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A Study on Association of Serum Osteocalcin and Adiponectin with Diabetic Markers in Genetically High Risk for Type 2 Diabetes Population

K. Subhash Chandra Bose[1*], Maninder Bindra[1], Shachin K Gupta[2] and Prerna Vyas[1]

[1]Department of Biochemistry, L. N. Medical College & Research Centre, Kolar Road, Bhopal – Madhya Pradesh, India.
[2]Department of Medicine, L. N. Medical College & Research centre, Kolar Road, Bhopal – Madhya Pradesh, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author KSCB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MB and SKG managed the analyses of the study. Author PV managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: In view of significant role of osteocalcin and adiponectin in the onset of insulin resistance and diabetes in rat model and cell line studies we aimed to study the influence of family history for diabetes on osteocalcin and adiponectin levels and their role in initiating the changes in diabetic markers in healthy adult springs of diabetic parents, thus a hypothesis can be drawn on their role in developing diabetes in high risk population.
Methodology: Age between 18 to 22 years was selected and divided into three groups. Group I: control group consists (n=81) with no family history of diabetes. Group II: (n=147) with one of their parents with history of type 2 diabetes. Group III: (n=47) with both parents having history of type 2 diabetes. In all the groups we estimated fasting plasma glucose, lipid profile, insulin and adiponectin and osteocalcin.
Results: We observed significant lower levels of adiponectin 8.7 ± 1µg/ml in group-III

**Corresponding author: Email: kscbreddy@gmail.com;*

and 9.5 ±1.3 µg/ml in group-II when compared to control 11.0 ± 1.2 µg/ml (p<0.01) and HOMA-IR in children of diabetic parents had a statistically significant correlation with plasma Adiponectin with Pearson's coefficient -0.504. Through linear regression analysis parental diabetes influences plasma adiponectin p <0.01 (B -1.50, 95% CI -1.79 - -1.20) but not osteocalcin P>0.05 (B .313, 95% CI -.114 - .740) levels in children of diabetic parents.

Conclusion: family history for diabetes does not influence osteocalcin levels but may influence adiponectin gene expression leading to a decrease in its plasma concentration, which might play a key role in developing diabetes in near future.

Keywords: Osteocalcin; adiponectin; HOMA-IR; diabetes.

1. INTRODUCTION

After the study done by Karsenty et al. [1] in 2007 on the role of osteocalcin an osteoblast derived protein on energy metabolism, the flow of scientific literature on the reciprocal interrelation of bone metabolism and energy homeostasis has increased tremendously. Many animal studies [1,2] showed lack of Osteocalcin displayed insulin resistance and obesity, moving osteocalcin into center on its endocrine like activity. Recent studies [3] documented osteocalcin can stimulate insulin secretion, acting directly on proliferation and secretion of pancreatic beta cells. It can also increase insulin sensitivity, probably by inducing the expression of adiponectin in adipocytes [4]. Human studies also revealed the decreased osteocalcin in type 2 diabetic patients and a negative correlation with fasting glucose and insulin resistance [5].

Many studies were done till date on the altered levels and association between osteocalcin [6] and adiponectin [7] either in well diagnosed type 2 diabetes or during anti diabetic drug therapy, but not even single study is available on the influence of family history for diabetes on plasma levels of osteocalcin, adiponectin and their prospective role in initiation and propagation of Insulin Resistance and diabetes in humans**.** In addition to older age and being overweight, family history is a well-known risk factor for type 2 diabetes, with risk estimates (relative risks [RRs]) ranging from 2 to 6 depending on study design and case definition [8] Thus the primary purpose of this study is to examine the association between serum osteocalcin, adiponectin and measures of insulin resistance in young non diabetic population with strong family history for type 2 diabetes.

2. MATERIALS AND METHODS

This cross sectional study is approved by institutional ethics committee with wide Ref No: IEC/3/2011 and written consent was taken from all participants. For this study we selected the participants and grouped in three groups based on their family history of type 2 diabetes. All the participants enrolled for our study were students of our flagship institutions comprising Medical, Dental and Nursing sciences**.** All the participants were voluntarily participated for this study. All were on vegetarian diet with identical ingredients as they were sharing same kitchen and residing in one campus. As physical activity influences osteocalcin levels we restricted the participants from doing exercise.

