ORIGINAL RESEARCH PAPER

Diabetology

PHARMACOLOGICAL EFFECT OF METFORMIN AND PIOGLITAZONE ON VISFATIN DERIVED FROM ADIPOCYTES IN PATHOGENESIS OF TYPE 2 DIABETES

KEY WORDS: Adipocytes; visfatin; mRNA expression; T2DM; Obesity; Lipid profile

Dr. Madhukar Saxena*	M.Sc., Ph.D. (Research Associate), Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareilly Road, Lucknow, India.,Institute for Industrial Research & Toxicology, UPSIDC, MG Road, Ghaziabad, Uttar Pradesh, India. *Corresponding Author
Yash Chikara	B.V.Sc. & A.H. (Research Scholar), Bombay Veterinary College, Goregaon (East), Mumbai, Maharashtra, India.
Mohseen	M.Pharm. (Research Scholar), Institute for Industrial Research & Toxicology, UPSIDC, MG Road, Ghaziabad, Uttar Pradesh, India.
Dr. Vandana Singh	M.V.Sc. (Scientist), Institute for Industrial Research & Toxicology, UPSIDC, MG Road, Ghaziabad, Uttar Pradesh, India.
Dr. Dinesh Raj Modi	M.Sc., Ph.D. (Professor), Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareilly Road, Lucknow, India

ABSTRACT

Adipocytes secreate many adipocytokines including visfatin. Many evidence either in direct relation or in in vitro showed that visfatin alters the state of type 2 diabetes (T2DM). To unfold the role of visfatin in response to metformin and pioglitazone we have investigated the lipid profile and levels of visfatin along with its mRNA expression in response to antidiabetic drugs metformin and pioglitazone in vitro adipocytes. Adipocytes were cultured for the estimation of lipid profile and secreted visfatin using ELISA and the response of mRNA visfatin gene expression by the use of metformin hydrochloride and pioglitazone hydrochloride and combination of both. The determination was performed by RT- PCR quantification. Differences were considered significant when P values were ≤0.05 calculated using SPSS software (ver. 19). In Glucose treated adipocytes the lipid profile showed significant change in HDL while highly significant change in other lipoproteins. However, the released level of visfatin also showed significant change in glucose treated adipocytes as compared to normal control adipocytes. No significant change was observed in metformin hydrochloride while pioglitazone hydrochloride showed significant change as concurred by mRNA level estimation. The present report examined whether visfatin is regulated by anti-diabetic drugs metformin and pioglitazone in glucose feed adipocytes mimicking the state of T2DM along with effect of these drugs in secreted lipid profile. Pioglitazone treatment showed highly significant association at higher concentration. Our finding suggest that the treatment with pioglitazone in glucose treated adipocytes could play a role in the regulation of visfatin in adipocytes.

INTRODUCTION

Adipocytes secreate many adipocytokines including visfatin. In obese individual the level of visfatin is increasing significantly. In obese state adipocytes depots and secreate visfatin due to hypertrophy and hyperplasia which alters the metabolism and homeostasis of the individual. Visfatin has many actions in the body and it is of two kinds extracellular and intracellular. Many evidence either in direct relation or in in vitro showed that visfatin alters the state of many metabolic syndrome including obesity and type 2 diabetes (T2DM) as well as some cancers [1].

