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RESEARCH ARTICLE

RELATIONSHIP OF FASTING PLASMA ADIPONECTIN WITH FASTING PLASMA INSULIN IN YOUNG NON DIABETIC INDIVIDUALS

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Abstract

Introduction: Adiponectin is a hormone secreted by the adipose tissue into blood circulation. Blood levels of adiponectin were found to be reduced in obesity and type 2 diabetes mellitus in humans. Adiponectin has also been linked to insulin resistance associated with high fat diet and obesity.

Aim: To evaluate the relationship between fasting plasmaadiponectin and fastinginsulin with each other, and with family history of diabetes mellitus in young non diabetic males and females.

Materials and methods: The present cross sectional study was conducted at the Department of Biochemistry, Amala Institute of Medical Sciences, Thrissur, Kerala. Clinically healthy males and females between 18 and 25 years of age, were included in the study. Participants were asked to fill out a questionnaire with personal information, family history of type 2 diabetes mellitus and other relevant information. Blood samples were collected and fasting serum glucose, serum insulin and serum adiponectin were estimated.

Results and Discussion: The relationship between adiponectin and fasting insulin were evaluated. Fasting adiponectin was found to be significantly and negatively correlating with fasting insulin and glucose in young non diabetic individuals between 18 – 25 years of age. Adiponectin also showed a negative relationship with factors that were directly related to insulin, such as fasting glucose, family history of type 2 diabetes mellitus.

Conclusion: Higher level of adiponectin and lower level of insulin seen in young non diabetic individuals show that plasma adiponectin levels have a strong genetic predisposition.

Introduction:-

Adiponectin is a hormone secreted by the adipose tissue into blood circulation.

In circulation, it exists as a homo-multimer[1, 2, 3].Adiponectin structure is similar to complement product C1q. Adiponectin can have a full-length structure or a smaller, globular fragment. Almost all adiponectin appears to exist as full-length adiponectin in plasma belongs to the C1q family of proteins

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Blood levels of adiponectin were found to be reduced in obesity and type 2 diabetes mellitus in humans[4,5]. Plasma adiponectin levels are also decreased in patients with cardiovascular diseases[6,7] hypertension[8], and metabolic syndrome[9.] Adiponectin was found to have an insulin sensitizing role in humans[11]along with leptin, which is also an insulin sensitizing adipokine[12]. Adiponectin has also been linked to insulin resistance associated with high fat diet and obesity[4]. Adiponectin has been used in the treatment of insulin resistance in type 2 diabetes mellitus. Adiponectin is known to activate PPAR α[4], and AMPkinase[15],

Adiponectin levels were found to be inversely related to incidence of type 2 diabetes mellitus and obesity. There is higher prevalence of type 2 diabetes in males at younger age than that in females. This may be related to the insulin sensitizing role of estrogen. As adiponectin is inversely related to insulin resistance, plasma adiponectin levels may also be inversely related to insulin levels .

Insulin resistance is generally associated with hyperinsulinemia but not always, especially when there is pancreatic beta cell secretory deficit. Hyperinsulinemia, metabolic syndrome (central obesity), increased BMI are early changes seen several years before the onset of type 2 diabetes mellitus. Clinically post prandial levels of insulin are highly variable. Therefore, it is important to see whether fasting insulin is inversely related to adiponectin. If adiponectin is altered in these conditions then it may be useful as an early predictive marker for type 2 diabetes mellitus.

Fasting insulin levels have lower variability and may be useful as a clinical marker of hyper insulinemia and insulin resistance. Fasting insulin levels are increased with family history of diabetes in pre diabetic conditions. If fasting adiponectin is inversely related to fasting insulin then it will be inversely related to clinical conditions associated with insulin resistance such as increased BMI, fasting glucose and family history of type 2 diabetes mellitus. In this study we are estimating fasting adiponectin in young healthy adults were beta cell secretory dysfunction may be low and in whom there is no diabetes mellitus or obesity.

