Role of Fetuin-A in Insulin Resistance in Type 2 Diabetes Mellitus

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Abstract

Objective: To determine the role of fetuin-A in triggering insulin resistance and leading to development of type 2 diabetes.

Methods: A cross sectional, case control study was conducted at Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Centre (JPMC). Time duration of the study was from February 2012 to September 2013. A total of 150 subjects were included by random sampling out of which 50 were known type 2 diabetics, 50 were having impaired fasting glycaemia (IFG) and 50 were normal healthy individuals. Detailed history taking, clinical examination and body mass index (BMI) calculation were done. Laboratory investigations included serum fasting glucose, measured by glucose oxidase method and serum insulin and serum fetuin-A levels that were measured by ELISA method. Insulin resistance was calculated by Homeostatic model assessment, (HOMA IR). Statistical analysis was done by using SPSS Version 16.

Results: We observed that serum fetuin-A levels were significantly higher in known type 2 diabetics as compared to impaired fasting glycaemics and controls (p<0.01). Serum insulin and HOMA IR were also significantly elevated in known type 2 diabetics when compared to impaired fasting glycaemics and healthy individuals (p<0.01). Body mass index was also significantly higher in known type 2 diabetics and impaired fasting glycaemics when compared to controls (p<0.01).

Conclusion: Our findings suggested that higher serum fetuin-A levels have a possible role in promoting insulin resistance and development of diabetes mellitus type 2.

Keywords: Type 2 diabetes, impaired fasting glycaemia (IFG), fetuin-A (AASH & KMDC 18(2):58;2013).

Introduction

Type 2 diabetes mellitus has become a major health threat worldwide. The projected prevalence in adults, 285 million in 2010, has increased to 438 million for the year 2030¹. In Asian countries the young to middle aged adults are affected more as compared to older individuals in Western countries². At present Pakistan stands on number seven among the top ten countries having an increased burden of diabetes mellitus and it is expected to go on the fourth position by the year 2030³.

Development of diabetes is nearly always preceded by the stage of pre-diabetes. Impaired fasting glycaemia (IFG) is a frequent glycaemic disorder in the general population and is considered as a pre-diabetic state⁴. IFG has received increasing attention in recent years because it is an intermediate stage in the development of diabetes and cardiovascular diseases⁵,⁶. IFG has thus come to be considered as a potential indicator of preventive importance for diabetes and CVD⁷.

Insulin resistance plays a major role in the development of type 2 diabetes⁸. Insulin mediates its actions through the insulin receptors (IR) that consist of two extracellular α subunits that bind to insulin and two transmembrane β subunits with intrinsic tyrosine kinase (TK) activity. Binding of insulin to the IR activates its intrinsic TK activity and results in autophosphorylation of tyrosine residues of the receptor which is then followed by subse-
quent phosphorylation of several insulin receptor substrates that mediate the effects of insulin.\(^9\)

Fetuin-A, a 60 kDa glycoprotein exclusively produced by liver, binds to insulin receptors in adipose and muscular tissue and inhibits insulin receptor tyrosine kinase activity as well as insulin receptor auto phosphorylation in vivo and in vitro.\(^10\) Therefore, it may be responsible for promoting insulin resistance and have a role in the pathogenesis of type 2 diabetes mellitus.

Thus, in view of the background of an increasing burden of type 2 diabetes mellitus in our population, we aimed to investigate the role of fetuin-A in causing insulin resistance in impaired fasting glycaemics and type 2 diabetes mellitus in the local population.

**Subjects and Methods**

The research protocol was approved by the Basic Medical Sciences Institute research ethics committee (no.f.1-2/2013/bmsi-e.comm/m04/Jpmc). All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. All the participants were volunteers who were explained about the minimal risk research procedure and were asked to complete a verbal and written informed consent.

This cross-sectional, case control study was done from February 2012 to September 2013. Calculation for sample size was performed by using the following formula and taking a reference study\(^11\)

\[
 n = \frac{Z^2 PQ}{d^2}
\]

Where \(n\) = sample size required in each group
\(Z\) = confidence level at 95% (standard value of 1.96)
\(P\) = estimated prevalence of disease in project area (11%)
\(Q\) = 1 - \(P\)
\(d\) = margin of error at 5% (standard value of 0.05)

Thus the sample calculated from the above formula was \(n = 150\).

A total of 150 subjects ranging in age from 35-60 years were recruited randomly for the study. The study was conducted at the Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Centre (JPMC). Out of these, 50 were known cases of type 2 diabetes mellitus (maintained on oral anti-diabetic therapy), 50 individuals were having impaired fasting glycaemia and 50 were healthy, non diabetics, who served as controls. Patients suffering from endocrine disorders (i.e. Cushing’s syndrome, hyperthyroidism), hepatic disease, renal diseases, alcoholism or other drug abuse were excluded. For female patients, those having pregnancy, on lactation or using oral contraceptive pills were excluded.

Considering the laboratory fasting blood glucose measurements, participants were categorized into three groups using American Diabetic Association (ADA) guidelines\(^12\).

- Control (normo glycaemic): fasting blood glucose level was < 100 mg/dl.
- Impaired fasting blood glucose level was 100-125 mg/dl.
- Known type 2 Diabetics: fasting blood glucose level was ≥ 126 mg/dl.

