The Egyptian Journal of Hospital Medicine (October 2013) Vol. 53, Page 874– 882

The Relation of Retinol-Binding Protein 4 (RBP4) With Insulin Resistance in Hemodialysis Patients

Eman I. Sarhan[1], Mohammed R. Halawa[2], Ahmed A. Abdelnaby[1], Hyam M. Aref[1], Yasser M. El Shahawy[1], Nahla M. Teama[3].

Ain Shams University,faculty of medicine,Internal Medicine&Nephrology[1], Internal Medicine&Endocrinology[2], Internal Medicine[3].

Abstract

Background: Insulin resistance (IR) is one of the important causes of abnormal glucose homeostasis in CKD even in the absence of overt diabetes mellitus. Retinol-binding protein 4 (RBP4) is a lipocalin superfamily molecule that is synthesized primarily by hepatocytes and adipocytes. CKD may contribute to the increased survival and retention of RBP4 in the circulation. RBP has recently been proposed as a novel adipokine involved in the metabolism of glucose.

Objective:

To evaluate Retinol Binding Protein 4 in stage 5 Chronic kidney disease patients on regular hemodialysis and its contribution to insulin resistance in those patients.

Patients &Methods:

The patients divided into 2 groups:

Group I: Patients group consist of 50 ESRD patients on regular hemodialysis; they were 20 females (40.0%) and 30 males (60.0%) with age ranged from 19 years to 52 years and with mean±SD (38.08±7.1 years).

Group II: Control group consists of 50 normal subjects; 26 females (52%) and 24 males (48%) with age ranged from 21 years to 51 years and with mean±SD (35.38±8.77 years).

Results: comparison between group I and group II show that there was highly significant increase in RBP4 (p 0.000) while no statistically significant difference found between the two studied groups regarding HOMA (p 0.260).

Conclusion:no correlation betweenRBP4 and HOMA in hemodialysis patients.

Keywords: Insulin resistance, Retinol binding protein.

Introduction:

Insulin resistance is defined as reduced sensitivity of target organs to the biologic effects of insulin. Insulin resistance is often distinguished as either hepatic insulin resistance or peripheral insulin resistance. Hepatic insulin resistance refers to impaired suppression of hepatic glucose production, whereas peripheral insulin resistance refers to impaired response to insulin in skeletal muscle and adipose tissue. [(1)]The site of insulin resistance in CKD is localized to skeletal muscles. [(2)] Insulin resistance is increasingly recognized as a 'nontraditional' risk factor contributing to cardiovascular disease through endothelial dysfunction, oxidative stress, dyslipidemia, systemic inflammation, and activation of the renin–angiotensin–aldosterone system. [(3)]

Insulin resistance is common in end-stage renal disease (ESRD), and possibly also in moderate-to severe stages of CKD. Therefore, insulin resistance may be an important therapeutic target for reduction of cardiovascular mortality in patients with CKD. Insulin resistance in dialysis patients has been linked to accelerated protein catabolism leading to protein energy wasting and malnutrition. [(4)]Numerous factors related to CKD have been implicated in the etiology of insulin resistance. [(5)] These include uremic toxins (enhanced protein carbamylation), chronic metabolic acidosis, intracellular ion homeostasis disequilibrium, as well as qualitative and quantitative disturbances of insulin receptors on adipocytes, skeletal muscle cells and hepatocytes, cytokines produced by adipocytes (adipocytokines), chronic inflammation as well as low physical activity. [(5)]

Initiation of renal replacement therapy by dialysis in patients with irreversible kidney failure has long been suggested to have a positive effect on the reduction of insulin resistance in this group of patients. [(6)]Adipose tissue-derived circulating hormones, or adipokines, may offer new opportunity to assess insulin resistance in CKD. [(7)] Retinol-binding protein 4 (RBP4) is a 21 kDa non-glycosylated, non-phosphorylated, and non-sulfated molecule. [(8)]It is a lipocalin superfamily molecule that is synthesized primarily by

DOI: 10.12816/0001650

hepatocytes and adipocytes. [9] This liver-derived transport protein retinol-binding protein (RBP) has recently been proposed as a novel adipokine involved in the metabolism of glucose. [10]

Elevated plasma levels of the insulin resistance–associated adipokines RBP4 and resistin was reported in CKD patients[11]. RBP4 promotes hyperglycemia through downregulation of the glucose transporter GLUT4 in adipocytes, upregulation of the hepatic gluconeogenic enzyme Phosphoenolpyrovate carboxy kinase, and attenuation of insulin receptor signaling in skeletal muscle. [12]

Aim of the Study:

This preliminary study aiming to valuate Retinol Binding Protein 4 in stage 5 Chronic kidney disease patients on regular hemodialysis and its contribution to insulin resistance in those patients.

