Study the Level of Interleukin -18 Binding Protein Alpha in Iraqi Arab female Patients with Diabetes Mellitus Type II

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ABSTRACT---- ***Diabetes mellitus consider as inflammatory disease associated with many inflammatory factors for that the present study aims to investigate the concentration of IL-18 binding protein alpha with classical risk factors to study their role in Iraqi female patients with diabetes mellitus type2. The study included 60 female patients newly diagnosed with type 2 DM and 28 healthy individuals. The patients mean age was (47.48±1.08) while control mean age was (39.46±1.86). Laboratory tests were include detection of IL18BPa in serum by ELISA kits, chemical parameters which included Fasting blood glucose, Glycosylated haemoglobin and Lipid profile using enzymatic and colorimetric methods, besides anthropometric parameters includes central obesity and body mass index. The present study showed a significant increasing in the serum level of IL-18binding protein alpha between DM type 2 patients and control. Fasting blood glucose, Glycosylated haemoglubin, total Cholesterol, Triglyceride, Low Density Lipoprotein, Very Low Density Lipoprotein and Atherogenic index showed significant increasing levels while a significant decreasing level of high density lipoprotein in sera of patients group compared to control group was observed also the mean of CO and BMI were non significant increased in patients group compared to control group. In conclusion, present study indicates that IL-18 binding protein alpha is an independent risk factor for diabetes mellitus type 2 in Iraqi Arab females. This result supported by the absence of significant correlation among IL-18 binding protein alpha and all others classical risk factors. While the increased level of IL-18 binding protein alpha associated with duration of disease and increased age over than fifty years old for patients and healthy females .Further studies are needed to understanding the relationship among present different factors.*** .

Keywords--- IL-18Binding Protein alpha, Diabetes mellitus type2

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1. INTRODUCTION

Diabetes Mellitus Type 2 (DM T2) is one of the severest public health problems worldwide. It is a common metabolic disease with a rapidly increasing prevalence in both developed and developing countries [1] Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs [2] development of disease related to interaction between genes and lifestyle ,where numerous susceptibility genes combined with an unhealthy lifestyle gradually lead to the development of manifest disease [3]. Type 2 diabetes acute-phase increase concentration of cytokines such as IL-6 , IL-12 and IL-18, which was originally identified as an interferon-γ –inducing factor [4]. It is a member of the proinflammatory IL-1 family and can induce either T helper 1 or T helper 2 immune response depending on the immunologic context [5]. Il-18 activity is determined in part by the action of an intrinsic inhibitor, IL-18 binding protein (IL-18BP) [6]. IL-18-binding protein (IL-18BP) has been previously described as a secreted protein that binds and neutralizes IL-18 [6]. As such, it regulates IL-18-induced IFN-γ production and consequently influences the Th1 and inflammatory responses. with a single Ig domain, IL-18BP resembles the extracellular segment of cytokine receptors. However, IL-18BP is a novel protein distinct from the IL-1 and IL-18 receptor families. Located in chromosome 11q13 at the inverted position of the nuclear mitotic apparatus protein-1[6]. The human IL-18BP gene encodes at least four distinct isoforms IL-18BP (a, b,c,d), which are derived by alternative splicing. The isoforms differ primarily in their carboxyl termini and biological activity. IL-18BP isoforms a and c neutralize the biological activity of IL-18, whereas b and d do not [7]. IL-18BPa is constitutively expressed in human spleen and, to a lesser extent, in colon, small intestine, and prostate [6].

2. MATERIALS AND METHODS

Sixty patients of Iraqi Arab females with newly diagnosed DM T2 were examined by physicians in Endocrinology and diabetes center in Al-kindy Hospital. Patients with chronic inflammation were not involved in this study. This reports involved women who suffer from diabetic type II less than six years and age between twenty to the end of fifty nine years, no history of any metabolic disorder, no medical history of hypertension, no thyroid dysfunction, no regular alcohol or smoking and no pregnancy, with twenty eight healthy individuals matched in age, sex and ethnic group as control. Laboratory test were include: anthropometric measurements included: weight (kg), height (m), Central Obesity (cm) measured by tool and body mass index (BMI) was calculated by dividing the body weight in (Kg the square of the height in (m) according to the following equation:-

BMI= Weight (kg)/ Height (m^2).