Insulin-sensitizing effects of adiponectin

The insulin-sensitizing effect of adiponectin was first identified in 2001[4,5] An insulin-resistant lipoatrophic diabetic mouse model that displays both adiponectin and leptin deficiency was employed, to confirm the insulin-sensitizing effect of adiponectin in vivo[13]. Addition of a physiological dose of recombinant adiponectin to the lipoatrophic diabetic mice decreased insulin resistance[4]. Besides that, insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone. An insulin-resistant lipoatrophic diabetic mouse model that displays both adiponectin and leptin deficiency was employed, to confirm the insulin-sensitizing effect of adiponectin in vivo[13]. Addition of a physiological dose of recombinant adiponectin to the lipoatrophic diabetic mice decreased insulin resistance[4]. Besides that, insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone. Replenishment of adiponectin significantly ameliorated high fat diet-induced insulin resistance and hypertriglyceridemia[4] .These data raise the possibility that the replenishment of adiponectin may provide a novel treatment modality for insulin resistance and type 2 diabetes. Adiponectin increased expression of molecules involved in fatty-acid transport (such as CD36), in fatty-acid combustion (such as acyl-coenzyme A oxidase) and in dissipation of energy (such as uncoupling protein 2)in the skeletal muscle. These changes led to reduced tissue triglyceride content in skeletal muscle[4].There has been reports that increased tissue triglyceride content interfere with insulin-stimulated phosphatidylinositol (PI) 3-kinase activation and subsequent glucose transporter 4 (glut 4)translocation and glucose uptake, resulting in insulin resistance[14]. The reduced tissue triglyceride content in muscle may contribute to improved insulin signal transduction. The group of Lodish and Ruderman also showed that muscle fat oxidation and glucose transport via AMPK activation and ACC inhibition were enhanced by the adiponectin /ACRP30 globular domain23. It was reported more recently that, AMPK is involved in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes [16].

Activation of AMPK may be a common mechanism by which insulin-sensitizing adipokines such as adiponectin and leptin increase insulin sensitivity, since leptin has also been shown to stimulate AMPK in skeletal muscle[17]. Reference interval of adiponectin showed difference with gender in the adult population .It was shown that plasma adiponectin levels were decreased in obesity. This reduction may play a causal role in the development of insulin resistance. Obesity decreased the expression levels of AdipoR1/R2, thereby reducing adiponectin sensitivity, which finally leads to insulin resistance, the so-called “vicious cycle”[18].Significant differences of fasting adiponectin in

groups with and without family history of type 2 diabetes mellitus and gender differences will also be studied. This study is to understand the physiological role of adiponectin seen in healthy individuals.

Materials And Methods:-

The study was conducted at the Clinical Biochemistry Lab, Department of Biochemistry, Amala Institute of Medical Sciences, Thrissur. The present cross sectional study included 24 healthy male and female participants between 18-25 years of age. Participants were students, staff and their relatives at Amala Institute of Medical Sciences, Thrissur selected at random, making sure that the number of males and females in the sample were close. In this study, we propose to evaluate the relationship between fasting levels of adiponectin in young adults between 18 and 25 years of age who do not have type 2 diabetes mellitus or obesity. They were earlier given instructions for sample collection, evaluated for the inclusion and exclusion criteria.

Inclusion and Exclusion Criteria.

Participants for the study will be clinically evaluated for any disease conditions. Clinically healthy males and females between 18 and 25 years of age, were included in the study. Any individual with a disease condition, injury or who is recovering from a disease will be excluded from the study. All individuals taking drugs/medicines, alcohol, or is a smoker will be excluded from the study. Samples having triglyceride value above 250 mg/dl, fasting glucose above 126 mg/dl were excluded. Other exclusion criteria are diabetes mellitus, obesity (BMI $\geq$ 30), pregnancy and lactating mothers.

After inclusion, the participants filled out a questionnaire with personal information, weight, height, waist circumference, family history of type 2 diabetes mellitus and other relevant information. Blood was then collected between 8.00 - 9.00 am in morning after overnight fasting. Immediately the blood sample was centrifuged to separate the serum and assays were done. This is been done to study the relationship between fasting adiponectin and fasting insulin healthy individuals.

Blood samples were collected in the Clinical Biochemistry Laboratory. Individuals were instructed to avoid heavy meals and exercise for 3 days before the test. The person should be taking regular diet and should not be taking any medication for a minimum of 2 weeks. The individual was requested to report to the laboratory between 7.30 am and 8.30 am so as to obtain the fasting sample 2 hours after waking from sleep. Following procedure was used to get a maximum yield of serum from the blood collected and to decrease hemolysis. Serum sample was found to be better for storage. 2 to 10 ml of blood sample is collected in plain plastic tube. It was centrifuged immediately at 3000 rpm for 5 minutes to separate plasma before clotting. Plastic tubes delay clotting. The separated plasma is transferred to a glass tube and allowed to clot for 20 minutes. Clot was separated and centrifuged to separate serum. Estimations were done immediately and the remaining serum sample was stored at -20^{o}C.