Patients and Methods:

This case control study was conducted on 100 individuals divided into two groups of comparable age and sex above 18 years old to study the relation between retinol binding protein 4 and insulin resistance in hemodialysis patients in Ain Shams University hospitals. These subjects were divided into two groups:

Group I: includes:

Fifty non diabetic patients CKD stage 5. All patients were receiving thrice-weekly HD with volumetric dialysis machines (Gambro AK95), bicarbonate-based dialysate and polysulfone dialyzers. The etiology of renal failure in this group was hypertention, Lupus nephritis, Chronic glomerulonephritis and Obstructive uropathy.

Group II: includes:

Fifty healthy control subjects.

Each group will be subdivided into two subgroups according to insulin resistance (HOMA level).

- Subgroup (A) when HOMA<2.
- Subgroup (B) when HOMA >2.

HOMA index = (plasma glucose (mg/dl) x plasma insulin (mU/L/405) ***(Wallace and Mathews, 2002).***

Exclusion:

We exclude diabetic patients, Obese individuals with BMI > 30,patients with chronic liver disease (HCV +ve, HBV +ve and liver cirrhosis by U/S), in efficient dialysis detected by urea reduction ratio < 60 and children less than 18 years old.

All individuals were subjected to the following after taking an informed consent.

1. Full history specially including hemodialysis data.
2. Clinical examination with special emphasis on (BMI).

Laboratory investigations :

1. Fasting plasma insulin.
2. Oral glucose tolerance test: 2 samples were taken from the subject, fasting and 2 hours post challenge with 75 gm glucose drink.
3. Homeostasis model assessment (HOMA index) as a measure of insulin sensitivity (plasma glucose (mg/dl) x plasma insulin (mU/L/405) ***(Wallace and Mathews, 2002).***
4. Biochemical analysis (S. creatinine, urea, Na, K, Ca, PTH, serum albumin, phosphorus and Hb level).
5. Serum retinol binding protein 4 using the Quantikine kit standards.

Radiological investigations :

Abdominal ultrasound.

- Method of assessment of RBP4level (reference range: 12.700 – 48.600 ng/mL).

Enzyme immunoassay of RBP4 Store at +2-8°C:

1. PRINCIPLE:

This assay employees the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for RBP4 has been pre coated onto a microplate. Standards and samples are pipetted into the wells and any RBP4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme linked monoclonal antibody specific for RBP4 is added to the wells. Following a wash to remove any unbound antibody enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of RBP4 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Method of assessment of insulin level (reference range: 2 µIU/mL to 25 µIU/mL)

Enzyme immune assay for insulin store at 2-8°C:

The insulin ELISA is an enzyme immunoassay for the quantitative in vitro

diagnostic measurement of insulin in serum and plasma.

Principle of the test:

The insulin ELISA kit is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtitre wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule.

An aliquot of patient sample containing endogenous insulin is incubated in the coated well with enzyme conjugate, which is an anti-insulin antibody conjugated with biotin. After incubation the unbound conjugate is washed off.

During the second incubation step streptavidin peroxidase enzyme complex binds to the biotin anti insulin antibody. The amount of bound HRP complex is proportional to the concentration of insulin in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of insulin in the patient sample.

Results:

This study was conducted on 100 individuals divided into two groups of comparable age and sex as follows:

Group I: Patients group consist of 50 end stage renal disease ESRD patients on regular hemodialysis; they were 20 females (40.0%) and 30 males (60.0%) with age ranged from 19 years to 52 years and with mean±SD (38.08±7.1 years).

Group II: 50 normal subjects as a control group; 26 females (52%) and 24 males (48%) with age ranged from 21 years to 51 years and with mean±SD (35.38±8.77 years).

Each group will be subdivided into two subgroups according to insulin resistance (HOMA level).

- Subgroup (A) when HOMA<2.
- Subgroup (B) when HOMA >2.