We prepared self designed questionnaire to know the family history for type 2 diabetes and divided the participants into 3 groups.

Group I: control group consists (n=81) both male and female of age group between 18 to 22 years, irrespective of BMI whose parents are non diabetic, non hypertensive and do not have any family history of coronary heart diseases.

Group II: (n=157) both male and female of age group between 18 to 22 years, irrespective of BMI with one of their parents with history of type 2 diabetes.

Group III: (n=47) both male and female of age group between 18 to 22 years, irrespective of BMI, with both parents having history of type 2 diabetes.

Exclusion criteria: by estimating fasting and post 2 hours OGTT (oral glucose tolerance test) blood glucose we excluded the diabetes in all participants. The diabetes in participants was excluded by applying WHO criteria [9]. By using detailed questionnaire and through consulting their family physician we excluded those whose parents were type 1 diabetics.

Measurements: all the subjects of above groups were on overnight fasting. In all the above subjects we measured serum Glucose, lipid profile and Hba1c by commercial kits for Biosystems A25 fully auto analyzer. Serum insulin with CV 6%, adiponectin with CV 5% and Total Osteocalcin with CV 8% were estimated by ELISA method with commercial kits (INVITROGEN). All the parameters were analyzed in ISO 9001: 2008 certified laboratory. Blood pressure was measured by Omron HEM-7203 automated BP monitor instrument and average of three measurements was taken into consideration.

Insulin resistance was measured by Homeostasis model for assessment (HOMA) based on formula [10]:

HOMA-IR = fasting serum insulin (µIU/ml) x fasting serum glucose (mg/dl)/405.

2.1 Statistical Analysis

Descriptive results of continuous variables are expressed as mean ± SD for normally distributed or as median for non parametrically distributed variables. Comparison between study groups and control was done by student t-test or Mann Whitney U test whichever is appropriate. Relationship between continuous variables was expressed by applying Pearson's correlation (r) for normally distributed variables and Spearman's correlation for non parametric distribution. P value <0.05 is considered significant and <0.01 as highly significant.

Linear regression was performed to evaluate the association among parental diabetes as independent and plasma adiponectin and osteocalcin of children of diabetic parents as dependent variables in the presence of confounders like blood glucose, lipid profile and BMI. P value <0.05 considered to be statistically significant and p<0.01 as highly significant. All the data were analyzed using statistical software SPSS version 19.

3. RESULTS

Baseline characteristics of the study population are presented in Table 1. in this study we observed high levels of fasting insulin 10.80 ± .99 µ IU/ml in children whose parents are diabetic when compared to control 8.06 ± 0.99 µ IU/ml (P<0.01) and the same rise in fasting glucose with 79.02 ± 10.2 mg/dl study population (parental diabetes) as compared to control

group with no family history for diabetes 73.4 ± 8.5 mg/dl (P<0.01) was also observed. The same statistically significant variation in HOMA-IR among control and study population (P<0.01) was also observed in this study. The same statistically significant (<0.05) high levels of lipid profile was also observed in study group when compared to control.

Table 1. Showing baseline anthropometric and biochemical characteristics of study and control groups

Parameters	Parents Non diabetic (N=81)	Parents diabetic (N=194)	P values
BMI (kg/m^2)	21.8 ± 3.3	22.7 ± 3.9	.078$^{\#}$
WHR	.81 (.79-.83)	.82 (.81-87)	.147$^{\#}$
Blood Pressure systolic (mmHg)	110.4 ± 5.6	110.3 ± 6.8	.894$^{\#}$
Blood Pressure diastolic (mmHg)	75 ± 3.9	74 ± 3.9	.204$^{\#}$
Plasma Glucose (mg/dl)	73.4 ± 8.5	79.0 ± 10.2	.000**
HOMA-IR	1.4 ± .23	2.1 ± .47	.000**
HbA1c (%)	4.3 + 5.5	4.5 + .55	.121$^{\#}$
Plasma Triglycerides (mg/dl)	97 ± 11.0	101 ± 13.5	.046*
Plasma Total Cholesterol (mg/dl)	132.1 ± 11.8	135.2 ± 11.0	.039*
Plasma HDL Cholesterol (mg/dl)	45.4 ± 4.5	44.0 ± 4.7	.028*
Plasma Insulin (µ IU/ml)	8.0 ± .99	10.8 ± 1.4	.000**
Plasma adiponectin (µg/ml)	11.0 ± 1.2	9.3 ± 1.3	.000**
Plasma total osteocalcin (ng/ml)	12.4 ± 1.4	12.7 ± 1.6	.141$^{\#}$

