Original Article - Role of Adiponectin and Inflammatory Markers CRP, IL-6 and TNF- Alpha in Diabetes Mellitus.

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Abstract
Introduction: *Type 2 diabetes patient have low level of adiponectin and this level correlates with insulin sensitivity level. Similarly inflammatory markers play a role in the development of type 2 diabetes. Elevated levels of interleukin-6 (IL-6) and C-reactive protein (CRP) with increased risk of type 2 diabetes.*
Aim: *The purpose of the study was to determine the association of adiponectin and inflammatory markers in clinically diagnosed diabetes mellitus patient.*
Material and method*: Total 70 cases of diabetes mellitus in age group of 10 years to 65 years, 25 normal healthy control of same group included in the study. 30 cases of complicated diabetic mellitus also collected. Level of adiponectin, TNF- α, CRP and IL-6 was determined by ELISA kit method.*
Result: *In type I DM cases compared with type II DM cases with serum adiponectin level was found to be significantly low in type II DM cases (8.018±4.23 vs 4.66±2.59 pg/ml, p_ <0.001).*
CRP titre was found significantly raised in DM patient in comparison to the control (p _0.009) with a mean value of (0.935±0.77mg/dl vs. 0.508±0.29mg/dl), but in complicated type II DM there was significant increase in CRP titre as compared to the control (1.30±0.85mg/dl vs. 0.5084±0.29mg/dl, p_<0.001).
IL-6 were more or less same to that of healthy control (7.625±8.24 vs 7.776±6.50 pg/ml), but in complicated cases IL-6 level was high in comparison to the control (11.160±7.65 pg/ml vs 7.776±6.50 pg/ml).
In type I DM without any complication TNF-α was comparable to control but in complication mean value was non-significantly higher than control (14.11±15.5pg/ml vs 8.275±3.60 pg/ml). Contrary to this in complicated type II DM mean value of TNF-α was significantly higher than control (p_ 0.009). ***Conclusion:*** *Various inflammatory markers (IL-6, CRP, TNF-α) were found to be increased in type II DM (complicated and uncomplicated) this suggest that infection and inflammation was more common in type II DM as compared to type IDM.* *Low level of serum adiponectin in type II DM as compared to type I DM suggest that insulin resistance play role in type II DM.*
Key words: *Adiponectin, Diabetes Mellitus, C - reactive protein, Tumour necrosis factor -α, Interleukin - 6.*

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I. Introduction

Adiponectin is a protein secreted by the adipocytes and this adiponectin protein play a major role in insulin sensitivity, lowering blood glucose levels and lipid metabolism (Ziemke, et al, 2010). The serum level of this adiponectin protein varies according to age, sex, and body mass index with obese individuals have lower levels of this protein (Lang, et al, 2011). It has been shown that type 2 diabetes (T2DM) patient have low level of adiponectin (Medina-Bravo, et al, 2011) and this level correlates with insulin sensitivity level (Pyrzak, et al, 2010). Consequently, low levels of plasma adiponectin might play a role in the etiology of insulin resistance and T2DM, thus implicating adiponectin gene (ADIPOQ) in the susceptibility of T2DM.

Circulating levels of TNF-α were elevated in individuals with impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) after adjusting for age, gender, ethnicity, clinic site , and body mass index in subjects with normal glucose tolerance, IGT , and T2D, respectively and differed by ethnicity (Oslon, et al, 2011, Chiodini, et al, 2010; Gong, et al, 2010; Siitonen, et al, 2011). There has been growing evidence that inflammatory markers play a role in the development of type 2 diabetes. Elevated levels of interleukin-6 (IL-6) and C-reactive protein (CRP) with increased risk of type 2 diabetes by conducting a meta-analysis and detected a significant dose-response association of IL-6 levels with type 2 diabetes risk (Wang, et al, 2013)

II. Method and material

Total 70 cases of diabetes mellitus in age group of 10 years to 65 years were included in the study and 25 normal healthy control of same age group were also collected. 30 cases of complicated diabetic mellitus also collected. Clinical details will be recorded in pretested performa in all cases.

Collection of sample

5 ml blood will be taken in plain vial for adiponectin, TNF-α, IL-6, and CRP.

Method for estimation

- IL-6 level was estimated by the Elisa kit of Neogin Corporation, supplied by Anand Brothers, New Delhi.
- Serum adiponectin level was determined by the Demetic Elisa kit. The Demetic Elisa for adiponectin DEE009 is a so-called sandwich -assay using two specific and high affinity antibodies.
- TNF-α level was estimated by Avibion human TNF-α Elisa Kit supplied by Orgenium laboratory.

