

Fetuin A: A Newer Marker for Pre Diabetes

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Authors' contributions

This work was carried out in collaboration between both authors. Author MM designed the study and wrote the protocol. Author SK wrote the first draft of the manuscript managed the literature searches and analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: To evaluate the role of Fetuin A levels in predicting glycemic outcome in individuals with impaired fasting glucose.

Research Design and Methods: A total of 742 young individuals were recruited for the study out of which 177 had impaired fasting glucose, 468 had normoglycemia and 97 individuals with diabetes. These individuals were offsprings of diabetics (either mother or father or both) and were siblings amongst themselves belonging to age group of 18-35 years. Various biochemical investigations such as fasting plasma glucose, glycosylated Hb, serum insulin, C-peptide and Fetuin A were carried out. People with impaired fasting glucose were followed and analyzed according to glycemic outcome and quartile of Fetuin A level.

Results: A total of 66 individuals with prediabetes reverted back to normal, 28 progressed to diabetes and 83 remained with prediabetes over a mean±S.D follow up of 24±4.1 months. People in the highest quartile of fetuin A had the highest Insulin, Insulin Resistance, Increased loss of beta cell activity, decreased sensitivity to insulin and a higher rate of progression to diabetes (relative risk 11.96, 95% CI 5.9 to 24.01, p<0.001) and a significantly lower rate of reversion to normoglycemia (relative risk 5.62, 95% CI 3.16 to 9.9, p<0.001) than those in other Fetuin A quartiles.

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fetuin A correlated positively with Insulin ($r = +0.289$, $p < 0.001$), C-peptide ($r = +0.177$, $p < 0.001$), β cell function ($r = -0.368$, $p < 0.001$), insulin resistance ($r = +0.436$, $p < 0.001$) and glycosylated Hb ($r = +0.958$, $p < 0.05$) and negatively with % sensitivity to insulin ($r = -0.287$, $p < 0.001$). Cox regression analysis showed that baseline fetuin A, insulin levels and fasting glucose levels were predictive of reversion to normoglycemia.

Conclusions: Increased fetuin A levels had an adverse impact on glycemic outcomes thus suggesting that fasting plasma glucose and Fetuin A can be used as a tool to determine the susceptibility of an individual to develop pre-diabetes and thus diabetes mellitus.

Keywords: Family history; pre diabetes; fetuin A; insulin resistance.

1. INTRODUCTION

Type 2 Diabetes Mellitus is a non communicable metabolic disorder resulting from a combination of impaired β - cell function, with marked increase in peripheral insulin resistance. It has been projected that type 2 diabetes will grow by 52% from 387 million in year 2014 to 592 million in the year 2035 especially in the developing countries [1], with a corresponding increase in prevalence of impaired fasting glucose thus making these individuals prone to complications as several years of life have passed away being asymptomatic but the raised levels of blood glucose are silently damaging the vital organs leading to development of complications.

Diagnosing pre diabetes has been a challenging task and till date it is done based on the levels of plasma glucose and glycosylated hemoglobin (HbA_{1c}) which are essentially affected by a number of factors which includes diet also. Fetuin A also known as α -2 HS glycoprotein or α -2 Heremans schmid glycoprotein (AHSG) has been known to play multifunctional role in normal and pathological processes like general metabolism, regulation of bone mineralization [2], biphasic role in cardiovascular system [3], central nervous system [4] and chronic kidney disease [5]. In metabolism it is an endogenous inhibitor of insulin stimulated insulin receptor [6]. It inhibits phosphorylation of β - subunit of insulin receptor and insulin receptor substrate-1, it decreases skeletal muscle glucose uptake by down regulating pAkt, pAS 160 and GLUT 4 translocation [7-9]. Experimentally it has been proven that knocking out the gene for fetuin A made the mouse completely fetuin A deficient, thus enhancing their glucose clearance and insulin sensitivity [10], these mice were resistant to weight gain and had decreased body fat thus suggesting that fetuin A may play a significant role in regulating post prandial glucose disposal, insulin sensitivity, weight gain and fat accumulation.