*Results are presented as mean ± SD or median (interquartile range, 25-75%). **p<0.01; *p<0.05; $^{\#}$p>0.05; BMI Body Mass Index; WHR waist to hip ratio; HOMA-IR homeostasis model assessment-insulin resistance; HbA1c glycated hemoglobin; HDL high density lipoprotein.*

In this study we observed no statistical difference in osteocalcin levels between control and study population (P>0.05), where as statistically significant lower plasma adiponectin (P>0.01) between study groups and control was observed (Table 1).

Of all the parameters (Table 2) statistically significant higher levels were observed only in fasting glucose, HOMA-IR with P<0.05 and lower adiponectin levels with P<0.01, when compared amongst both parent diabetic and single parent diabetic group.

In observing the sexual dimorphism in the levels of plasma osteocalcin and adiponectin (Table 3), we observed no statistical difference between male and females p >0.05.

Correlation of adiponectin and osteocalcin with HOMA-IR and parental diabetes and association of parental diabetes with plasma adiponectin and osteocalcin in study group:

From Table 4, adiponectin showed statistically significant correlation with HOMA-IR (Pearson correlation -.502, P<0.01) where as osteocalcin does not show any correlation (Pearson correlation .037, P>0.05).

Table 2. Showing mean difference in serum adipocytokines and other biomarkers between single parent diabetic and both parents' diabetic groups

Parameters	Single parent diabetic group (n=147)	Both parents diabetic group (n=47)	P value
BMI	22.48 ± 4.1	23.63 ± 3.5	.086#
WHR	.82 (.80-.86)	.82 (.83-.88)	.935#
Systolic BP	110.29 ± 6.7	110.49± 4.9	.879#
diastolic BP	74.43 ± 3.7	75.68 ± 4.1	.074#
Fasting serum insulin	10.79 ± 1.5	11.12 ± 1.3	.192#
Fasting serum Glucose	78.0 ± 10.9	82.44 + 9.7	.010**
HbA1c	4.5 ± .49	4.53 ± .510	.136#
HOMA-IR	2.09 ± 0.49	2.26 ± 0.41	.030*
Adiponectin (µg/ml)	9.5 ± 1.3	8.7 ± 1.0	.000**
Osteocalcin (ng/ml)	12.76 ± 1.71	12.65 ± 1.66	.764#
Plasma Triglycerides (mg/dl)	100.98 ± 13.1	102.7 ± 14	.448#
Plasma Total Cholesterol (mg/dl)	135.22 ± 11.2	135.26 ± 10.4	.984#
Plasma HDL Cholesterol (mg/dl)	44.98 ± 4.8	43.85 ± 4.5	.686#

*Results are presented as mean ± SD, or median (interquartile range, 25-75%). **p<0.01; *p<0.05; #p>0.05; BMI Body Mass Index; WHR waist to hip ratio; BP blood pressure; HbA1c glycated hemoglobin; HOMA-IR homeostasis model assessment-insulin resistance; HDL high density lipoprotein*

Table 3. Showing gender difference of plasma osteocalcin and adiponectin levels

Parameters	Females (N= 126)	Males (N=149)	P value
Plasma Adiponectin (µg/ml)	9.71 ± 1.3	10.02 ± 1.5	.093#
Plasma Osteocalcin (ng/ml)	12.56 ± 1.5	12.7 ± 1.7	.444#

Results are presented as mean ± SD; #p<0.05;

Table 4. Showing correlation between HOMA-IR with adiponectin and osteocalcin in study groups