Presentation of result and statistics:

Statistical analysis was performed using SPSS version-16. The various parameters studied during observation period were compared using chi-square test for non-continuous variable. For continuous variables Mann Whitney U test and Anova test was used. The results were presented in absolute frequencies, percentages, averages, standard deviation, relative risk. The critical value of "p" indicating the probability of significant difference was taken as <0.05 for comparison.

III. Observations

Table 1: Distribution of Diabetes mellitus cases according to the age and sex.

Age (years)	Subgroup			
	Total cases of DM		Control	
	No.	%	No.	%
10-20	9	12.9	0	0
21-30	10	14.3	16	64
31-40	5	7.1	5	20
>40	46	65.7	4	16
Total	70	100	25	100

Total 70 Cases of diabetes mellitus were studied with in a period 1 year. 9 Cases of type 1 DM were detected between 10 years to 20 years. 10 Cases were Seen in third decade (14.3%) and maximum Cases were of type II DM occurring after 40 year**. (Table -1)**

Table 2: level of IL-6 in diabetes mellitus cases & control.

Groups	IL-6 level (pg/ml)					
	<12pg/ml	12-20pg/ml	>20pg/ml	Mean±SD	A vs. B t-value	p-value
(A) Diabetic cases (n=70)	41 (58.6)	14 (20)	15 (21.4)	10.007±8.60	1.549	0.125
(B) Control (n=25)	20 (80)	3 (12)	2 (8)	7.776±6.50		

Cytokine levels are increased in DM patients, Serum IL-6 level were raised above 12pg/ml in 41.4% diabetics as compared to 20% in control. Although mean value of IL-6 was high in diabetic patients (10.007±8.60 pg/ml) as compared to control (7.776±6.50 pg/ml) but statistically it was not found significant. (Table-2)

Table 3: Correlation of IL-6 with different type of diabetes mellitus and its complication.

Groups	IL-6 mean+SD	p-value
(A) Type I without complication	10.3750±9.87	0.500 (A vs. E)
(B) Type I with complication	14.0000±14.14	0.635 (B vs. E)
(C) Type II without complication	7.6250±8.24	0.999 (C vs. E)
(D) Type II with complication	11.16071±7.65	0.230 (D vs. E)
(E) Control	7.776±6.50	

IL-6 were analyzed according to the types of DM with control. In type I DM without complication level of IL-6 was high as compared to control (10.37±9.87 pg/ml vs. 7.776±6.50 pg/ml). Further in type I DM with complication level of IL-6 was found to be more elevated in comparison to the control (14.00±14.14 pg/ml vs 7.776±6.50 pg/ml) but statistically it was not found to be significant. In type II DM without complication IL-6 were more or less same to that of healthy control (7.625±8.24 vs 7.776±6.50 pg/ml), but in complicated

cases IL-6 level was high in comparison to the control (11.160±7.65 vs 7.776±6.50 pg/ml) but that was not found to be statically significant.(Table-3)

Table 4: Correlation of CRP titer with diabetes mellitus cases and control.

Groups	CRP titer(mg/dl)			A vs. B t-value	p-value
	<0.6mg/dl	>0.6mg/dl	Mean±SD		
(A) Diabetic cases (n=70)	33 (47.1)	19 (76)	0.93514±0.77	2.672	0.009
(B) Control (n=25)	37 (52.9)	6 (24)	0.50840±0.29		

CRP titer was found significantly raised in DM patient in comparison to the control (p _0.009) with a mean value of (0.935±0.77mg/dl vs. 0.508±0.29mg/dl). 76% of DM cases showed CRP titer >0.6mg/dl in comparison to the control in which only 24% have CRP titer >0.6mg/dl. (Table-4)

Table 5: Correlation of CRP titer with different type of diabetes mellitus and its complication.

Groups	CRP titer (mean+SD)	p-value
(A) Type I without complication	0.518±0.53	1.000 (A vs. E)
(B) Type I with complication	2.245±0.17	0.001 (B vs. E)
(C) Type II without complication	0.6985±0.46	0.699 (C vs. E)
(D) Type II with complication	1.308571±0.85	<0.001 (D vs. E)
(E) Control	0.5084±0.29	

In type I DM cases without complication there was no significant increase in CRP titer in comparison to the control (0.518±0.53 vs 0.508±0.29 mg/dl) but in complicated type I DM cases CRP titer was significantly raised in comparison to the control (p_0.001) and mean value of 2.24±0.017mg/dl and 0.5084±mg/dl respectively. Similarly in type II DM patient CRP titer was more than control (0.698±0.46 mg/dl vs 0.584±0.25mg/dl) but this correlation statistically not found significant but in complicated type II DM there was significant increase in CRP titer as compared to the control (1.30±0.85 vs. 0.5084±0.29 mg/dl , p_<0.001**). (Table-5)**

Table 6: Correlation of TNF_α with diabetes mellitus cases and Control.