Chemical parameters includes: Fasting blood glucose (FBG), Glycosylated haemoglubin (HbA1c) and Lipid profile (T-c, TG-c, LDL-c, VLDL-c and AI) have been evaluated among patients using enzymatic and colorimetric methods by biolyzer 300 Analytion. While LDL detected by using the formula:

LDL-cholesterol=Total cholesterol –triglycerides/5 –HDL-cholesterol,

S.VLDL-C =TG-C % 5

Atherogenic index (AI) =serum-total cholesterol /HDL-cholesterol.

IL-18BPa have been measured using enzymatic and colorimetric methods by Elisa kit from (Ray Biotech /USA). The Statistical Analysis System- SAS (2010) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

3. RESULTS

Characteristic of IL-18BPa (pg/ml) in patients and control show in table (1) . The mean of IL-18BPa was significantly higher in patients group (12.07 ± 1.26) compared to control group (7.71 ±0.84) .

Table -1.Statistical analysis of IL-18BPa (pg/ml) in the sera of patients and control.

Inflammatory marker	Patients group Mean ± SE	Control group Mean ± SE	t- test	P –value
IL-18 BPa (pg/ml)	12.07 ± 1.26	7.71 ± 0.84	3.867	0.027 *

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$

The statistical analysis of Anthropometric parameters in study groups is shown in table (2), the mean of both Central Obesity and BMI were non significantly higher in patients group (101.18 ± 1.67, 29.23 ± 0.57) compared to control group (95.75 ± 3.06, 27.75 ± 0.82) respectively.

Table-2. Statistical analysis of Anthropometric parameters in patients and control.

Characteristics	Patients group Mean ± SE	Control group Mean ± SE	t- test	P -value
CO (cm)	101.18 ± 1.67	95.75 ± 3.06	6.386	0.094 NS
BMI (Kg/ m^2)	29.23 ± 0.57	27.75 ± 0.82	1.996	0.143 NS

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$

As The results showed in table (3) the comparison between patients and control groups according to the chemical parameters. The mean of all chemical parameters includes(FBG, T-c, TG-c, LDL-c, VLDL-c,HbA1c and AI)were significantly higher in patient group (199.48 ± 9.04 , 195.05 ± 5.43, 159.07 ± 10.47, 118.63 ± 5.52 , 31.51 ± 2.09 , 9.30 ± 0.24 , 4.66 ± 0.22) compared to control group (86.46 ± 2.27 , 157.11± 6.48 , 100.35 ± 4.79, 86.42 ± 7.17 , 19.50 ±

0.85 ,5.21 ± 0.15 , 3.14 ± 0.12) respectively ,except the level of HDL-c showed significant decreased in patients group (44.10 ± 1.90) compared to control group (50.60 ± 1.36).

Table- 3: Statistical analysis of chemicals parameters in patients and control:

Chemical parameters	Patients group Mean ± SE	Control group Mean ± SE	t- test	P -value
FBG (mg/dl)	199.48 ± 9.04	86.46 ± 2.27	26.704	0.0001 **
HbAIC (%)	9.30 ± 0.24	5.21 ± 0.15	0.738	0.0001 **
T-c (mg/dl)	195.05 ± 5.43	157.11 ± 6.48	18.121	0.0001 **
TG- c (mg/dl)	159.07 ± 10.47	100.35 ± 4.79	31.239	0.0003 **
HDL-c (mg/dl)	44.10 ± 1.90	50.60 ± 1.36	5.858	0.029 *
LDL-c (mg/dl)	118.63 ± 5.52	86.42 ± 7.17	18.822	0.001 **
VLDL-c(mg/dl)	31.51 ± 2.09	19.50 ± 0.85	6.232	0.0002 **
AI (%)	4.66 ± 0.22	3.14 ± 0.12	0.673	0.0001 **

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$

The distribution of IL-18BPa according to age groups in study groups show in table (4). patient of age group more than 50 years showed the highest significant level of IL-18BPa (16.36 ± 2.87) compared to both age groups of less than 40 years and 40-50 years (10.26 ± 1.39 ,9.34 ± 1.16) while no significant differences between both others group. Control age group showed no significant increase with increase age. The comparison between patients and control for each age group showed significant increase level of IL-18BPa for both patient age groups of 40 -50 years and more than 50 years but the differences increase non significantly in patient age group less than 40 years.