As regard glucoparameters and RBP4 there was highly statistically significant increase in RBP4 with best cut off point > 26000 ng/ml with sensitivity 93.18%, specificity 100.00% (p 0.000) and FBG (0.005) in hemodialysis patients compared control group and there was statistically significant increase in fasting blood insulin FBI in hemodialysis group compared to control group (p 0.043), while there was no statistically significant difference found between the two studied groups regarding HOMA and 2h post challenge blood glucose (p 0.260)

Discussion:

Insulin resistance is defined as reduced sensitivity of target organs to the biologic effects of insulin. [1] ***In 2002 Shinohara et al.,*** demonstrated for the first time that insulin resistance is an independent predictor of mortality from cardiovascular causes in patients with end‑stage renal disease without coexisting diabetes.

Numerous factors related to CKD have been implicated in the etiology of insulin resistance. [5]These include uremic toxins (enhanced protein carbamylation), chronic metabolic acidosis, intracellular ion homeostasis disequilibrium, as well as qualitative and quantitative disturbances of insulin receptors on adipocytes, skeletal muscle cells and hepatocytes, cytokines produced by adipocytes (adipocytokines), chronic inflammation as well as low physical activity. [5]

The relative amounts of apo-RBP4 are increased during acute renal failure and RBP4-L and RBP4-LL have been shown to be increased in hemodialysis patients. [13]

RBP4 was shown to induce expression of the gluconeogenic enzyme phosphoenol pyruvate carboxy kinase and decrease insulin signaling in muscle. [14] There are multiple mechanisms described by which RBP4 can affect changes in glucose metabolism. Retinol-dependent mechanisms may use different retinol isomers to affect insulin sensitivity and insensitivity, [15] whereas retinol-independent mechanisms may affect metabolism through cell surface receptors. [14] The cell surface protein megalin (gp320) is the only RBP4 receptor known so far, but the reported binding affinity is low. However, RBP4 binds to a wide range of retinoids and is likely to transport other lipophilic molecules that may mediate its effect on glucose. [16]

This case control study included one hundred subjects above 18 years old to study the relation of retinol binding protein 4 and insulin resistance in

hemodialysis patients in Ain Shams University hospitals. These subjects were divided into 2 groups:**Group I:**50 ESRD patients on regular hemodialysis 20 females (40.0%) and 30 males (60.0%) with mean age ±SD (38.08±7.1 years). **Group II:** 50 healthy individuals as **a** Control group 26 females (52%) and 24 males (48%) with mean age ±SD (35.38±8.77 years). According to insulin resistance (HOMA level) each group will be subdivided into two subgroups:

- Subgroup (A) when HOMA<2.
- Subgroup (B) when HOMA >2.

In this study there was highly significant increase in RBP4 in hemodialysis patients compared with the control group which agreed with **Ziegelmeier *et al.*** [17] who studied RBP4 in diabetic and non diabetic ESRD on regular hemodialysis and compared its level in normal controls and found significant increase in RBP4 in HD patients than in healthy controls **and Frey *et al.*** [18] who studied serum levels of RBP4, apo-RBP4, holo- RBP4, RBP4-L, RBP4-LL, retinol and transthyretin in hemodialysis patients and in healthy controls and found that HD patients had elevated levels of RBP4 than in normal controls.

In this study we found highly statistically significant increase in FBG in hemodialysis patients compared with the control group which agreed with **Becker *et al.*** [19] who studied insulin resistance in chronic kidney disease patients not on hemodialysis and compared it with normal population and found significant increase in fasting blood glucose in CKD patients than in normal.

Also we found significant increase in fasting blood insulin in hemodialysis patients in comparison to controls which agreed with **Becker *et al.*** [19] who studied insulin resistance in chronic kidney disease patients not starting hemodialysis and compared it with normal population and found significant increase in fasting blood insulin in comparison to control group but our results were not compatible with **Ziegelmeier *et al.*** [17] who studied insulin resistance in HD patients and compared it with normal healthy controls and found that fasting blood insulin was not increased in HD patients than in controls**.**

There was no statistically significant difference between the two studied groups regarding 2h post challenge blood glucose which is in contrast with **Fliser *et al.*** [20] who examined patients with IgA glomerulonephritis (IgAGN) and patients with adult polycystic kidney disease (ADPKD) in different stages of renal failure, and in addition, healthy age-matched subjects. Insulin sensitivity and other variables of glucose metabolism were assessed using a frequent sampling intravenous glucose tolerance test and found statistically significant difference between the studied groups regarding 2h post challenge blood glucose and document that insulin resistance and concomitant hyperinsulinemia present early in the course of renal disease, that is, even in patients with GFR within the normal range, irrespective of the type of renal disease.