Groups	TNF_α level (pg/ml)			Mean±SD	A vs. B t-value	p-value
	<5pg/ml	5-10 pg/ml	>10pg/ml			
(A) Diabetic cases (n=70)	22 (31.4)	19 (27.1)	29 (41.4)	12.01143±9.41	1.929	0.057
(B) Control (n=25)	4 (16)	14 (56)	7 (28)	8.27560±3.60		

TNF-α was done in 70 diabetic patients and 24 healthy control. In 41.4% patient of DM serum TNF-α was more than 10pg/ml while in healthy control only 28% have raised value above 10pg/ml. Mean value of TNF-α was raised (12.011±9.41pg/ml) as compared to control (8.27±3.60pg/ml) which was borderline significant (p_ 0.057) (Table- 6).

Table 7: Correlation of TNF-α with diabetes mellitus cases and its complication.

Groups	TNF-α (mean+SD)	p-value	p-value
(A) Type I without complication	8.6900±8.50	1.000 (A vs. E)	1.000 (A vs. B)
(B) Type I with complication	14.110±15.52	0.755 (B vs. E)	
(C) Type II without complication	10.6330±8.09	0.762 (C vs. E)	0.556 (C vs. D)
(D) Type II with complication	15.2185±9.99	0.009 (D vs. E)	
(E) Control	8.27560±3.60		

Level of TNF-α was correlate with DM with and without complication and subtype of DM. In type I DM without any complication TNF-α was comparable to control but in complication mean value was higher than control (14.11±15.5pg/ml vs 8.275±3.60 pg/ml) but statically not significant. Contrary to this in complicated type II DM mean value of TNF-α was significantly higher than control (p_0.009). (Table-7)

Table 8: Correlation of Adiponectin with diabetes mellitus cases & control.

Groups	Adiponectin level (pg/ml)			Mean±SD	A vs. B t-value	p-value
	<5pg/ml	5-10pg/ml	>10pg/ml			
(A) Diabetic cases (n=70)	39 (55.7)	21 (30)	10 (14.3)	5.71770±3.53	0.075	0.940
(B) Control (n=25)	13 (52)	10 (40)	2 (8)	5.65874±2.78		

Adiponectin level was analyzed with total DM cases and control .In 55.7% patient of DM serum adiponectin level was <5pg/ml while in healthy control 52% have adiponectin level <5pg/ml. Total DM cases shows a mean value (5.71±3.53pg/ml) which was more or less same to the control (5.65±2.78pg/ml) .(Table-8)

Table 9: Correlation of Adiponectin with different type of diabetes mellitus and its complication.

Groups	Adiponectin mean+SD	p-value
(A) Type I without complication	8.04599±4.44	0.045 (A vs. E)
(B) Type I with complication	7.74500±0.70	0.801 (B vs. E)
(C) Type II without complication	4.292245±2.15	0.428 (C vs. E)
(D) Type II with complication	4.928032±2.87	0.835 (D vs. E)
(E) Control	5.658740±2.78	
(F) Total Type I DM cases	8.0186±4.23	<0.001
(G) Total Type II DM cases	4.6631±2.59	(F vs. G)

In Type I DM cases serum adiponectin level was more than healthy control (8.045±4.44 pg/ml vs 5.65±2.78 pg/ml). Although type 2 DM cases showed adiponectin level less then healthy control (4.92±2.87 pg/ml vs 5.65±2.78 pg/ml) but this was not found statistically significant. When type I DM cases compared with type II DM cases with relation to serum adiponectin level, this correlation was statistically found significant (p <0.001) with mean value of 8.0186±4.23 pg/ml and 4.663±2.59 pg/ml respectively. **(Table- 9)**

IV. Discussion
IL-6 in diabetes mellitus-
Many previous studies have shown that pro-inflammatory marker IL-6 is increased in type 2 DM cases and in patient with impaired glucose tolerance (Spranger, et al, 2003 and Hu, et al, 2003). But Choi, et al, in 2003 showed that IL-6 neither increases in subject with impaired glucose tolerance nor closely correlate with component of metabolic syndrome or type 2 DM.