		HOMA-IR	Adiponectin	osteocalcin
HOMA-IR	Pearson correlation	1	-.504**	.037#
	Sig. (2-tailed)		.000	.546
	N	275	275	275
Adiponectin	Pearson correlation	-.504**	1	-.048
	Sig. (2-tailed)	.000		.426
	N	275	275	275
Osteocalcin	Pearson correlation	.037#	-.048	1
	Sig. (2-tailed)	.546	.426	
	N	275	275	275

*** Correlation is significant at P <0.01; # correlation is insignificant*

In assessing the association of parental diabetes with plasma osteocalcin and adiponectin in children of diabetic population as dependent variable (Tables 5 and 6) when compared to osteocalcin only adiponectin showed significant association with beta -1.502 and 95% confidence interval CI -1.79 - -1.20 (P<0.01) and also had a significant association with BMI and plasma glucose levels (p<0.01).

Table 5. Showing linear regression analysis with plasma adiponectin of children with parental diabetes as dependent variable

Model	Unstandardized coefficient		Standardized coefficient	P value	95% CI	
	B	Std. Error	Beta		Lower	higher
1 (constant)	15.68	1.347		.000	13.031	18.335
Diabetes	-1.413	.162	-.431	.000**	-1.732	-1.094
BMI	-1.149	.188	-.299	.000**	-1.519	-.778
Serum triglyceride	.005	.006	.043	.364#	-.006	.016
Serum total cholesterol	-.007	.006	-.057	.229#	-.020	.005
Serum HDL	-.015	.015	-.048	.308#	-.045	.014
Serum glucose	-.029	.007	-.197	.000**	-.044	-.014

***p<0.01; # p>0.05*

Table 6. Showing linear regression analysis with plasma levels of osteocalcin of children with parental diabetes as dependent variable

Model	Unstandardized coefficient		Standardized coefficient	P value	95% CI	
	B	Std. error	Beta		Lower	higher
1 (constant)	12.114	1.903		.000	8.367	15.860
Diabetes	.360	.229	.101	.117#	-.091	.811
BMI	.214	.266	.051	.421#	-.309	.738
Serum triglyceride	-.009	.008	-.072	.242#	-.024	.006
Serum total cholesterol	.009	.009	.062	.314#	-.008	.026
Serum HDL	.008	.021	.023	.713#	-.034	.049
Serum glucose	-.008	.011	-.047	.472#	-.28	.013

#p>0.5;

4. DISCUSSION

In the current study by measuring fasting glucose, insulin, lipid profile, BMI, blood pressure, osteocalcin, adiponectin and through mathematical model for insulin resistance HOMA-IR in population with strong family history of diabetes, we tried to assess the association of osteocalcin and adiponectin in future onset of metabolic syndrome a distinguishing feature for the onset of diabetes and we observed statistically significant higher levels of fasting glucose and insulin and lipid profile in study population than in control population (Table 1). As the study done by Anastassios G. Pittas et al. [11] observed the statistically significant variation in serum osteocalcin with BMI, we in this study did attempt to minimize the effect of BMI confounder by enrolling participants (all the three groups) with uniform BMI (P .078 Table 1). From the baseline anthropometric and biochemical characteristics of study population and control group, of osteocalcin and adiponectin levels, we observed statistically significant low adiponectin levels but not in osteocalcin.

Though few studies showed the sexual dimorphism in levels of adiponectin between males and females like study done by Altan Onat et al. [12], we observed no statistical difference of adiponectin (Table 3) which may be because of the selection of particular age. No data is available on gender difference of adiponectin levels at this particular age group we selected. The same insignificant difference in levels of osteocalcin is also observed (Table 3), in support to our observations, in the study done by Selda Celik et al. [13] they observed

gender variation in levels only between postmenopausal women but not with premenopausal women when compared with male counterparts.