Fetuin A has been suggested to be a link between obesity and insulin resistance [11]. It has been suggested to be a risk factor for type 2 diabetes in people with normoglycemia [12,13].

Thus keeping in view the role of fetuin A in regulating glucose homeostasis and a possible role in development of insulin resistance and diabetes the present study was planned to evaluate glycemic status of genetically predisposed individuals belonging to the age group of 18-35 years, and to evaluate the role of fetuin A in predicting pre diabetes and its progression to diabetes or reversion to normoglycemia in individuals with Impaired Fasting Glucose over a period of two years.

2. METHODOLOGY

The present study was conducted in the Department of Biochemistry Government Medical College Amritsar (India). It was a randomized prospective study on high risk group consisting of offsprings of diabetics (who were siblings amongst themselves). The sample size was collected by conducting survey of villages of Amritsar and Tarn Taran district. Each individual was considered as one family unit and his/ her offsprings who belonged to young age group of >18-35 years were included in the present study. These siblings were apparently healthy asymptomatic individuals. Detailed history in the form of dietary and life style pattern and any associated problem was noted. Informed consent was taken from the head of the family for inclusion of his family in the study. The purpose of the study was indicated clearly to the individuals participating in the study in their vernacular language. Samples of all the registered families of one particular area, after an overnight fast was collected on a fixed date at a common place of the village.

The collected samples were transported under controlled conditions of temperature to the

Department of Biochemistry on the same day for performing the various investigations. In vitro determination of fasting plasma glucose was estimated by glucose oxidase-peroxidase method as described by Trinder P, [14], Glycosylated Hb was estimated by the method based on ion exchange chromatography as described by Klenk et al. [15] using kits from Transasia, Serum Insulin was estimated by direct solid phase enzyme immunoassay as described by Boehm TM, [16] by using commercially available kit from Dia Metra Italy, C-peptide in serum was estimated by direct solid phase immunoassay as described by Kuzuya H, [17] by using commercially available kit from Dia Metra Italy. Homeostasis model assessment was taken as a measure of insulin sensitivity (HOMA-IR 2) using the equation $(\text{HOMA-IR} = \text{Glucose (mg \%)} \times \text{Insulin } (\mu\text{IU/ml})/405)$ as described by Mathew et al. [18]. Fetuin A was estimated by sandwich enzyme immunoassay technique as described by Ombrellino M, [19] using kits from R and D systems USA on Lisacan by ERBA Mannheim. The serum samples were allowed to clot for 30 min and centrifuged at 1000 rpm for 15 minutes and was diluted 4000 times using 10 μl of sample and 990 μl of calibrator diluents. Out of this 25 μl of sample was added to 975 μl of calibrator diluents to achieve the desired dilution. The assay was run using six calibrators and four controls. A standard curve was generated for each set of samples analyzed. The intra assay CV was 3.9% and inter assay CV was 8.2%

2.1 Exclusion Criteria

The subjects with liver disease, renal disease, thyroid disorder, tuberculosis, hypertension, pancreatitis, Coronary artery disease (CAD, previous history) Stroke, individuals on drugs like gluco-corticoids, Nicotinic acid, Thyroid hormones, β -adrenergic antagonists and thiazide diuretics, drug addicts, patients with endocrinopathies such as acromegaly, patients with down syndrome were excluded from the present study. The project was cleared by the institutional ethical committee.

2.2 Data Analysis

The data thus collected was analyzed using ANOVA (computer software SPSS 16.0 version). Students t test was used to calculate significance of variance amongst various groups. Pearson's coefficient of correlation was calculated to study the significance of correlation between different parameters. Cox regression analysis was performed using baseline characteristics to

evaluate their contribution to the development of study end point. p values <0.05 were taken as significant.

3. RESULTS

A total of 742 individuals belonging to age group of >18 -35 years were screened of whom 177 were selected as having pre diabetes (Fig. 1), this classification was done as per the guidelines of ADA (2014) i.e. taking into consideration the levels of fasting plasma glucose and glycosylated hemoglobin.