Assay Methodology

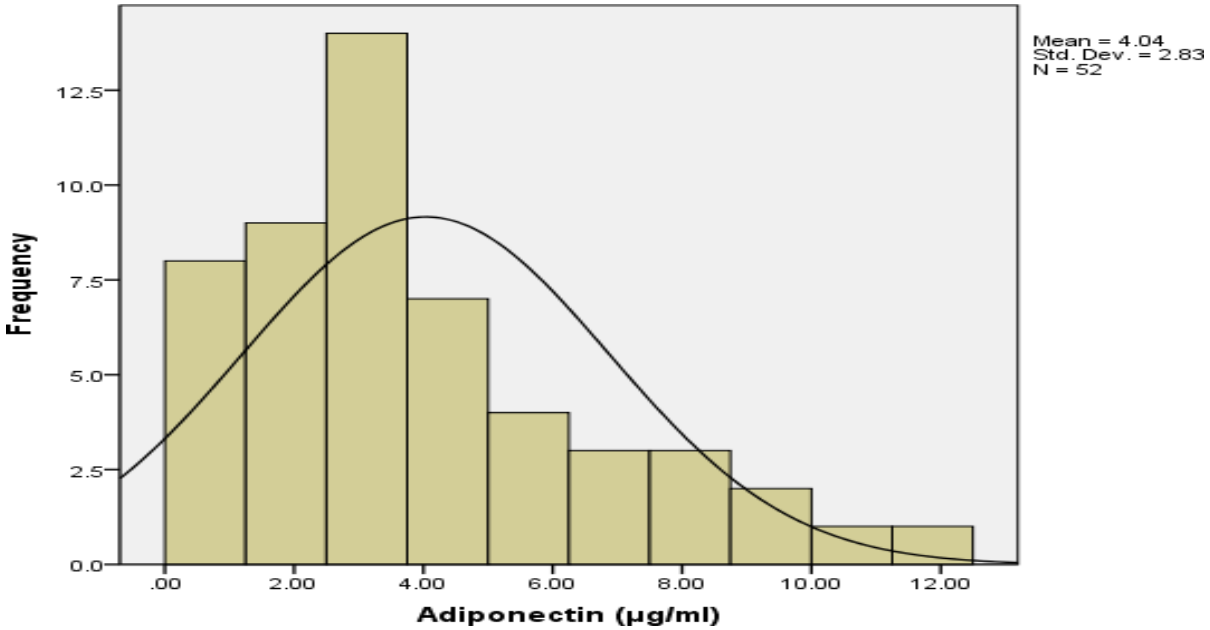
Manual enzyme immunoassay for Adiponectin (Demeditec DEE009) using antibody coated plate and detection by absorbance method of the color formed, was used. The method was Sandwich immunoassay using two specific and high affinity antibodies. The adiponectin in the serum sample binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-adiponectin antibody binds in turn to the immobilized adiponectin. The second antibody is biotinylated and will be applied in a mixture with a streptavidin peroxidase enzyme conjugate. In the final substrate reaction the colour formed will be catalysed quantitatively depending on the adiponectin-level of the samples.

Sandwich or immunometric immuno assay was done using mouse monoclonal anti insulin antibody coated on paramagnetic particles and mouse monoclonal anti insulin antibody conjugated with alkaline phosphatase. Detection was by chemiluminescence using chemiluminescence reagents and luminometer[30]. Beckman Coulter Access II Immunochemistry Analyser was used for estimation of serum insulin.