In contrast to the observations of study done by Confavreux et al. [14], in the present study we found insignificant correlation between osteocalcin and HOMA-IR with Pearson correlation .037, p >0.05 (Table 4). In accordance to our findings, study done by You-Cheol Hwang et al. [15] to assess the association of osteocalcin with incidence of type 2 diabetes in 8.4 years follow-up study, they also observed same non association of osteocalcin with onset of diabetes. In a recent study done by Chunyan Lu et al. [16] in analyzing the association of osteocalcin with glucose metabolism and leptin across generations of non diabetic population they also observed insignificant association of osteocalcin (both total and carboxylated osteocalcin) with HOMA-IR and with fasting glucose levels. In an experimental study done by Ferron et al. [2] in wild mice, they observed osteocalcin enhances the expression of insulin gene and cyclin D2 gene for beta cell proliferation. But in human study done by kanasawa et al. [17] they observed insignificant correlation between serum osteocalcin levels and fasting C-peptide, which is a surrogate marker for endogenous insulin secretion. Our results clearly affirm no association of osteocalcin with levels of diabetic markers in children of diabetic parents. Our statement strongly supported by large scale population based long term cohort study (n=1,03,562) by Peter Vestergaard [18], in his study he observed amazing fact of pronounced reduction in risk of developing diabetes up to 80% in patients using antiresorptive drugs against osteoporosis like alendronate. These agents improve osteoporosis by lowering bone turnover by inhibiting osteoclasts. By this mechanism the osteoblasts secret less osteocalcin and theoretically should lead to impaired glucose homeostasis and insulin levels, thus leading to diabetes. In agreement to these findings we in this study through linear regression analysis with parental diabetes as independent variable and osteocalcin as dependent variable, observed insignificant association with B .313, 95% CI -.114 - .740 and P >0.05. Though we observed significant higher levels of blood glucose and lipid profile in children of diabetic parents P<0.05 (Table1), through regression analysis observed no significant association of these biomarkers with plasma osteocalcin levels (Table 6).

From this study of osteocalcin and adiponectin later emerged as independent risk factor which may play an important role in initiating and developing future onset of diabetes in genetically high risk for type 2 diabetes, with highest correlation (Pearson correlation -.504) HOMA-IR (Table 4), and also have strongest association (B -1.502, 95% CI -1.79 - -1.20 and P<0.01) with parental diabetes (Table 5). Though through the Table 1 we observed merely any difference in BMI between control and study population with P>0.05, in evaluating the association between parental diabetes and plasma adiponectin levels in their children through linear regression analysis we observed it is BMI (B -1.149, 95% CI -1.519 - -.778) and blood glucose (B -.029, 95% CI -.044 - -.014) dependent, indicating even at normal BMI adipose tissue of children of diabetic parents may produce less adiponectin and this low adiponectin levels may increases insulin resistance and decrease uptake of blood glucose in peripheral tissue. This observation of significant impact of adiponectin on insulin resistance and blood glucose homeostasis in genetically high risk for diabetes population may have clinical relevance. As per Lucy S. Jun et al. [19] adiponectin activates adenosine monophosphate (AMP) activated protein kinase (AMPK) and peroxisome proliferator activated receptor-α, both of which promote fatty acid oxidation and glucose uptake in skeletal muscle while decreasing inflammation, because decrease in adiponectin expression in this population might lead to development of hyperglycemia and insulin resistance. The hypoglycemic effect of adiponectin was also supported by Berg et al. [20] in their study on administration of recombinant adiponectin they observed reduction in serum glucose in

normal and diabetic rodents without stimulating insulin secretion. Our observation positively correlates with Yamauchi et al. [21] findings. In their study fat derived hormone adiponectin reversed the insulin resistance associated with both lipoatrophy and obesity.

5. CONCLUSION

From this study, of adiponectin and osteocalcin, adiponectin levels were grossly reduced in genetically high risk for diabetes population, and only adiponectin showed inverse correlation with HOMA-IR and from linear regression analysis parental diabetes and blood glucose has highest association with adiponectin indicating its probable role in future onset of diabetes in this population. Though some animal studies showed association of osteocalcin with diabetes, its role in humans may be very limited.

CONSENT

All authors declare that written informed consent was obtained from all participants of this present study.

ETHICAL APPROVAL

All authors declare that required approval was obtained from Institutional Ethics committee with wide approval No: IEC/03/2011.

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CONFLICT OF INTEREST STATEMENTS

We state that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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