Despite significant increase in FBI and FBG in hemodialysis patients than in controls there was no statistically significant difference in insulin resistance (HOMA level) between the two studied groups which agrees with **Kobayashi *et al*** [6] who studied non diabetic ESRD as regard insulin resistance before and after start of hemodialysis and compared the results with normal population and found that mean duration of hemodialysis therapy of 4-9 weeks completely normalize insulin sensitivity also our results were compatible with **Defronzo *et al.*** [21] who studied also non diabetic ESRD as regard insulin resistance before and after start of hemodialysis and compared the results with normal population and demonstrated that 10 weeks of hemodialysis therapy resulted in a marked improvement in glucose metabolism but not completely normalize glucose utilization.

And this may explain the absence of difference in HOMA level between HD patients and controls as mean duration of hemodialysis therapy in our patients were 8 years also this may be explained by HOMA primarily reflects hepatic IR rather than peripheral IR [22] and estimates of IR based on fasting insulin concentration may

not be adequate in patients with CKD as it largely reflects hepatic defects, whereas CKD impairs insulin catabolism .[23].

Mak *et al.*[24]. also studied insulin resistance in hemodialysis patients and compared it to normal population and demonstrated that insulin resistance improved markedly after treatment with 1,25 dihydroxycholecalciferol and this was compatible with our results as all our patients were on 1,25 dihydroxycholecalciferol with acceptable Ca and P.

Comparison between subgroups Ia&Ib in our results as regard FBI show highly significant increase when HOMA >2 and as regard 2hPP showed significant increase when HOMA >2.

Comparison between subgroups IIa&IIb as regard FBI&2hPP show highly significant increase when HOMA >2 and significant increase in serum albumin when HOMA> 2.

In this study we found no correlation between RBP4 and HOMA, BMI, FBI and FBG in hemodialysis patients which is agreed with **Ziegelmeier *et al.* [17],** who studied RBP4 in HD patients and compared it in normal healthy controls and its relation to insulin resistance in both groups and found that no correlation between RBP4 and HOMA, BMI, FBI and FBG.

We found highly significant positive correlation between HOMA, FBI and glucose tolerance (2 hs post challenge blood glucose) and significant positive correlation between HOMA and FBG in hemodialysis patients and in controls which agrees with **Qu H-Q *et al.*[25]** who studied insulin resistance in normal healthy individuals and found positive correlation between HOMA, FBI and glucose tolerance (2 hs post challenge blood glucose) and significant positive correlation between HOMA and FBG**.**

As regard the Comparison of laboratory results between hemodialysis patients and control group there was statistically significant increase in Na in control group (P 0.031) and also a highly significant difference was found regarding Hb, Ca and Alb which showed a decrease in group I (P 0.000), while P, PTH and K were increased in patient group (P 0.000).

The comparison between HOMA levels in the subgroups Ia & Ib showed a highly statistically significant increase in FBI (P 0.000) and 2h post challenge blood glucose (P 0.012) in subgroup Ib (HOMA level $\geq$ 2),but there was no statistically significant difference regarding RBP4.

Comparison between subgroup IIA and subgroup II B regarding glucoparameters and RBP4 showed a highly significant increase in FBI and 2h post challenge blood glucose (P 0.000) when HOMA level $\geq$ 2, but there was no statistically significant difference regarding RBP4 according to HOMA level.

The correlation between HOMA and RBP4 and the studied parameters in the hemodialysis patients showed statistically significant positive correlation between HOMA and BMI (P 0.31) and FBG (P 0.028), and also showed a highly significant positive correlation with FBI (P 0.000) and 2 hours post challenge blood glucose (P 0.000).

While the RBP4 showed no significant relation with any of the studied parameters.

Conclusion:

Elevated levels of RBP4 in hemodialysis patients than in controls with cut off point > 26000 ng/ml with sensitivity 93.18%, specificity 100.00%.than in controls also we found significant increase in FBG and FBI in HD patients than in controls but no difference between the two studied groups as regard insulin resistance measured by HOMA. HOMA was positively correlated with FBI, 2hPP and BMI in both HD patients and in controls. No correlation between RBP4 and HOMA, BMI, FBI and FBG in hemodialysis patients.

