantly higher (p<0.001) than the HOMA-IR index of diabetic subjects in Group-A (Table-3 and 4).

Table-2: Fasting insulin level and HOMA-IR of the two groups (Mean±SEM)

Groups	Fasting insulin (µIU/ml)	HOMA-IR Index
Group A (Insulin deficient)		
Male 14 (18.7%)	6.98±0.737	3.35±0.411
Female 7 (9.3%)	5.21±0.885	2.48±0.876
Total 21 (28.0%)		
Group B (Insulin resistant)		
Male 24 (32%)	48.06±3.331	23.18±1.79
Female 30 (40%)	40.46±2.131	18.89±1.02
Total 54 (72%)		
Control		
Male 37 (24%)	11.51±0.42	2.36±0.06
Female 38 (25%)	11.21±0.46	2.24±0.79
Total (75)		

Table-3: Comparison of fasting insulin level and HOMA-IR index of male subjects (Group A, B) and controls (Mean±SEM)

Groups	Fasting insulin µIU/ml	HOMA-IR index
Group A (14)	6.98±0.737††	3.35±0.411
Group B (24)	48.06±3.33†	23.18±1.79†
Controls (37)	11.51±0.42*	2.36±0.067**

*p<0.001 compared to Group A and B, **p<0.001 compared to Group B, $^{\dagger}p$<0.001 compared to Group A, $^{\dagger\dagger}p$<0.001 compared to controls

Table-4: Comparison of fasting insulin level and HOMA-IR index of female subjects in Group-A, B and controls (Mean±SEM)

Groups	Fasting insulin µIU/ml	HOMA-IR Index
Group A (7)	5.21±0.885††	2.48±0.876**
Group B (30)	40.46±2.13†	18.89±0.791
Controls (38)	11.21±0.465*	2.24±0.79**

*p<0.001 compared to Group-A & B, **p<0.001 compared to Group-B, $^{\dagger}p$<0.001 compared to Group-A, $^{\dagger\dagger}p$<0.001 compared to controls

The mean age of control groups was not statistically different from subjects in Group-A and B. When compared with each other, male subjects in Group-B had mean age of 50.29±1.39 year which was significantly higher (p<0.05) than the male subjects in Group-A whose mean age was 44.46±1.79 year. The mean BMI in male control group was 25.01±0.32 Kg/m^2 which was not significantly different from the male subjects in Group-A. Male subjects in Group-B had mean BMI 30.01±0.83 Kg/m^2 which was significantly higher (p<0.05) than the male control group and also significantly higher (p<0.05) than the male subjects in Group-A. The mean BMI of female control group was 24.89±0.48 Kg/m^2 which was not significantly different from female subjects in Group-A who had a mean BMI of 24.11±1.47 Kg/m^2. Female subjects in Group-B had mean BMI 29.97±0.79 Kg/m^2 which was significantly higher (p<0.05) than the female control group and subjects in Group-A (Table-5).

Table-5: Age and BMI of subjects in Group-A, Group-B and controls (Mean±SEM)

Groups	Age (years)	Weight (Kg)	BMI (Kg/m^2)
Group A: (Insulin deficient)			
Male (14)	44.46±1.79	74.38±4.16	26.51±1.39
Female (7)	43.71±1.89	63.14±3.32	24.74±1.47
Group B: (Insulin resistant)			
Male (24)	50.29±1.39*	83.13±2.59**	30.01±0.83†
Female (30)	46.96±1.22	76.69±2.00**	29.97±0.79†
Controls			
Male (37)	48.27±1.00	70.68±1.21	25.01±0.32
Female (38)	47.26±0.88	62.87±1.18	24.89±0.48

*p<0.05 compared to males in Group-A, **p<0.01 compared to their respective control group and Group-A, $^{\dagger}p$<0.05 compared to respective control group and Group-A

Pearson coefficient of correlation was calculated for fasting serum insulin with age and BMI. Fasting serum insulin did not show any significant correlation with age in male controls but a significant (p<0.05) positive correlation (r=0.468) of fasting insulin level with age was seen in female control group. Diabetic subjects in Group-A and B did not show any significant correlation between these two parameters. Fasting insulin showed a highly significant negative correlation (r= -0.042) with BMI in female controls. Female subjects in Group-A and in Group-B, male control group and male subjects in Group-A and B did not show any significant correlation between these two parameters (Table-6).