Results:-

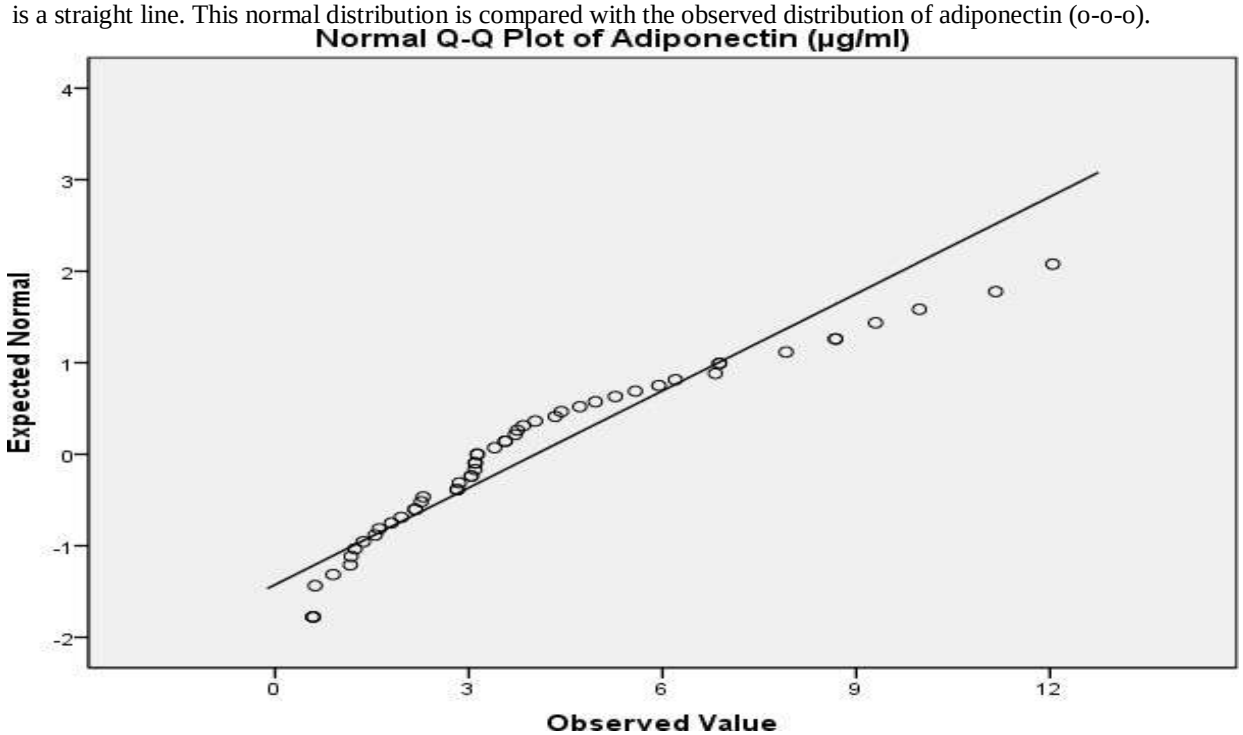
The relationship between adiponectin and fasting insulin were evaluated in young adults. Statistical estimations and hypothesis testing can be done by parametric or nonparametric methods. Parametric statistical estimations required a Gaussian normally distributed sample. As insulin is related to a large number of other parameters such as glucose, type 2 diabetes mellitus, the relationship of adiponectin indirectly with these parameters were also be evaluate

Fig. 1:- Distribution of fasting adiponectin in the total sample analyzed by histogram. N = 52; mean±SD = 4.04±2.83.



The Q-Q plot of the total sample showed that the distribution of adiponectin was away from the normal distribution represented as a straight line in the Q-Q plot (Fig 2).The box whisker plot of the total sample showed that there were 2 outliers and the adiponectin sample was positively skewed.

Fig.2:- Distribution of fasting adiponectin in the total sample analyzed by Quantile-Quantile (Q-Q) plot. The observed adiponectin value is plotted against the expected normal distribution as z value and the plot obtained is a straight line. This normal distribution is compared with the observed distribution of adiponectin (o-o-o).



The total sample was partitioned according to gender as males and females and evaluated by Box-Whisker plot for their distribution (Figure 3). There were four positive outliers in the male sample but there was only one positive outlier in the female sample. The male sample distribution segments of fasting adiponectin in the Box Whisker plot were at a lower range than that in the female adiponectin sample.

Before log10 transformation, the male fasting adiponectin was positively skewed and the female adiponectin sample was normally distributed. Log10 transformed male and female fasting adiponectin samples were analysed by Q-Q plot (Fig. 5.4a and b). Log10 transformation converted the male adiponectin samples to normal distribution (Fig. 5.4a) as seen by Q-Q plot. The female sample was distributed normally without log transformation by both K-S method (P=0.200) and by Shapiro-Wilk method (P=0.132).