References:

(1) Stumvoll M, Goldstein BJ and van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. Lancet, 365:1333–1346.

(2)**DeFronzo RA, Alvestrand A, Smith D et al. (1981).** Insulin resistance in uremia. J Clin Invest., 67: 563-568.

(3) Siew ED and Ikizler TA (2008). Determinants of insulin resistance and its effects on protein metabolism in patients with advanced chronic kidney disease. Contrib Nephrol., 161:138–144.

(4) Siew ED and Ikizler TA.(2010). Insulin resistance and protein energy metabolism in

patients with advanced chronic kidney disease. Semin Dial., 23:378–382.

(5) Kobayashi S, Maesato K, Moriya H, et al. (2005). Insulin resistance in patients with chronic kidney disease. Am J Kidney, 45: 275-280.

(6) Kobayashi S, Maejima S, Ikeda T, et al. (2000). Impact of dialysis therapy on insulin resistance in end-stage renal disease: comparison of haemodialysis and continuous ambulatory peritoneal dialysis. Nephrol Dial Transplant., 15: 65-70.

(7) Hung AM, Sundell MB, Egbert P, et al. (2011). A comparison of novel and commonlyused indices of insulin sensitivity in African American chronic hemodialysis patients. Clin J Am Soc Nephrol., 6:767–774.

(8) Jaconi S, Rose K and Hughes GJ (1995). Characterization of two post-translationally processed forms of human serum retinol-binding protein: altered ratios in chronic renal failure. J.Lipid Res., 36: 1247–1253.

(9) Blomboff R,Green M.H and Norum K.R. (1992). VitaminA: physiological and biochemical processing,Annu Rev.Nutr.,12:37-57.

(10) Axeisson J, O'Byrne SM, Blander WS, et al. (2009). Serum retinol-binding protein concentration and its association with components of the uremic metabolic syndrome in nondiabetic patients with chronic kidney disease stage 5 . Am J Nephrol.,29(5):447-53.

(11) Yaturu S, Reddy RD and Rains J et al. (2007). Plasma and urine levels of resistin and adiponectin in chrnic kidney disease. Cytokine,37(1):1-5.

(12) Klöting N, Graham TE and Berndt J et al (2007). Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell Metab., 6:79–87.

(13) Jaconi S, Saurat JH and Siegenthaler G. (1996). Analysis of normal and truncated holo- and apo-retinol-binding protein (RBP) in human serum: altered ratios in chronic renal failure. Eur J Endocrinol .,134:576-582.

(14) Yang Q, Graham TE, Mody N, et al. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature ,436:356–362.

(15) Chambon P. (1996). *A decade of molecular biology of retinoic acid receptors. FASEB J .,10:*940–*954.*

(16) Graham TE, Yang Q, Bluher M, et al. (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med., 354:2552–2563.

(17) Ziegelmeier M, Bachmann A, Seeger J et al. (2007) Serum level of adipokine reinol – binding protein 4 in relation to renal function Diabetes Care, 30 (10): 2588-2592.

(18) Frey SK, Henze A, Nagl B et al. (2008). Effect of renal replacement therapy on retinol-binding protein 4 isoforms.Clin Chim Acta.,401(1-2):46-50.

(19) Becker B, Kronenberg F, Kielstein J T et al. (2005). Renal Insulin Resistance Syndrome,Adiponectin and Cardiovascular Events in patients with Kidney Disease:The Mild and Moderate Kidney Disease Study. J Am Soc Nephrol.,16:1091-1098.

(20) Fliser D, Pacini G, Engelleister R et al. (1998). Insulin resistance and hyperinsulinaemia are present already in patients with incipient renal disease. Kidney int .,53:1243-1247.

(21) DeFronzo RA, Tobin JD, Rowe JW et al. (1978). Andres R. Glucose intolerance in uremia. Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. *J Clin Invest*., 63: 425–435.

(22) Shoji T, Emoto T and Nishizawa Y. (2001)."HOMA index to assess insulin resistance in renal failure patients"Nephron, 98 (3):348-349.

(23) Pham H, Utzscneider K M and De Boer H. (2011)." measurement of insulin resistance in chronic kidney" current opinion in Nephrology and hypertention,20 (6): 640-646.