It has been proposed that visfatin mimics the role of insulin apart from their role as proinflammatory cytokine [2]. Previously visfatin was known as pre-B cell colony-enhancing factor (PBEF) [3]. Apart from its action on immune system high dose of visfatin decreases level of glucose by inhibiting the production of endogenous glucose production and increasing the uptake of glucose [4]. Although it is established fact that human brown adipocytes are involved in the burning of fat as well as glucose. Due to this thermogenic effect it is now considered to create panic to metabolic disorders including obesity and T2DM. Visfatin, a nicotinamide phosphoribosyl transferase is involved in nicotinamide dinucleotide synthesis and is involved in the insulin production from cells of pancreas [5]. Studies have been established to understand the role of visfatin how it exert insulin mimicking effects in vivo as well as in vitro [6]. The molecular mechanistic behavior of visfatin was also established that visfatin activates the insulin signaling cascade involving tyrosine phosphorylation of the insulin receptor and insulin receptor substrate 1/2 (IRS1/2) and downstreaming of protein kinase B/Akt [6]. Additionally visfatin activates insulin receptor in different manner as that of insulin. To understand this mechanism and the effect of visfatin in response to lipid profile we speculate that the mRNA gene expression and secretion level of visfatin will clear the sight of it and their involvement in the progression of T2DM. However, now visfatin is targeted for the treatment of obesity and T2DM and the development of new antidiabetic therapies. Apart from this our major queries are that whether visfatin mRNA gene expression in adipocytes is regulated by traditional antidiabetic drugs viz. metformin and pioglitazone and how they can improve the insulin sensitivity. To unfold this we have investigated the lipid profile and levels of visfatin along with its mRNA expression in response to antidiabetic drugs metformin and pioglitazone in vitro adipocytes.

MATERIALS AND METHODS

ADIPOCYTES

Wharton's Jelly Mesenchymal Stem Cells (Himedia) were cultured for 0-48 h in DMEM (Dulbecco's modified Eagle's medium)/ Ham's F12 (1:1) media supplemented with 10% FCS (fetal calf serum) having antibiotics and normal glucose level (5 mM). The cell line proliferated in the medium containing DMEM/F-12 medium (1:1, v/v), HEPES, FBS/FCS and antibiotics and upon the addition of Insulin Transferrin selenium (ITS), Sodium bicarbonate, biotin and pantothenate begins the differentiation phase. These cells were maintained and at ~7 day abundant lipid droplets were accumulated in the cell which was checked by using lipophilic/ fatty acid soluble dye, the Oil Red O staining. Cultured cells were then fixed in a 10% formaldehyde solution in PBS (Phosphate buffer saline) for 5 min at RT (room temperature) followed by

washing with 60% isopropanol. The cells then stained with Oil Red O solution in 60% isopropranol for 10 min; then wash the cells with 10 ml water at least four times. Stained cells were then immediately viewed under phase contrast inverted microscope and images were captured using inbuilt mounted digital camera.

BIOCHEMICAL ASSAYS

Supernatant from Adipocytes (centrifuged at 300g for 2 min.) were used for biochemical assays. Ferric chloride-acetic acid reagent was used for the estimation of Total Cholesterol (TC), the reagent mixed gently and centrifuged at 3354xg for 10 min. at 4 0C. Supernatant was poured in conc. H2SO4 and incubated at 50-60 0C for 10 min. When the solution reached room temperature the absorbance was read at 560 nm. Similarly, Phosphotungustate reagent and Magnesium Chloride (MgCl2) was used initially for the estimation of High Density Lipoprotein (HDL) followed by centrifugation (3354xg for 30 min at 4 0C). Supernatant then mixed with Ferric chloride-acetic acid reagent and sulphuric acid (0.033N H2SO4) and incubation was done at 50-60 ^{0}C for 10 min. The absorbance was read at 560 nm at room temperature. Similarly, Triglycerides (TGL) was measured in adipocyte cells using n-heptane, Iso-propanol, sulphuric acid (0.08N H2SO4), potassium hydroxide (KOH, 6.25 mol/L), sodium metaperiodate and acetyl acetone. Incubated the mixture at 70 ^{0C} for 20 min. and absorbance was taken at 425 nm. Low Density Lipoprotein-Cholesterol (LDL) and Very Low Density Lipoprotein-Cholesterol (VLDL) were calculated by known formulae [LDL=TC – (TGL / 5 + HDL); VLDL=TC – (LDL + HDL)]. The readings were randomly checked Inoline kits (Merck) in a double-beam UV-vis spectrophotometer. All readings were recorded in triplicates.

Metformin hydrochloride and pioglitazone hydrochloride treatment and visfatin estimation Control Adipocytes along with adipocytes incubated with 20mM of glucose. Glucose incubated adipocytes were treated with metformin hydrochloride (50-200 mg/ml) and pioglitazone hydrochloride (5-20 mg/ml) (Sigma Aldrich, USA) and combination of both. The standardization of drugs was as per our previous report [7]. Cells were centrifuged at 300g for 2 min. to remove supernatant which was used for the estimation of visfatin by enzyme linked immunosorbent assays (ELISA) (invitrogen (USA)).