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Table- 4.the distribution of IL-18BPa (pg/ml) in study groups according to age groups

Study group	Mean ± SE		
	less than 40 year(10)	40-50 year (28)	More than 50 year (22)
Patients	10.26 ± 1.39 a	9.34 ± 1.16 a	16.36 ± 2.87 b
Control	7.98 ± 1.66 a	5.66 ± 0.80 a	8.92 ± 1.43 a
P-value	0.462 NS	0.052 *	0.047 *

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$ The different letters at the same row mean significant differences.

The distributions of IL-18BPa in patients according to the duration of disease are shown in table (5). The patients were divided into three groups according to the duration of disease for groups less than 1 year, group with 1-3 years and 4-6 years duration .the level of IL-18BPa showed gradually non significant increased level in each patients group.

Table- 5. The distribution of IL-18BPa (pg/ml) in patients according to duration of disease.

parameter	Mean ± SE			LSD Value	P-value
	Less than 1 year	1-3 year	4 -6 year		
IL-18BPa (pg/ml)	10.31±2.62	11.35 ±0.98	13.76 ± 2.83	7.319	0.80 NS

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$.

As shown in table (6) mean IL-18BPa positively significant correlate with urea in patients (r= 0.547,p< 0.0001) while no significant correlation between means of IL-18BPa and urea in control. also there was negative correlation between the mean of IL-18BPa and HbA1c in patients .there was a positive significant correlation between means of IL-18BPa and central obesity (r=0.590, p< 0.001) and with BMI (r=0.513, p< 0.005) in control groups while there was no significant correlation between them in compared with patients group. also there was no significant correlation between means of IL-18BPa and all other parameters.

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Table -6.Correlation between IL-18BPa and study parameters:

Parameters	IL-18BPa (pg/ml)			
	Patients		Control	
	R	p-value	R	p-value
Age	0.198	0.128	0.286	0.139
C O (cm)	-0.096	0.464	0.590	0.001*
BMI (Kg/ m²)	0.033	0.798	0.513	0.005*
Fbg (mg/dl)	-0.173	0.186	0.246	0.206
HbA1c (%)	-0.259	0.04*	0.303	0.116
Urea	0.547	< 0.0001*	-0.265	0.189
Uric acid	0.207	0.165	0.138	0.498
T-c (mg/dl)	0.081	0.533	-0.215	0.270
TG- c (mg/dl)	-0.056	0.666	-0.273	0.158
HDL(mg/dl)	-0.015	0.905	0.068	0.729
LDL(mg/dl)	0.106	0.417	-0.176	0.369
VLDL(mg/dl)	-0.042	0.749	-0.256	0.187
AI (%)	-0.006	0.962	-0.245	0.208

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$

Distribution of IL-18BPa in study groups according to the percentage level of HbA1c shown in table (7), the patients with less than 7.3 level of HbA1c showed the highest significant mean of IL-18BPa (21.25 ±9.04) compare to other groups 7.9-9.3 and less than 9.3 (11.66 ±1.45 , 10.51 ± 1.30) respectively, but there were no significant differences between other patients groups ,while control group showed IL-18BPa level just at the first level of HbA1c (7.71 ± 0.84) with significant differences between patients and control at the same level.

Table -7.Distribution of IL-18BPa in study groups according to the percentage level of HbA1c .

IL-18BPa (pg/ml)	Mean ± SE		
	Less than 7.3	7.3-9.3	More than 9.3
Patients	21.25 ±9.04 b	11.66 ±1.45 a b	10.51 ±1.30 a
Control	7.71 ± 0.84	--	--
P-value	0.028 *	--	--

Significant differences $p \leq 0.05$* , $p \leq 0.01$** , non significant $p > 0.05$
The different letters at the same row mean significant differences.