(24) Mak RHK. (1995). Insulin resistance in uremia: effect of dialysis modality. *Pediatr Res* .,40: 304–308.

(25) Qu H-Q, Li Q, Rentfro AR, Fisher-Hoch SP et al. (2011). The Definition of Insulin Resistance Using HOMA-IR for Americans of Mexican Descent Using Machine Learning. PLoS ONE 6(6): e21041. doi:10.1371.

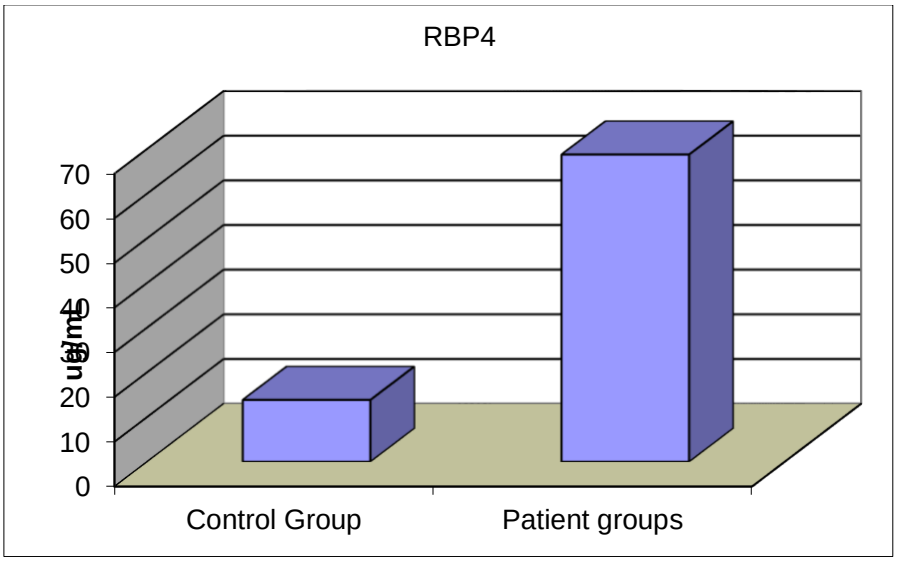

Fig. (1): Comparison between the two groups regarding to RBP4 (µg/ml).

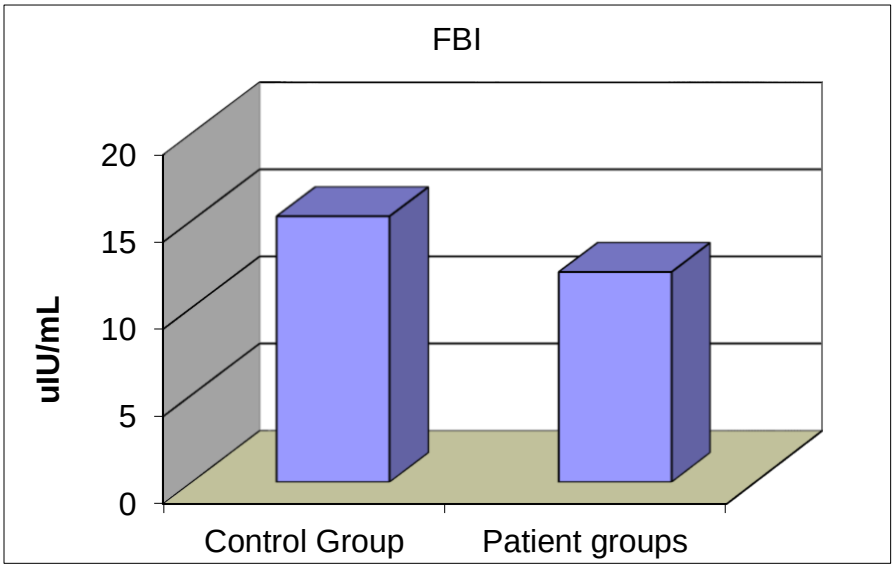

Fig. (2): Comparison between the two groups regarding to FBI (µIU/mL).