Present study also was did not find any significant association between IL-6 level in type 2 DM cases (7.62±8.24 pg/ml) in comparison to control (7.776±6.50 pg/ml). However increased level of IL-6 was found in type 2 DM with complication (11.16±7.65 pg/ml) in comparison to the control (7.776±6.50 pg/ml), however this association was not found to be statistically significant.

CRP titer in diabetes mellitus
Choi, et al, 2003 and Wang, et al, 2013, showed that CRP was one of important inflammatory marker increased in patient of type 2 diabetes mellitus and impaired glucose tolerance. Present study also found the significant increase in CRP titer in DM patient in comparison to the control (mean±SD ,0.935±0.77 vs 0.508±0.29 mg/dl, p < 0.009). There was significant increase in CRP titer in complicated type 2 DM patient in comparison to the control (mean±SD, 1.30±0.85 vs 0.508±0.29 mg/dl, p <0.001). CRP titer also found to be increased in type 2 DM patient without complication in comparison to the control (mean±SD, 0.698±0.46 vs 0.508±0.29 mg/dl, p-value 0.699) although it was not found to be statistically significant.

TNF-α in diabetes mellitus
Many studies have found the association of inflammatory marker with impaired glucose tolerance and type 2 DM. Circulating TNF-α was elevated in individual with impaired glucose tolerance and type 2 DM (Oslon, et, al,2011, Nadeem, et al,2013).

Present study also showed that circulating TNF-α level was more elevated in type 2 DM cases (10.63±8.09 pg/ml) in comparison to the control (8.27±3.60 pg/ml) , although this association was not found statistically significant but when type II DM patient with complication compared with control there was significant increase in TNF-α level in complicated type II DM (15.21±9.99 pg/ml) in comparison to the control (8.27±3.60 pg/ml) with a p-value of 0.009. Contrary to it in type 1 DM TNF-α level was more or less same to that of healthy control. This suggest that infection ,inflammation and tissue necrosis is more common in type II DM then type I.

Adiponectin in diabetes mellitus

Adiponectin is an adipocyte-secreted protein playing a major role in regulating blood glucose levels, insulin sensitivity, and lipid metabolism (Ziemke, et al, 2010). Adiponectin levels vary according to age, sex, and body mass index with lower levels in obese individuals (Lang, et al, 2011).

Low level of serum adiponectin is associated with insulin resistance and type 2 diabetes mellitus (Vandramini, et al ,2006, Jalovaara, et al, 2008). Present study also showed that serum adiponectin level decreases in type II DM patient (mean±SD ,4.92±2.87 pg/ml) in comparison to the control (mean±SD, 5.65±2.78 pg/ml) although this association was not found to be statistically significant. But when type 1 DM cases compared with type II DM cases there was statistically significant decrease in serum adiponectin level in type II DM cases in comparison to the type 1 DM (4.66±2.5 pg/ml Vs 8.01±4.23 pg/ml) with a p-value of <0.001. These data indicate that low level of serum adiponectin is associated with insulin resistance and type II DM. Contrary to it in type I DM there was no significant increase in adiponectin as compared to the control. This suggest that low level of adiponectin is associated with insulin resistance which found in type II DM.

In present study prevalence of type 1 DM was more in male patient ((68.18%) in comparison to the female (31.8%). Majority of type 1 DM cases (86.36%) were below 30 years of age. However, there was no major difference in mean age of diagnosis between male and female patient (20.6 yrs vs 21.42 years). Only 3 cases were found >30 years of age which could be due to late diagnosis of disease in these patients. Youngest patient was 10 years old female.

V. Summary and Conclusion

In diabetic patients serum IL-6 level was non-significantly high. Correlation of serum IL-6 level with types of diabetes mellitus showed that in type II DM without complication IL-6 were more or less same to that of healthy control (7.625±8.24 vs 7.776±6.50 pg/ml), but in complicated cases IL-6 level was high in comparison to the control (11.160±7.65 pg/ml vs 7.776±6.50 pg/ml). In type I DM without complication level of IL-6 was high as compared to control but statistically it was not significant.