DETERMINATION OF MRNA LEVELS

For RNA determination, the oligonucleotide primer pairs Sense (5'->3') CCAGAGCTCCCAGACTGC and Antisense (5'->3') GTGGCCAGGAGGATGTTGAA were used; β-Actin was used as housekeeping gene: 5'-ACGGGGTCACCCACACTGT GC-3' and 5'-CTAGAAGCATTTGCGGTGGACGATG-3'. Total RNA was isolated from adipocytes (~107 cell) by GeneJET RNA Purification kit (Thermo Scientific, India) followed by cDNA amplification using random hexamer primers (Verso cDNA synthesis kit (Thermo Scientific, India)).

mRNA gene expression was performed by DyNAmo ColorFlash SYBR Green qPCR kit (Thermo Scientific, India). Relative quantification was done between all the groups. Quantification was performed with a SYBR-Green real-time PCR assay of target gene mRNA which was expressed relative to the housekeeping gene mRNA. Amplification was performed with PCR master mix-containing target primers, DNA polymerase, SYBR-Green I, 5mM $MgCl_2$, dNTP mix including dUTP and PCR buffer in duplicates. Amplification cycle starts with initial denaturation at 95^{0}C for 7 min, followed by 40 PCR cycles each consisting of 95^{0}C for 10 sec, 62^{0}C for 30 sec and 74^{0}C for 60 sec. Relative gene expression of visfatin was calculated.

Statistical Analysis

The values were plotted using Microsoft excel. Student t-test was used for statistical analysis. Differences were considered significant when P values were ≤0.05. Results were expressed as means standard deviations. P values were calculated using SPSS software (ver. 19).

RESULTS

Table 1 shows the mean ± standard deviation of lipid profile in adipocytes and level of visfatin before and after treatment of variable concentration of metformin and pioglitazone along with their combinations. In glucose treated adipocytes there was a significant increase in the lipid profile including total cholesterol (TC), triglyceride (TGL), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) levels ($p<0.05$). However, the released level of visfatin also showed significant ($p = 0.010$) decrease in glucose treated adipocytes as compared to normal control adipocytes (Table 1). During the treatment of antidiabetic drug metformin hydrochloride and pioglitazone the change in the levels of lipid profile as well as visfatin is remarkable. When we treat the cultured glucose treated adipocytes with metformin hydrochloride (50 mg/ml) we found that the no significant decrease in it while metformin hydrochloride (100 mg/ml) treatment only HDL showed significant decrease ($p = 0.039$) (Table 1). Similarly no significant association was observed during the treatment of adipocytes at metformin hydrochloride (150 mg/ml). While HDL showed significant decrease was observed in the treatment of adipocytes with metformin hydrochloride (200 mg/ml) (Table 1). Moreover to this the behavior on the lipid profile is interesting in case of pioglitazone treatment. During the treatment of adipocytes with pioglitazone hydrochloride (5 mg/ml) significant increase was observed in lipid profile except HDL and LDL and no association was observed in pioglitazone hydrochloride (10 mg/ml) treatment (Table 1). While pioglitazone hydrochloride (15 mg/ml) TGL, HDL and VLDL showed significant difference ($p = 0.016$) as similar in pioglitazone hydrochloride (20 mg/ml) ($p = 0.004$) except HDL (Table 1). Although, in case the relation of visfatin with metformin hydrochloride no change was observed when compared with the glucose treated adipocytes while showed significant release as compared to normal control adipocytes. However, during the treatment of pioglitazone hydrochloride significant increase was observed at concentration of 10 mg/ml and 20 mg/ml having $p = 0.025$ and $p = 0.012$ respectively. This showed that the treatment with pioglitazone in glucose treated adipocytes regulate the visfatin level.

Table 1: Mean ± Standard Deviation Of Lipid Profile In Adipocytes And Level Of Visfatin Before And After Treatment Of Variable Concentration Of Metformin And Pioglitazone Along With Their Combinations.