A total of 177 individuals with pre diabetes with a mean follow up of 24 ± 4.1 months were analyzed, of whom 66 reverted to normoglycemia, 23 progressed to diabetes and 83 remained with pre diabetes. Individuals progressing to diabetes had the higher baseline fasting glucose and fetuin A levels as compared to other groups. People in the highest quartile of fetuin A had highest insulin, insulin resistance, increased loss of β -cell function, decreased sensitivity to insulin.

Cox regression analysis showed that fetuin A and insulin along with levels of fasting plasma glucose were predictive of reversion to normoglycemia. Each unit increase in fetuin A was associated with 4.5% increase in the levels of fasting plasma glucose and 5.04% increase in insulin thus indicating increase in progress towards hyperglycemia.

Increase in levels of fetuin A in prediabetes was observed to be independent of inheritance pattern i.e. whether mother or father was diabetic but when both parents were diabetic the levels were much more as compared to when either parent was diabetic (Table 2).

Mean values of fasting glucose did not show a statistically significant variation in various quartiles of fetuin A, similar observation was made for glycosylated Hb whereas insulin and c-peptide varied significantly in the various quartiles of fetuin A, β cell function decreased significantly with a decreased sensitivity to insulin and increased insulin resistance.

People in the highest quartile of Fetuin A had the highest Insulin, insulin resistance, Increased loss of beta cell activity, decreased sensitivity to insulin and a higher rate of progression to diabetes which approached statistical significance (relative risk 11.96, 95% CI 5.9 to

24.01, $p < 0.001$) and a significantly lower rate of reversion to normoglycemia (relative risk 5.62, 95% CI 3.16 to 9.9, $p < 0.001$) than those in other Fetuin A quartiles (Table 1).

Fetuin A correlated positively with Insulin ($r = +0.289$, $p < 0.001$), C-peptide ($r = +0.177$, $p < 0.001$), % β cell function ($r = -0.368$, $p < 0.001$), glycosylated Hb ($r = +0.958$, $p < 0.05$) and insulin resistance ($r = +0.436$, $p < 0.001$) and negatively with % sensitivity to insulin ($r = -0.287$, $p < 0.001$). Cox regression analysis showed that baseline Fetuin-A ($p = 0.05$) and insulin ($p = 0.02$) levels and fasting glucose levels ($p = 0.05$) were predictive of reversion to normoglycemia. Each unit increase in fetuin A levels was associated with a 4.5% increase in levels of fasting glucose and 5.04% increase in the levels of insulin thus

indicating an increase in progress towards hyperglycemia.

Kaplan Meier analysis showed that people with prediabetes in the highest fetuin A quartile had the highest progression to diabetes which was statistically significant ($p = 0.05$, log rank test) and lowest rate of reversion to normoglycemia ($p = 0.013$, log rank test).

4. DISCUSSION

Fetuin A levels, insulin resistance values increased across the spectrum of glycemia and were highest in subjects with diabetes, followed by those with pre diabetes and were lowest in subjects with normoglycemia similar to previous studies [20].