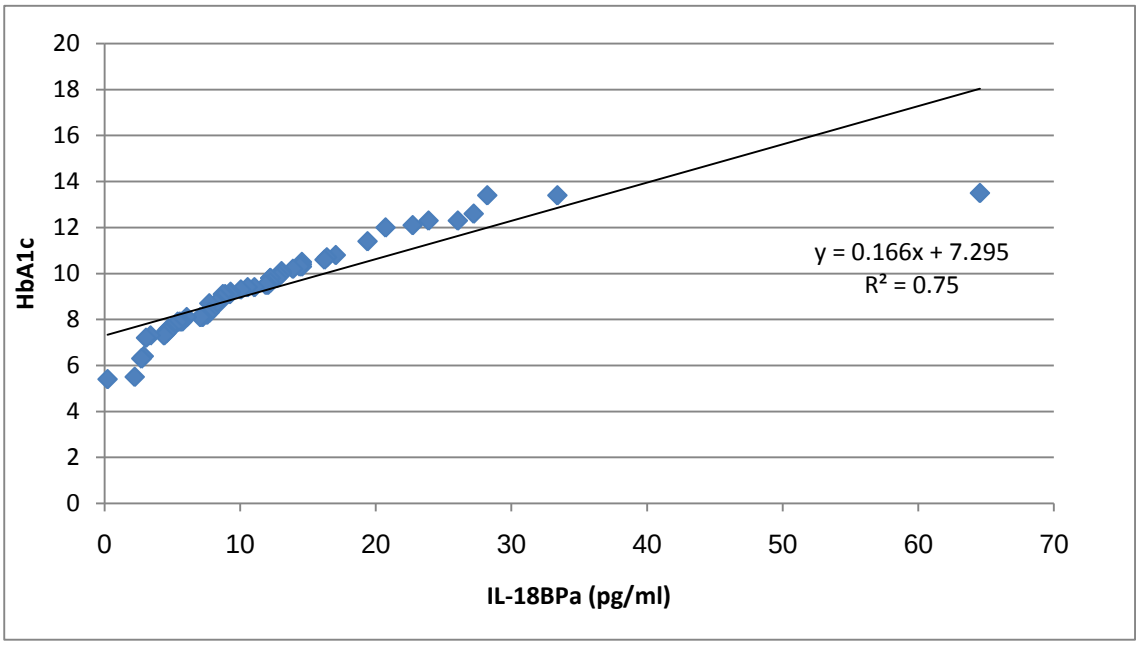

Figure 1: Correlation between IL-18BPa levels and HbA1c in sera of patients with Diabetes mellitus type2.

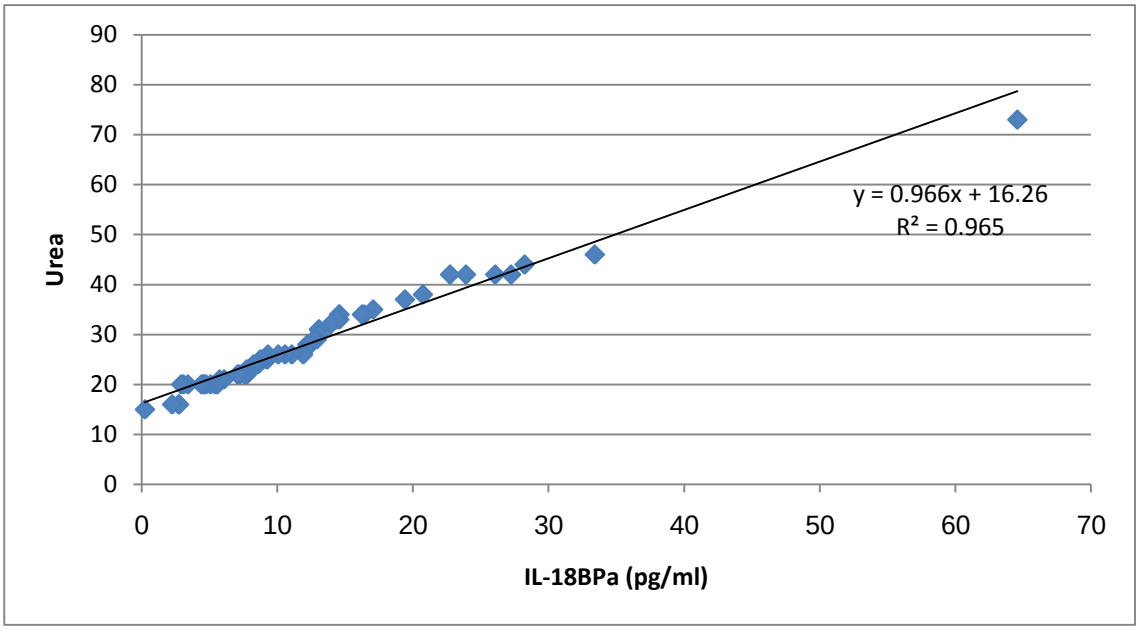

Figure3: Correlation between IL-18BPa levels and HbA1c in sera of patients with Diabetes mellitus type2.