	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Visfatin (ng/ml)
Control	212.67 ± 3.06	132.00 ± 2.65	31.00 ± 2.00	155.27 ± 2.00	26.40 ± 0.53	20.37 ± 0.71
Glucose Treated	250.00 ± 3.00^{a}	158.00 ± 2.00^{a}	35.33 ± 0.58^{a}	183.07 ± 3.70^{a}	31.60 ± 0.40^{a}	17.63 ± 0.75^{a}
Glucose+Met50	253.67 ± 2.52^{a}	158.67 ± 2.52^{a}	35.17 ± 0.76^{a}	186.77 ± 1.79^{a}	31.73 ± 0.50^{a}	18.63 ± 0.15^{a}
Glucose+Met100	246.33 ± 1.53^{a}	155.67 ± 1.53^{a}	34.00 ± 0.50^{b}	181.20 ± 1.73^{a}	31.13 ± 0.31^{a}	17.27 ± 0.25^{a}
Glucose+Met150	252.00 ± 1.00^{a}	157.83 ± 0.29^{a}	34.43 ± 0.21^{a}	186.00 ± 0.82^{a}	31.57 ± 0.06^{a}	17.03 ± 0.06^{a}
Glucose+Met200	247.83 ± 1.26^{a}	158.17 ± 0.76^{a}	36.77 ± 0.25^{ab}	179.43 ±1.46^{a}	31.63 ± 0.15^{a}	16.97 ± 0.21^{a}
Glucose+Pio5	243.33 ± 1.53^{ab}	150.17 ± 1.04^{ab}	34.23 ± 0.78	179.07 ± 2.30^{a}	30.03 ± 0.21^{ab}	17.27 ± 0.25^{a}
Glucose+Pio10	250.67 ± 1.53^{a}	156.00 ± 0.20^{a}	34.93 ± 0.70^{a}	184.53 ± 1.99^{a}	31.20 ± 0.04^{a}	16.03 ± 0.25^{ab}
Glucose+Pio15	251.33 ± 2.52^{a}	151.33 ± 2.08^{ab}	36.87 ± 0.32^{ab}	184.20 ± 1.95^{a}	30.27 ± 0.42^{ab}	16.43 ± 0.31^{a}

Glucose+Pio20	252.83 ± 1.76[a]	150.17 ± 1.04[ab]	34.37 ± 1.00	188.43 ± 1.00[a]	30.03 ± 0.21[ab]	15.47 ± 0.42[ab]

TC: Total-cholesterol; TGL: Triglyceride; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein cholesterol; VLDL: Very low density lipoprotein cholesterol; Met50: Metformin hydrochloride (50 mg/ml); Met100: Metformin hydrochloride (100 mg/ml); Met150: Metformin hydrochloride (150 mg/ml); Met200: Metformin hydrochloride (200 mg/ml); Pio5: Pioglitazone hydrochloride (5 mg/ml); Pio10: Pioglitazone hydrochloride (10 mg/ml); Pio15: Pioglitazone hydrochloride (15 mg/ml); Pio20: Pioglitazone hydrochloride (20 mg/ml). All the readings were recorded in triplicate. [a] $P < 0.05$ vs control adipocytes; [b] $P < 0.05$ vs glucose treated adipocytes.

We also examined the mRNA level of visfatin which was significantly released as compared to normal controls ($p < 0.001$). The quantified the mRNA level of visfatin in adipocytes with respect to the metformin hydrochloride showed significant decrease in the state of visfatin level at 100 mg/ml, 150 mg/ml and 200 mg/ml having $p = 0.004$, $p = 0.007$ and $p = 0.001$ respectively (Figure 1a) as compared to control adipocytes. However, during the treatment of pioglitazone hydrochloride only 20 mg/ml showed significant decrease ($p = 0.042$) (Figure 1b). Furthermore, in combination of both the drugs i.e. metformin hydrochloride and pioglitazone hydrochloride significant association of mRNA level was observed at low concentration of metformin hydrochloride (50 mg/ml) and pioglitazone hydrochloride (5 mg/ml) ($p = 0.030$). While at 150 mg/ml metformin hydrochloride and 15 mg/ml pioglitazone hydrochloride and 200 mg/ml metformin hydrochloride and 20 mg/ml pioglitazone hydrochloride showed highly significant association ($p = 0.006$ and $p = 0.002$ respectively) (Figure 1c).