Table-6: Correlation (r) of fasting serum insulin with age and BMI

	Control		Group-A Diabetic: Low insulin		Group-B Diabetic: High insulin	
Group compared	**M (37)**	**F (38)**	**M (14)**	**F (7)**	**M (24)**	**F (30)**
Fasting insulin with age	-0.210	-0.061	-0.107	-0.426	0.180	0.468*
Fasting insulin with BMI	-0.083	-0.402**	-0.307	-0.230	0.288	0.202

*p<0.05, **p<0.5

DISCUSSION

Our study was conducted to see the prevalence of insulin resistance and insulin deficiency in newly diagnosed type 2 diabetic subjects who were not on any medication for diabetes. The international prevalence of insulin deficient individuals among newly diagnosed type 2 diabetics is 10–15%.

Out of 75 diabetic subjects, 18.7% males and 9.3% females had fasting insulin levels

significantly lower than that of the control group. On the other hand, 32% males and 40% females had fasting insulin level significantly higher than that of the control. Regardless of gender, 20 (28%) subjects had insulin deficiency while 54 (72%) subjects had insulin resistance. These values are in close agreement with the previous studies that have reported that some of the persons diagnosed with type 2 diabetes, actually had lower blood insulin levels and were not insulin resistant when compared with their other counterparts in the same age group, i.e., above 40 years of age. Moreover in previous studies twenty percent of newly diagnosed diabetics were found to have relative insulin deficiency and were not insulin resistant and hence were diagnosed to have LADA.[14–16]

Although all the diabetic subjects in this study were above 40 years of age, the diabetic male subjects with relative insulin deficiency were younger compared to the male subjects with insulin resistance. The prevalence of younger age in insulin deficient diabetics is also evident from a study by Gerich.[17]

Mean weight of diabetic male and female subjects in Group-B was significantly higher ($p<0.01$) than their respective control groups as well as the subjects in Group-A. Mean BMI of male and female diabetic subjects in Group-B was significantly higher ($p<0.05$) than the control group and subjects in Group-A. Thus the present study indicated that insulin resistant individuals were obese while the subjects with relative insulin deficiency had BMI within normal limits.

The prevalence of obesity in diabetics with insulin resistance and the prevalence of normal BMI in diabetic subjects with relative insulin deficiency is also evident from previous studies.[18,19] According to Sidharta, some of the type 2 diabetic subjects were found to have lower than normal BMI.[20]

Pearson coefficient of correlation was calculated for fasting serum insulin with age and BMI. When fasting serum insulin was correlated with age, non-significant negative correlation between the two parameters in all the groups of subjects and controls was seen except in female subjects with insulin resistance (Group-B) who showed a significant ($p<0.05$) positive correlation of fasting serum insulin with age.

Many researchers[21–23] have reported that insulin was negatively correlated with age, but Nasution IR[24] reported no correlation between age and insulin resistance. On the other hand according to Fawwad *et al* (2006), correlations of fasting insulin with clinical parameters of metabolic syndrome are lost when people develop diabetes.[25]

Fasting serum insulin showed significant ($p<0.5$) negative correlation with BMI in female control group and in male subjects with insulin deficiency. These results are in agreement with a study of Bano *et al*[26], who reported that insulin showed a negative correlation with weight and BMI in non-obese and non-diabetic subjects. Male and female subjects with insulin resistance, in the present study showed non-significant positive correlation between fasting insulin and weight and BMI while Yatsuya *et al*[27] have reported a significant positive correlation of fasting insulin with weight and BMI in subjects with insulin resistance. Lukshmy *et al*[28] have reported that there is no significant correlation of insulin with BMI when diabetes is uncontrolled.

CONCLUSION

There are a considerable number of persons who develop diabetes after 40 years of age but are not insulin resistant. Twenty-eight percent subjects have relative insulin deficiency and 72% subjects have insulin resistance. Further studies may be carried out with a larger sample size so that population based results may be derived. C-peptide levels and auto-antibodies detection may be performed on subjects with insulin deficiency to confirm the diagnosis of latent autoimmune diabetes of adults (LADA).

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