The relationship of adiponectin with two parameters, fasting insulin and fasting glucose were analyzed (Table 1). Fasting adiponectin was found to be significantly and negatively correlating with fasting insulin by non parametric Spearman's rho (rs = -0.547; P=0.000) and by parametric Pearson correlation (r = -0.531; P= 0.004. Fasting total adiponectin was found to be significantly and negatively correlating with fasting glucose by the non parametric Spearman's rho (rs = -0.0336; P= 0.015)

Table 1:- Comparison of fasting s.adiponectin with fasting s. insulin and fasting s. glucose.

Variables	**Total Sample**		**Male (log10 transformed)**		**Female**	
	Spearman 's rho (rs)	P value	Pearson Correlation (r) P	P value	Pearson Correlation (r)	P value
Adiponectin vs Insulin	-0.547	0.000	-0.441	0.031	0.531	0.004
Adiponectin vs Glucose	-0.336	0.015	-0.131	0.542	-0.180	0.360

But the male and female sample of fasting insulin showed lower negative correlation by parametric method, but the correlation was not significant. X-Y scatter diagram of the male fasting adiponectin sample with fasting insulin showed a negative relationship with fasting insulin (Figure 5.5a) (Figure 5.5b).X-Y scatter 41 diagram of the female fasting adiponectin sample with fasting insulin showed a negative relationship with fasting insulin (Table 2).

Fig 3:- X-Y scatter diagram of fasting adiponectin with fasting insulin in male sample.

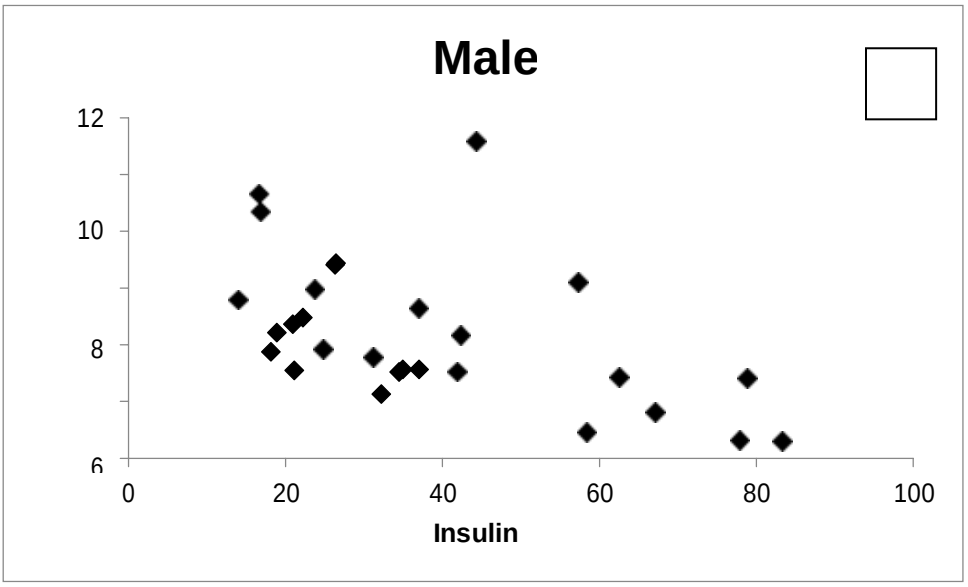
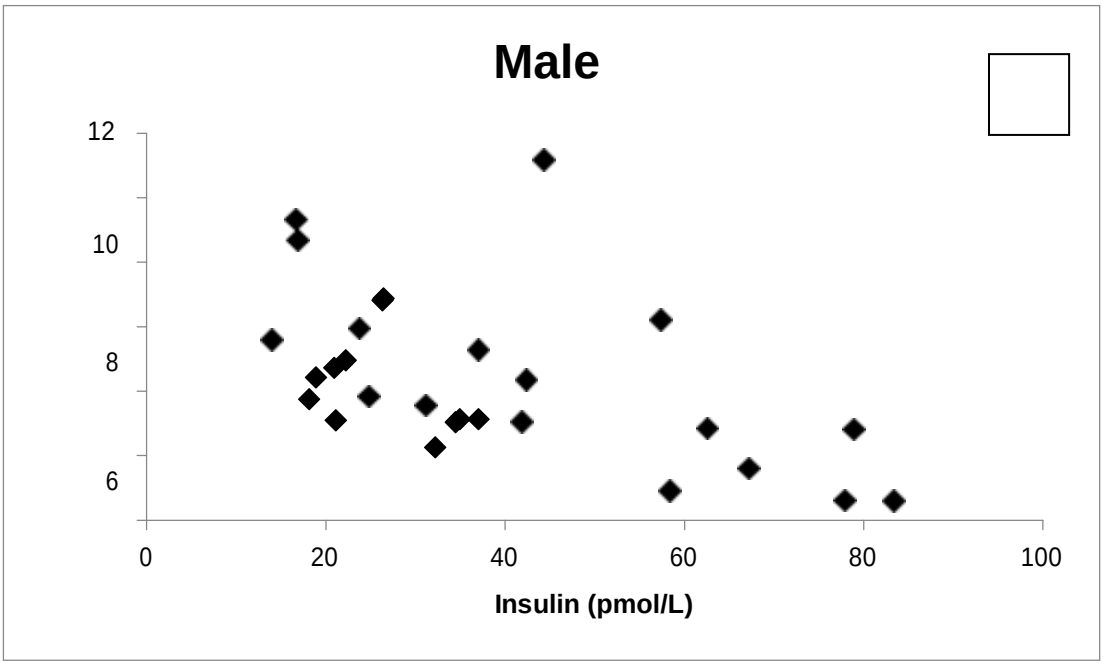