4. DISCUSSION

The main finding in the present study were the significant increase level of IL-18BPa in Iraqi Arab females with DM T2 (table 1), it act as a risk factor. This result supported by the fact that several proinflammatory cytokines are found to be elevated in diabetes and its complications [8] such as IL-6 and IL-18 which is a proinflammatory cytokine known to cause tissue injury by inducing inflammation and cell death [9]. a local study demonstrated elevated level of IL-18 in Iraqi Arab population with type 2 diabetic patients [10], also other studies have shown that elevated levels of IL-18 are associated with higher risk of diabetes [11,12]. Therefore the highly level of IL-18BPa provided protection against inflammatory stimuli in the body [13]. The comparison among study groups according to anthropometric parameters showed non significant increased C.O and BMI in patients compared to control. While the same parameters showed increased level in Iraqi Arab female patient in previous report [14],Other studies [15,16] whish associated with increased diabetic complication as BMI increases above about 25 kg/m² [17, 18]. The present data indicated that the mean of FBG and HbA1c were significantly higher in diabetic patient compared to control, these result agree with previous Iraqi study [14]. While study find an elevated FBG level (≥126 mg/dl,ie, 7.0 moll/L) and HbA1c levels above 7% (HbA1c ≥ 0.07) has been suggested as evidence of disease [19]. The mean of lipid profile and Atherogenic index in present data was significantly higher in diabetic group compared to healthy control, these results agree with previous Iraqi studies showed the same result for lipid profile in Iraqi female diabetic patients [10,14] Further more, It agree with other study demonstrated that Type II diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including increased serum TG, VLDL, LDL and reduced HDL cholesterol, [20] and the precise cause of the low HDL–C in type 2 diabetes may be the consequence of insulin resistance, increased very low density lipoprotein production and increased activities of cholesterol ester transfer protein (CETP) and endothelial lipase [21]. The present data showed an increased level of IL-18BPa with increased age even in healthy individuals especially more than fifty age, this result agrees with previous Iraqi report which demonstrated that IL-18 increase in healthy individuals with increase age, versely with patients [10]. Other studies show that DM T2 is a chronic inflammatory disease in which increased levels of cytokines are produced under various stimuli such as over nutrition, increasing age, genetic or fetal metabolic preprogramming [22,23]. Present study illustrated that the level of IL-18BPa increased gradually with increased duration of disease. This finding supported by a previous study demonstrated that multiple metabolic complications occur in diabetic patient with long duration of disease [24]. A multivariate statistical analysis revealed that the level of IL-18BPa was non significant positively associated with some parameters and non significant negatively with others in both control and patients group. The absence of correlation between the level of IL-18BPa and level of FBG, HbA1c observed in present study was an expected, increasing level of HbA1c was associated with decreasing level of IL-18BPa that may occur because high level of HbA1c consume significant quantities of IL-18BPa which seems to be the only constitutively secreted protein that impacts upon the Th1 response after infection or immune stimulation. Because IL18BP is a natural product, it is an attractive treatment for treating diseases that mediated, in part, by IL12, IFNγ or IL18 itself [25]. As a result IL-18BPa act as independent risk factor in Iraqi Arab female with DM T 2 ,this result supported by the absence of significant correlation among IL-18BPa and all others classical risk factors. While the increased level of IL-18BPa associated with duration of disease and increased age over than fifty years old for patient and healthy females. Further studies are needed to understanding the relationship among different factors.