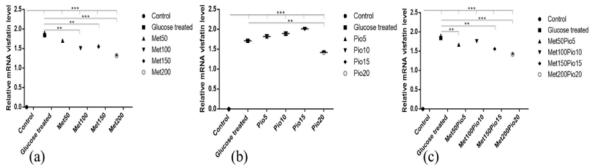

Figure 1: The relative visfatin mRNA expression in response to anti diabetic drugs (a) metformin hydrochloride (b) pioglitazone hydrochloride (c) combination of both metformin hydrochloride and pioglitazone hydrochloride. ** showed significant association and *** showed highly significant association when campared with glucose treated adipocytes and control adipocytes respectively. The p values were calculated using one way ANOVA.

DISCUSSION AND CONCLUSIONS

Adipocytes have many functions apart from triglycerides storage. The importance of adipocytes in the pathogenesis and manifestation of T2DM is attracting the interest of researchers around the globe. Adipocyte released variety of cytokine including visfatin [6]. This is well established fact that the secretion of adipokines from adipocytes plays an important role in the development of T2DM as well as in insulin resistance and it was also proposed that the level of many of adipocytokines including adiponectin and visfatin has been altered [8]. In the present report we have examined whether visfatin is regulated by anti-diabetic drugs metformin hydrochloride and pioglitazone hydrochloride and their combinations in glucose feed adipocytes mimicking the state of T2DM and also on the effect of these drugs in secreted lipid profile. It is well established fact that visfatin was clearly expressed in adipocytes and is a true adipocytokine. We found that the level of secreting visfatin in glucose treated adipocytes is highly increased ascompared to the controlled adipocytes. This showed that the visfatin enhances the state of inflammation and may be involved in the progression of insulin resistance and T2DM manifestation. The same was analyzed in the mRNA levels of visfatin. The report was supported by other report which showed that the mRNA levels of visfatin were higher in the isolated adipocytes than in the adipocytes of biopsies [9]. However, this report also concluded that the treatment with thiazolidinediones for 3–4 weeks did not increase the mRNA level of visfatin along with the circulating levels of it and no significant association was observed [9]. However, visfatin was highly expressed in visceral fat as that of subcutinous adipocytes and involved in creating insulin resistance [10]. However, one of the report which showed the association of IL-6 on visfatin gene in vitro adipocytes using 3T3-L1 cell line and concluded that IL-6 is negative regulator of visfatin gene expression and no effect was observed with the use of thiazolidinediones [11]. Thus thiazolidinediones did not enhance the mRNA gene expression in vivo as well as in vitro studies. Inflammation avalanche in obesity and T2DM coincide with insulin resistance [12]. Other reports on 3T3-L1 adipocytes showed that differentiated adipocytes were incubated with TNF-alpha did not influence adiponectin receptor (AdipoR1 and AdipoR2) synthesis in vitro [13]. The difference in the results may be due to the change in kind of adipocytes. Our present report is first report to find the association of metformin hydrochloride and pioglitazone hydrochloride in the lipid profile and the regulation of visfatin level and mRNA gene expression using adipocytes. Our results suggest that the visfatin is regulated by pioglitazone in adipocytes. Metformin addition in glucose treated cells did not change significantly visfatin levels although there were reduced compared to control adipocytes. It is supported in other studies that this kind of expression regulation is tissue specific [14-16]. A adipocytokine that is known as PBEF earlier and now known as visfatin and their expression level is quite higher in visceral fat than that of subcutaneous adipocytes and is involved in lowering the glucose level of blood stream by stimulating the uptake of glucose and bind to the receptors of insulin [6]. Previous reports showed that visfatin plays an important role in inflammation by enhancing the pre-B cell colony formation [3], modulating the gene expression of monocytes and neutrophil [17]. It has been reported that visfatin mRNA expression is increased in the monocytes from T2DM individuals independent of their BMI strengthening more the role of visfatin in inflammation [18]. In present report we found that the in glucose treated adipocytes the lipid profile and visfatin release showed significant association. During the treatment of antidiabetic drug metformin hydrochloride at low concentration we found no significant change while HDL showed significant change at high concentration. However, pioglitazone treatment showed significant change in lipid profile except HDL and LDL at low concentration and showedhighly significant association at higher concentration. The level of visfatin showed no change with metformin hydrochloride treatment. Significant change was observed at higher concentration of pioglitazone hydrochloride. Our finding suggest that the treatment with pioglitazone in glucose treated adipocytes could play a role in the regulation of visfatin in adipocytes and regulate the visfatin level. The results were supported by the examination of the mRNA level of visfatin. The result is further conferred by the use of combination of both the drugs, showed highly significant association. Our findings are interesting as this is first kind of report to find the association of visfatin regulation in response to metformin and pioglitazone. More studies are required to understand the exact mechanism and regulation of visfatin in the pathogenesis and manifestation of T2DM and obesity.