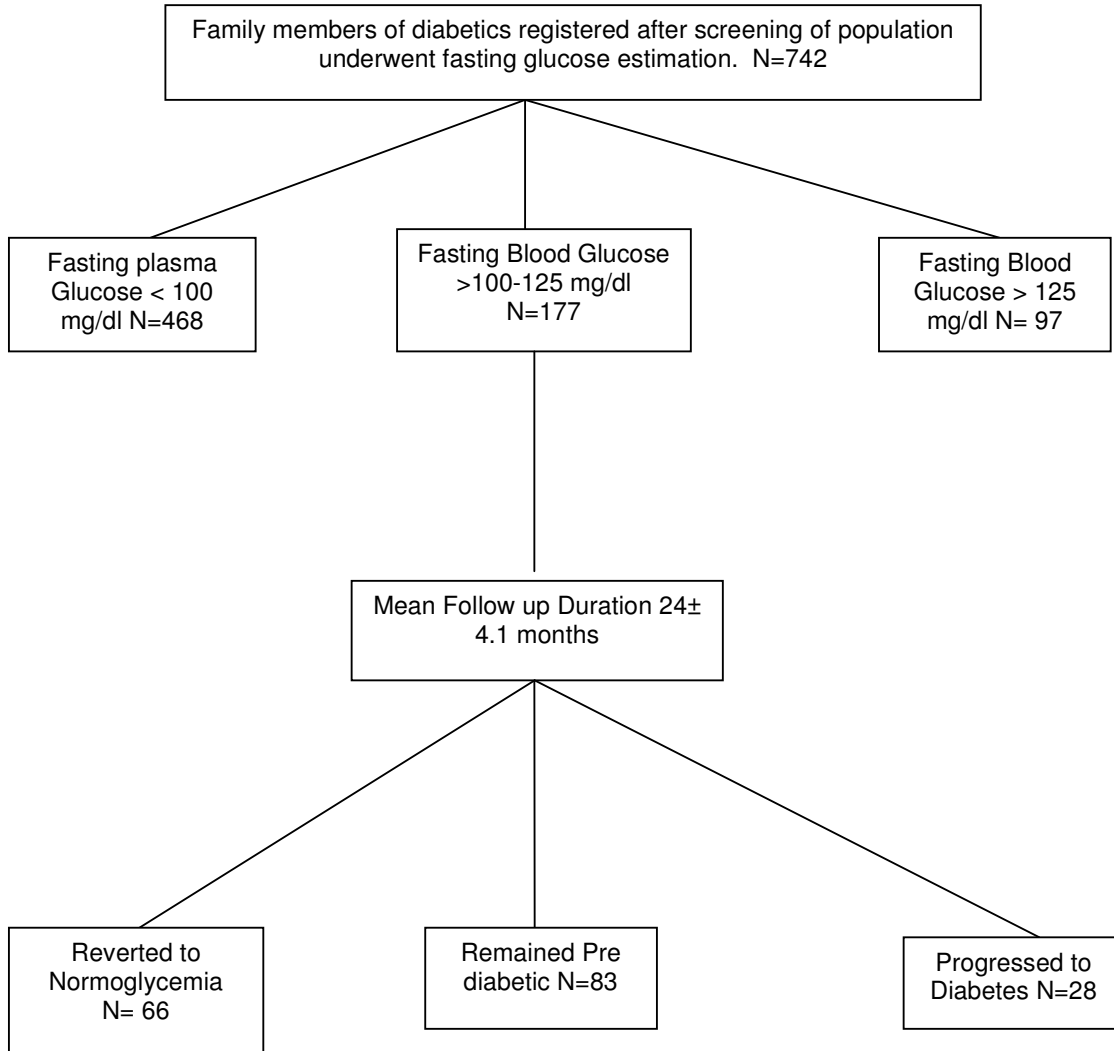


Fig. 1. Flowchart showing the study protocol

Table 1. Baseline characteristics and glycemic outcomes of individuals with prediabetes stratified by Fetuin A quartiles

Parameters (mean ± S.D)	Fetuin A quartiles n=177				p value
	0-300 µg/ml	301-600 µg/ml	601-900 µg/ml	901-1200 µg/ml	
Fetuin A (µg/ml)	168.73±86.39	463.43±75.37	735.61±71.97	1088±99.21	0.001*
Fasting plasma glucose (mg %)	107.2±2.8	108±2.3	111.1±6.3	124.0±0.15	0.58
Glycosylated Hb (HbA _{1c}) (mmol/mol)	37±0.12	39±0.49	40±0.48	43±0.25	0.53
Insulin (µIU/ml)	13.7±1.8	16.7±2.3	19.3±3.0	21.3±3.6	0.01
C-peptide(ng/ml)	1.13±0.6	1.27±0.72	1.41±0.84	2.65±0.92	0.004*
%β	135.6±51.2	136.3±36.8	106±43.8	97.48±18.1	0.004*
%S	80.26±46.7	60.29±32.6	53.6±19.2	51.3±18	0.001*
HOMA-IR2	1.6±0.92	2.01±0.4	2.82±0.71	3.13±1.16	0.007*
Duration of follow up(months)	23.7±4.1	24.8±4.4	24.9±4.2	23.9±3.8	0.067
Final outcome normoglycemia	30	28	18	12	0.081
n					
Pre diabetes	12	21	15	18	0.584
Diabetes	1	2	7	13	0.023