All study participants were requested to come with 8-10 hours of fasting for sample collection. Fasting glucose was estimated by GOD-PAP (GlucoseOxidase-Phenol-Aminophenazone) method (Merck, France). Fasting insulin was measured using an ELISA kit (DIA source Immuno Assay S.A., Belgium). Serum fetuin-A levels were measured with an enzyme immunoassay kit (DIA source Immunoassay S.A., Belgium), using ELISA plate reader equalizer ER 2005, (EQUIpar, Italy). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index \([\text{fasting insulin (units per milliliter)} \times \text{fasting glucose milligram/deciliter)}/405]\)\(^13\). BMI was calculated by dividing weight by height meter squared (kg/m\(^2\))\(^14\).

A descriptive statistical analysis of continuous variables was performed using SPSS (version 16; SPSS Inc., Chicago, IL, USA). Data on continuous variables i.e. biophysical (age, height, weight, BMI,
waist circumference, hip circumference, waist hip ratio and blood pressure etc.) and biochemical (Serum fasting blood glucose, fasting insulin and serum fetuin-A etc.) parameters were calculated as mean ± standard deviation (SD). Statistical comparisons were computed using one-way analysis of variance (ANOVA) for continuous/quantitative variables. In all statistical analysis performed p<0.05 was considered significant.

**Results**

The demographic and biophysical characteristics of study participants are presented in table 1. The mean age of healthy controls was 51.9 ± 4.8 years, impaired fasting glycaemias was 52.2 ± 4.9 years and diabetic was 53.8 ± 4.6 years. There were 52% male and 48% female in control group, 56% male and 44% female in IFG group and 48% male and 52% female in known type 2 diabetics group. Systolic blood pressure, weight and BMI were significantly increased in IFG and known type 2 diabetics when compared with controls (p<0.001).

Biochemical variables among study groups are shown in table 2. Fasting blood glucose, insulin and HOMA IR were significantly increased among subjects with known type 2 diabetes and IFG (p<0.001). Post hoc test showed that type 2 diabetics and impaired fasting glycaemias subjects had significantly higher fetuin-A concentrations than the healthy control subjects (p<0.001) table 2.

**Discussion**

Insulin resistance is one of the key factors that is not only responsible for the development of diabetes mellitus but also for cardiovascular diseases as well\[^{15}\]. Various factors, including fatty acids and cytokines, have been shown to influence the effect of insulin-signaling molecules or through other pathways that interfere with the insulin-signaling pathways\[^{16}\]. Fetuin-A is also thought to be in-
involved in pathogenesis of insulin resistance\textsuperscript{17}. To the best of our knowledge, there was no study done on serum fetuin-A levels in healthy, impaired fasting glycaemics and type 2 diabetics in Pakistan. We aimed to investigate the possible role of serum fetuin-A in the development of insulin resistance.

We found that serum fetuin-A concentrations were significantly higher in type 2 diabetics as compared to impaired fasting glycaemics and healthy controls. Fetuin-A inhibits insulin action on target tissues through its interaction with insulin receptor\textsuperscript{18}. Different prospective studies have investigated the association between fetuin-A and risk of diabetes mellitus. These studies have shown that fetuin-A is associated with incidental diabetes mellitus in older persons in 6 years of follow up\textsuperscript{11}. A large prospective study with 7 years follow up, has also shown significant association of fetuin-A with increased risk of future diabetes in those individuals who had elevated glucose levels but not in the diabetic range\textsuperscript{19}. These earlier studies together with our cross sectional study support the hypothesis that elevated fetuin-A might be responsible for development of future diabetes in those individuals who have impaired fasting glycaemia. On the other hand, Mori et al.\textsuperscript{10} found no difference in fetuin-A level in diabetics and non-diabetics. This might be due to existence of glucose toxicity and or protein modifications such as non-enzymatic glycation that may overcome the effect of fetuin-A on insulin resistance.

Obesity is a most common risk factor for the development of diabetes mellitus. In the present study, we also observed a significant increase in the BMI of type 2 diabetic patients and impaired fasting glycaemics as compared to the healthy controls. These findings are in line with Ishibashi et al. 2010\textsuperscript{20} and Stefan et al. 2008\textsuperscript{19}. This study may suggest that increased BMI in type 2 diabetics and impaired fasting glycaemics may increase the fetuin-A levels which in turn induces insulin resistance. The HOMA-IR levels were increased significantly in the diabetic group as compared to impaired fasting glycaemics and controls. Our results were consistent with the results of Jung et al.\textsuperscript{21}. These findings support the hypothesis that fetuin-A might be involved in the pathogenesis of insulin resistance.

Limitations of this study include a small sample size and effect of hypoglycemic drugs in diabetic patients was not studied. Further studies needed to elaborate on these aspects.

**Conclusion**

Fetuin-A concentrations are higher in type 2 diabetics and impaired fasting glycaemics as compared to controls. Fetuin-A might be related to insulin resistance and may play a role in the pathogenesis of type 2 diabetes mellitus. These findings together with previous human and animal studies increase the possibility that fetuin-A could be the potential therapeutic target in the treatment of type 2 diabetes mellitus. Further prospective studies with large sample size are required to establish a direct relationship between serum fetuin-A levels and development of type 2 diabetes mellitus.

**References**