Fig 4:- X-Y scatter of fasting adiponectin with fasting insulin in female samples.



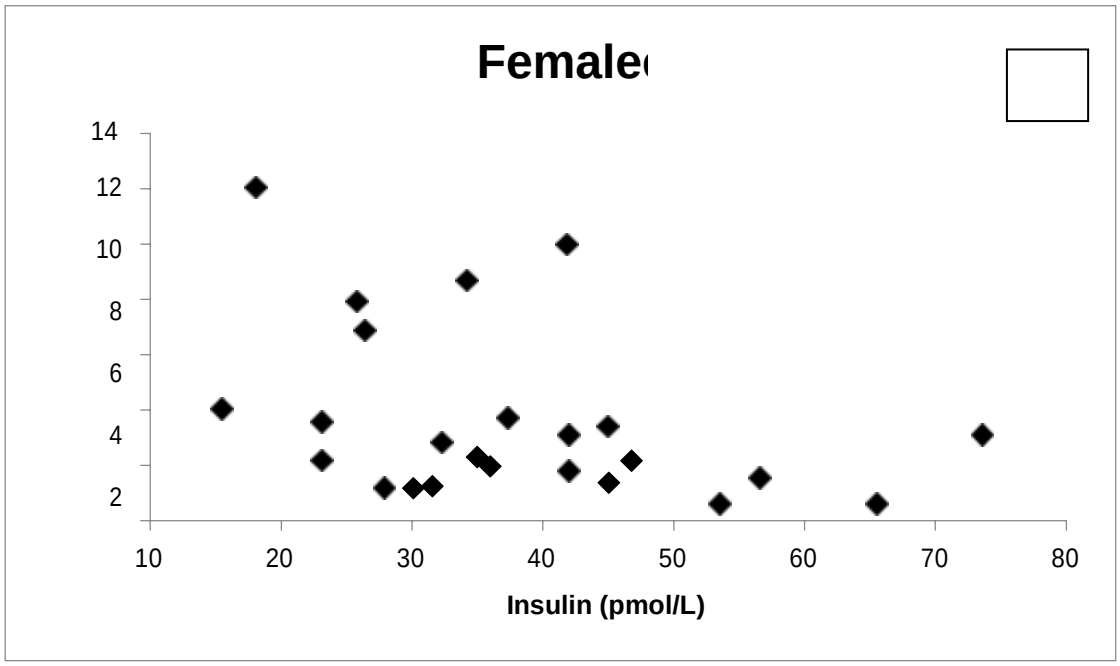


Table 2:- Comparison of fasting s. adiponectin between male and female samples.

Variables	Mean ±SD	Mann-Whitney U test
Male (n = 24)	3.64±3.13	1.698 (0.089)
Female (n = 28)	4.38±2.56	

Similarly total fasting adiponectin was partitioned according to family history of diabetes into group with family history of diabetes and group without family history of diabetes. Fasting adiponectin was higher in the group without family history of diabetes mellitus (P=0.041) (Table 5.3 and Fig. 5.7). These results indicated that fasting

adiponectin showed a negative relationship with fasting insulin. Adiponectin also showed a negative relationship with factors that were directly related to insulin, such as fasting glucose, family history of type 2 diabetes mellitus .