Conflict Of Interest

There are no conflicts of interest.

Acknowledgements

MS is thankful for Research Associate from ICMR (IRIS: 2019-5194), New Delhi, India.

REFERENCES:

[1]. Kumari B, Yadav UCS. Adipokine Visfatin's Role in Pathogenesis of Diabesity and Related Metabolic Derangements. Curr Mol Med 2018; 18(2):116-125.

[2]. McGee KC, Harte AL, da Silva NF, et al. Visfatin Is Regulated by Rosiglitazone in Type 2 Diabetes Mellitus and Influenced by NF B and JNK in Human Abdominal Subcutaneous Adipocytes. PLoS One 2011; 6(6): e20287.
[3]. Samal B, Sun Y, Stearns G, et al. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony enhancing factor. Mol Cell Biol 1994; 14: 1431- 1437.
[4]. Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin . Science 2005; 307: 426-430.
[5]. Pisani DF, Dumortier O, Beranger GE, et al. Visfatin Expression Analysis in Association With Recruitment and Activation of Human and Rodent Brown and Brite adipocytes. Adipocytes 2015; 5(2): 186-95.
[6]. Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307: 426-430.
[7]. Saxena M, Ali D, Modi DR, et al. Association of TNF-α Gene Expression and Release in Response to Anti-Diabetic Drugs from Human Adipocytes in vitro. Diabetes Metab Syndr Obes 2020; 13: 2633-2640.
[8]. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; 7: 941 -946.
[9]. Hammarstedt A, ki JP, Sopasakis VR, et al. Visfatin Is an Adipokine, But It Is Not Regulated by Thiazolidinediones. J Clin Endocrinol Metab 2006; 91(3): 1181-1184.
[10]. Brendt J, Kloting N, Kralisch S, et al. Plasma visfatin concentrations and fat depot-specificmRNA expression in humans. Diabetes 2005; 54: 2911-2916.
[11]. Kralisch S, Klein J, Lossner U, et al. Interleukin-6 is a negative regulator of visfatin gene expression in 3T3-L1 adipocytes. Am J Physiol 2005; 10: 1152.
[12]. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specifi c protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; 20: 1595-1599.
[13]. Fasshauer M, Klein J, Kralisch S, et al. Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3-L1 adipocytes . FEBS Lett 2004; 558: 27-32.
[14]. Inukai K, Nakashima Y, Watanabe M, et al. Regulation of adiponectin receptor gene expression in diabetic mice . Am J Physiol Endocrinol Metab 2005; 288: 876-882.
[15]. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects . Nature 2003; 423: 762-769.
[16]. Civitarese AE, Jenkinson CP, Richardson D, et al. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of type 2 diabetes. Diabetologia 2004; 47: 816-820.
[17]. Jia SH, Li Y, Parodo J, et al. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental infl ammation and clinical sepsis . J Clin Invest 2004; 113: 1318-1327.
[18]. Tsiotra PC, Tsigos C, Yfanti E, et al. Visfatin, TNF-aand IL-6 mRNA expression is increased in mononuclear cells from Type 2 Diabetic women. Horm Metab Res 2007; 39: 758 – 763.