CRP titer was found significantly raised in DM patient in comparison to the control (p _0.009) with a mean value of (0.935±0.77mg/dl vs. 0.508±0.29mg/dl). In type I DM cases without complication there was no significant increase in CRP titer in comparison to the control (0.518±0.53mg/dl vs. 0.508±0.29 mg/dl) , but in complicated type II DM there was significant increase in CRP titre as compared to the control (1.30±0.85mg/dl vs. 0.5084±0.29mg/dl, p_<0.001**).**

In type I DM without any complication TNF-α was comparable to control but in complication mean value was non-significantly higher than control (14.11±15.5pg/ml vs 8.275±3.60 pg/ml). Contrary to this in complicated type II DM mean value of TNF-α was significantly higher than control (p_ 0.009).

In Type I DM cases serum adiponectin level was more or less same to the healthy control (8.045±4.44 pg/ml vs 5.65±2.70 pg/ml) while in type II DM cases adiponectin level was non-significantly less then healthy control. when type I DM cases compared with type II DM cases with serum adiponectin level was found to be significantly low in type II DM cases (8.018±4.23 vs 4.66±2.59 pg/ml, p_ <0.001).

VI. Conclusion

Various inflammatory markers (IL-6, CRP, TNF-α) were found to be increased in type II DM (complicated and uncomplicated) this suggest that   infection and inflammation was more common in type II DM as compared to type I DM.

Low level of serum adiponectin in type II DM as compared to type I DM suggest that insulin resistance play role in type II DM.

References

[1].   Ziemke, F., Mantzoros, C.S, 2010. Adiponectin in insulin resistance: lessons from translational research. Am. J. Clin. Nutr. 91 (1), 258S–261S.
[2].   Lang, H.F., Chou, C.Y., Sheu, W.H., Lin, J.Y., 2011. Weight loss increased serum adiponectin but decreased lipid levels in obese subjects whose body mass index was lower than 30 kg/m2. Nutr. Res. 31, 378–386.
[3].   Medina-Bravo, P., et al., 2011. Decrease in serum adiponectin levels associated with visceral fat accumulation independent of pubertal stage in children and adolescents. Arch. Med. Res. 42, 115–121.
[4].   Pyrzak, B., Ruminska, M., Popko, K., Demkow, U., 2010. Adiponectin as a biomarker of the metabolic syndrome in children and adolescents. Eur. J. Med. Res. 15 (Suppl. 2), 147–151.
[5].   N Olson N C, Callas P W, Hanley A J, Festa A, Haffner S M, Wagenknecht L E, and Tracy R P **.** Circulating Levels of TNF-α are associated with Impaired Glucose Tolerance, Increased Insulin Resistance, and Ethnicity : The Insulin Resistance Atherosclerosis Study. J. *Clin EndocriClin Metab-2012,* 97:1032–1040.
[6].   Chiodini, B.D., et al., 2010. Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population. Ther. Adv. Cardiovasc. Dis. 4, 223–230.
[7].   Gong, M., Long, J., Liu, Q., Deng, H.C., 2010. Association of the ADIPOQ rs17360539 and rs266729 polymorphisms with type 2 diabetes: a meta-analysis. Mol. Cell. Endocrinol. 325, 78–83.

[8]. Siitonen, N., et al., 2011. Association of ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin concentrations: the Finnish Diabetes Prevention Study. BMC Med. Genet. 12, 5.

[9]. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Lui LG. Inflammatory markers and risk of type 2 diabetes: A systematic review and meta-analysis. Diabetic care. 2013, 36: 166-75.

[10]. *Spranger J , Kroke A, Möhlig M, Bergmann M M, Ristow M, Boeing H, Pfeiffe A FH.* Adiponectin and protection against type 2 diabetes mellitus. . *Lancet* -2003; 361: 226–28

[11]. Spranger J, Kroke A, Mo¨hlig M,Hoffmann K, Bergmann M M, Ristow M, Boeing H, and Pfeiffer A F .   Inflammatory Cytokines   and   the   Risk   to Develop Type 2 Diabetes. Results of the Prospective   Population-Based European Prospective Investigation into Cancer and Nutrition (EPIC)- Potsdam Study . *Diabetes-2003*,52:812–817.

[12]. Choi KM,   Leeb J,   Lee KW,   Seo JA, Oha JH, Kima SG, Kima NH, Choi DS, Baik SH .Comparison of serum concentrations of C-reactive protein, TNF-α, and interleukin 6 between elderly Korean women with normal and impaired glucose tolerance. Diabetes Research and Clinical Practice,2004, 64; 99–106.

[13]. Jalovaara K, Santaniemi M, Jokelainen J,Ukkola O, Kesaniemi Y.A, Rajjala U, Kiukaanniemi SK, Low serum adiponectin level as a predictor of impaired glucose regulation and type 2 diabetes mellitus in a middle-aged Finnish population,2008.