Table 3:- Comparison of fasting adiponectin between individuals with and without family history of type 2 diabetes mellitus.

Variables	Mean ±SD	Mann-Whitney U test (P Value)
With FH of type 2 diabetes mellitus (n = 44)	3.73±2.70	2.042 (0.041)
Without FH of type 2 diabetes mellitus (n = 8) 5	5.70±3.12	

Discussion:-

In this study, fasting adiponectin was found to be inversely related to fasting insulin and glucose levels in healthy young adults (Table 1).Higher levels of adiponectin are found in more healthy individuals, and lower levels in individuals having tendency for type 2 diabetes mellitus, obesity and atherosclerosis. Therefore, the distribution of fasting adiponectin levels in serum is important. The histogram of distribution showed more number of individuals at lower levels and less number of individuals at higher levels in the fasting sample of adiponectin at the range from lowest to 6µg/ml (Fig. 1). Beyond 6 µg /ml, there is positive skewing of the fasting adiponectin distribution with 2 positive outliers higher level. Adiponectin mRNA expression is exclusive to adipose tissue. The mRNA expression of adiponectin was reduced in obese diabetic murine model db/db mice. Blood levels of adiponectin in humans and obese/diabetic mice have been found to be reduced.

The heterozygous PPAR (peroxisome proliferator-activated receptor)γ knockout mice remained insulin-sensitive on a high fat diet[10]. From the increased expression levels of PPARα target genes such as CD36, acyl-coenzyme A oxidase and uncoupling protein 2, it was hypothesized that adiponectin could activate PPARα [4]. Consistent with this hypothesis, adiponectin increased the expression levels of PPARα in vivo [4].These data suggested that adiponectin increased fatty-acid combustion and energy consumption, presumably mediated by PPARα activation at least in part, which led to decreased triglyceride content in the liver and skeletal muscle, and thus co-ordinately increased in vivo insulin sensitivity. Hyperinsulinemia is directly related to insulin resistance, therefore, fasting adiponectin will be inversely related to fasting insulin (Table 1).

Globular adiponectin and full-length adiponectin stimulated phosphorylation and activation of AMPK in skeletal muscle, whereas only full-length adiponectin did so in the liver [15].In parallel with its activation of AMPK, adiponectin stimulated phosphorylation of acetyl coenzyme-A carboxylase (ACC), fatty-acid combustion, glucose uptake, and lactate production in myocytes, and also stimulated phosphorylation of ACC and caused a reduction in molecules involved in gluconeogenesis in the liver, which can account for the acute glucose-lowering effects of adiponectin in vivo[15]. We have observed that adiponectin is inversely related to glucose (Table 1).

Partitioning of fasting adiponectin showed lower levels of adiponectin in young adult males than in females (Table 2). The lower levels indicated the higher prevalence of type 2 diabetes mellitus in males. Both the male and female samples were also positively skewed with positive outliers. Estrogens (E2) are produced in the adipocytes (via aromatization from androgenic precursors) and increase in proportion to total body adiposity. This is in addition to the estrogens produced by the ovary. Estrogen Receptor is expressed in adipose tissue. Mice of both sexes with a targeted deletion of the Estrogen Receptor gene have increased adiposity, with a near doubling of visceral adipose depots relative to their age-matched wild-type counterparts[22]. E2 also suppresses white adipose tissue accumulation by decreasing fatty acid and triglyceride synthesis and lipogenesis. Estrogens are a likely contributor to the reduced incidence of type 2 diabetes observed in premenopausal women [31,32].

As the sample were positively skewed with positive outliers it was necessary to evaluate their distribution and transform the distribution to normal before parametric calculations Log transformation could change the distribution of total sample and male sample of fasting adiponectin to normal distribution. Intra abdominal adipose tissue is thought to be metabolically and functionally different from subcutaneous adipose tissue. Indeed, intra-abdominal adipose tissue has more capillaries and efferent sympathetic axons per unit volume than subcutaneous fat, and unlike subcutaneous fat, it drains into the hepatic portal vein.

Conclusion:-

The inverse and significant correlation of fasting adiponectin and fasting insulin showed that higher level of adiponectin and lower level of insulin are more seen in healthy individuals. But there was a significant difference in fasting adiponectin with and without family history of diabetes mellitus in parents. This showed that plasma adiponectin levels have strong genetic predisposition .

Declarations

We would like to thank all the students for their cooperation and time for making this research successful.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research , authorship and /or publication of this article.

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