5. REFERENCES

1-Wild S.Roglic G,Green A,et al. ?(2004) Global prevalence of diabetes:estimates for the year 2000 and projections for 2030.diabetes care 27:1047-1053.
2- American Diabetes Association; (2008); Diagnosis and classification of Diabetes mellitus; *Diabetes Care*; 31 (suppl.1): S55- 60.
3- Romaol Roth J (2008) Genetic and environmental interactions in obesity and type 2 diabetes.J Am Diet Assoc 108:S24-28.

4- Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K: Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378:88 –91, 1995.
5- Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* 12:53–72, 2001.

6- Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity*. 1999;10:127–136
7- Kim, S. H., M. Eisenstein, L. Reznikov, G. Fantuzzi, D. Novick, M. Rubinstein, and C. A. Dinarello. 2000. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc. Natl. Acad. Sci. USA 97:1190.*
8- Kado S, Nagase T, Nagata N. Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. Acta Diabetol 1999;36:67–72

9-Wang, M.; Tan ,J.; Wang.Y.; Meldrum, K. K.; Denarello, C.A.; Meldrum,D. R.;(2009) IL-18 binding protein-expressing mesenchymal stem cells improve myocardial protection after ischemia or infarction. Indiana University School of Medicine, Indianapolis, IN, 46202;
10- Agha, Z .K.A. (2013). A Thesis "Immunological and Genetical study for Iraqi patients with Type 2 Diabetes Mellitus". College of Science for women . Baghdad U
11- Hivert MF, Sun Q, Shrader P, Mantzoros CS, Meigs JB, Hu FB. Circulating IL-18
and the risk of type 2 diabetes in women. Diabetologia 2009;52:2101–2108
12- Thorand B, Kolb H, Baumert J, et al. Elevated levels of interleukin-18 predict the
development of type 2 diabetes: results fromtheMONICA/KORA Augsburg Study,
1984-2002. Diabetes 2005;54:2932–2938
13- Fantuzzi G, et al. (2003) Generation and characterization of mice transgenic for human IL-18-binding protein isoform a. *J Leukoc Biol* 74:889–896.
14- Ali ,R .M.K.(2013) .A thesis "Study the association between ABO/Rh Blood groups and Endothelial inflammatory parameters in Iraqi Arab females with Diabetes Mellitus Type 2" .College of Science for women .Baghdad U.
15- Kumar, S., Mukherjee, S., Mukhopadhyay, P. 2008. Prevalence of diabetes and impaired fasting glucose in a selected population with special reference to influence of family history and anthropometric measurements – The Kolkata policeman study. *JAPI*, **56:** 841-844.
16- Daousi, C., Casson, I.F., Gill, G.V. 2006. Prevalence of obesity in type 2 diabetes patients in secondary care: association with cardiovascular risk factors. *Postgrad. Med. J*. **82:** 280-284.

17- Chan JM, Stampfer MJ, Ribb EB *et al*. Obesity, fat distribution and weight gain as risk factors for clinical diabetes in man. *Diabetes Care* 1994;**17**:961-9.
18- Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 1995;**122**:481-6.
19- Stumvoll M, Goldstein B, van Haeften T. Type 2 diabetes: principles of
pathogenesis and therapy. *Lancet*. 2005;365:1333–1346
20- American Diabetes Association: Management of dyslipidemia in adults with diabetes (Position Statement). *Diabetes Care* 26 (Suppl. 1):S83–S86, 2003a
21- Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, Thomas WG, Mukhamedova N, de Courten B, Forbes JM, Yap FY, Kaye DM, van Hall G, Febbraio MA, Kemp BE, Sviridov D, Steinberg GR, Kingwell BA. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009;119:2103-11.
22- Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004; 25: 4-7.
23- Lumeng C, Maillard I, Saltiel A. T-ing up inflammation in fat. Nat Med 2009; 15: 846-7.
24-Shih,L.Y.; Ho,T.K.; Taso,H.C.; Chang,H.Y.; Shian,Y.M.; Huang,N.C.;Yang,C.S.; (2013) Role of Cyotkines in Metabolism and Type 2 Diabetes Mellitus. International Journal of Biomedical Laboratory Science (IJBLS) 2013 Vo1. 2, No. 1:1-6
25- Dinarello,A.C.; Targeting interleukin 18 with interleukin 18 binding protein. *Ann Rheum Dis* 2000; **59** (suppl I): i17–i20) .