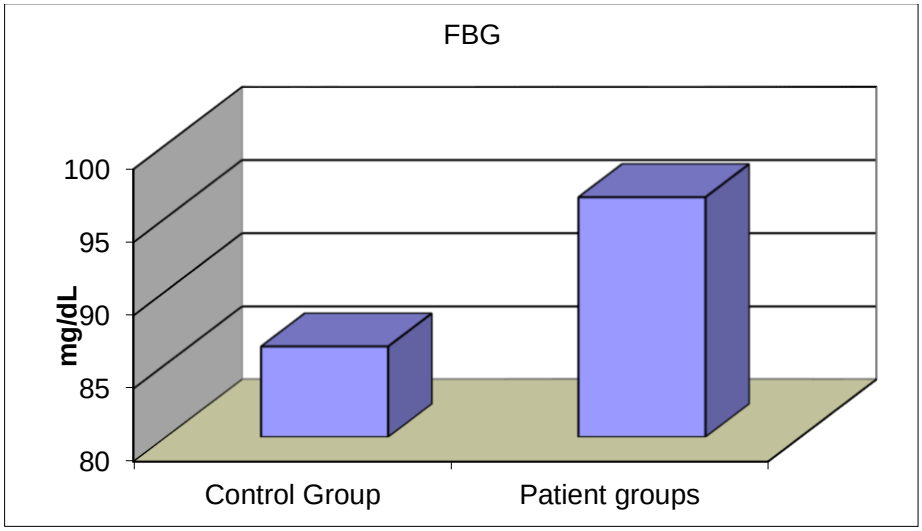

Fig.(3): comparison between the two groups regarding to FBG(mg/dl).

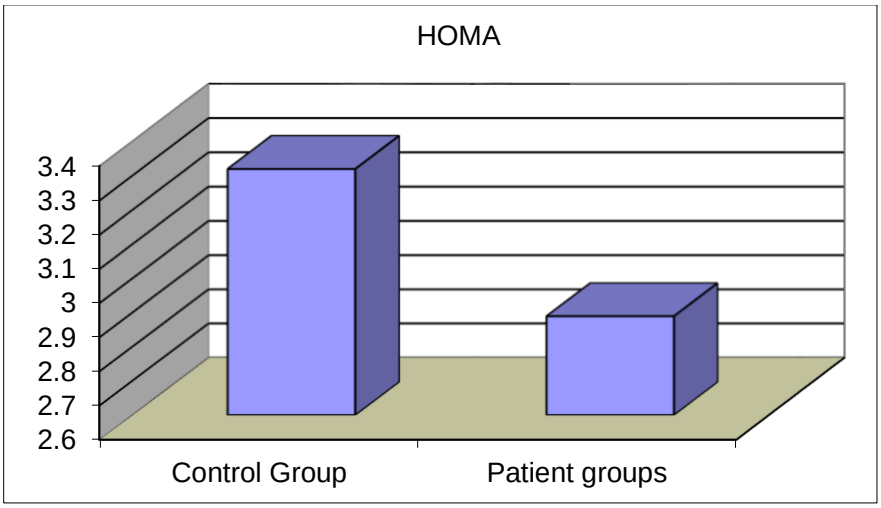

Fig. (4): Comparison between the two groups regarding to HOMA.

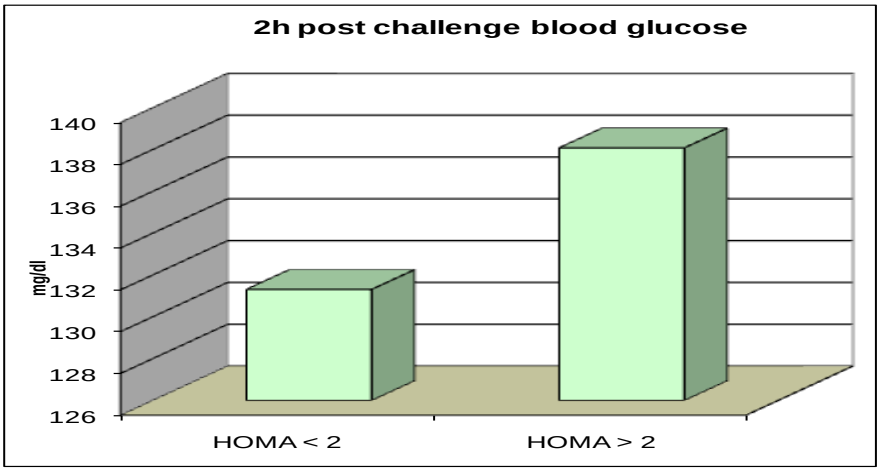

Fig. (5): Comparison between subgroup I A and subgroup I B regarding 2h post challenge blood glucose.