* Significant p value

Table 2. Comparison of Fetuin A in individuals having father, mother or both parents diabetic

S. no	Category	Mean Fetuin A (µg/ml)		
		Normal	Impaired fasting glucose	Diabetic
1	Father diabetic	309.89±14.5	626.95±14.7 *	1019.91±28.46 ††
2	Mother diabetic	354.75±15.6	640.36±22.6 *	1087.90±24.8 ††
3	Both parents diabetic	-----	997.38±19.2	-----

* p<0.001 when IFG and normal individuals were compared with each other

†p<0.001 when IFG and Diabetics were compared with each other

††p<0.001 when Normal and Diabetics were compared with each other

People with pre diabetes in the highest fetuin A had a higher rate of progression to diabetes which approached statistical significance (RR 11.96, 95% CI 5.9-24.01, p= 0.001) and a significantly lower rate to reversion to normoglycemia (RR 5.62, 95% CI 3.16-9.6, p=0.001). In the EPIC postdam study [9] subjects with normoglycemia in the highest fetuin A quartile had a significantly higher risk of diabetes than those in the lowest quartile (RR 1.75, 95% CI 1.32-2.31 p = 0.001), similar results have been reported in another study.

In nurses health study [13] fetuin A was a predictor of diabetes where with each 100 µg/ ml rise in fetuin A was associated with a 27% higher risk of diabetes. Increase in fetuin A was observed to be independent of inheritance pattern i.e. whether mother or father was diabetic, but when both parents were diabetic the levels were much more as compared to when either parent was diabetic.

The increase in Fetuin A levels, a physiological modulator of insulin receptor function at a younger age (>18-24 years) could therefore be responsible for decline in age of onset of diabetes and insulin resistance [21]. fetuin A

correlated positively with fasting plasma glucose and glycosylated Hb thereby suggesting that fetuin A can be a better marker of Insulin resistance instead of glycosylated Hb, as Glucose and glycosylated Hb are affected by a number of factors like Diet [22,23], Plasma Albumin content, Alcoholism, Hemoglobinopathies, Chronic Renal Failure and Hyperbilirubinemia [24]. It is also affected by levels of Iron, B₁₂, Aspirin, Vitamin C and E [25]. All these modifiable factors affecting the levels of HbA_{1c} were ruled out in the present study at the time of inclusion of the individual in the present study.

Serum Insulin, c-peptide levels and insulin resistance (as calculated by HOMA-IR score) were much higher in Impaired Fasting Glucose individuals than normal persons. In addition there was observed a significant positive correlation between fetuin A and insulin r= +0.289, p<0.001, fetuin A and c-peptide r=+ 0.177, p<0.001, fetuin A and %β cell function r= -0.368, p<0.001, Fetuin A and % sensitivity to insulin r= -0.287, p<0.001, fetuin A and insulin resistance r= +0.436, p<0.001). These correlations clearly define the role of fetuin A in controlling the action of insulin and maintenance of glucose homeostasis.

Further support for a potential role of fetuin A in regulation of glucose metabolism is its correlation with inheritance pattern as the gene encoding for fetuin A has been found to be located on chromosome 3q27, the region that was previously mapped as a susceptibility locus for metabolic syndrome [26] or insulin resistance. Thus the present study highlights the importance of fetuin A as a biomarker for assessing the status of an individual and an effective indicator of the disease.

5. CONCLUSION

Increased fetuin A levels had an adverse impact on glycemic outcomes thus suggesting that fasting plasma glucose and Fetuin A can be used as a tool to determine the susceptibility of an individual to develop pre-diabetes and thus diabetes mellitus.

PRIOR PRESENTATION

A part of this article was presented as oral presentation at 41st Annual conference of Association of Clinical Biochemists of India. Jodhpur India 10-13 December 2014.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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