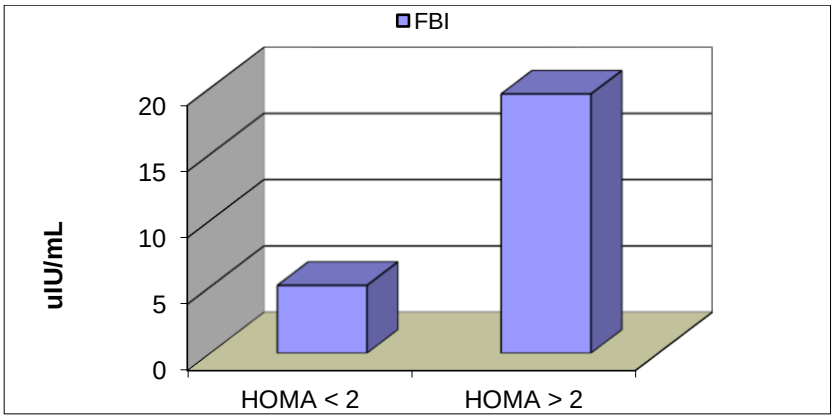

Fig. (6): Comparison between subgroup IA and subgroup IB regarding FBI.

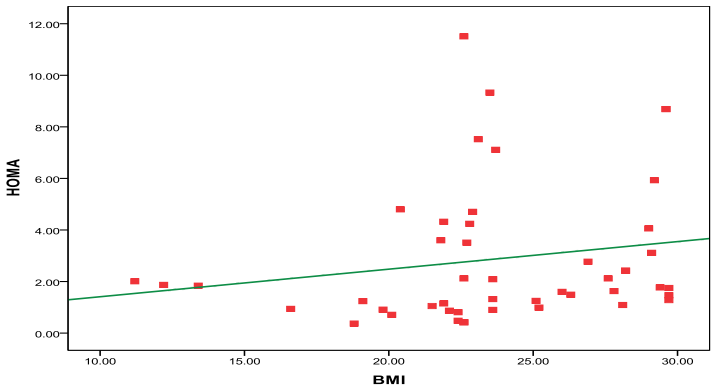

Fig. (7): Correlation between HOMA and BMI in hemodialysis patients.

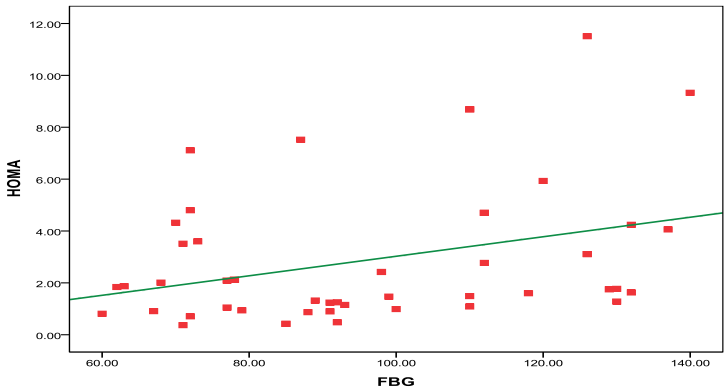

Fig. (8): Correlation between HOMA and FBG in hemodialysis patients.

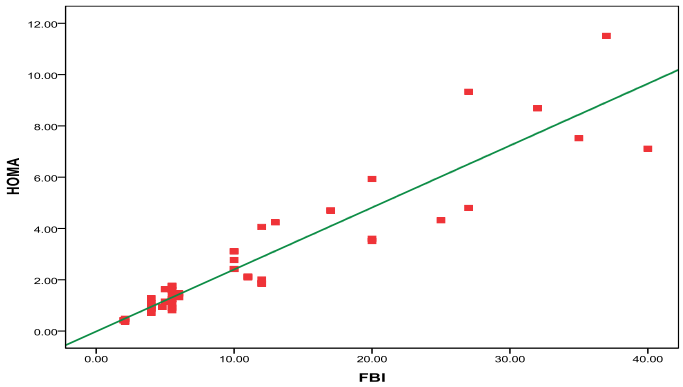

Fig. (9): Correlation between HOMA and FBI in hemodialysis patients.