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Anew Correlation Between C- Reactive Protein, Vit. C and glutathione In Diabetic patients

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Abstract

The study done to explain the role of hyperglycaemia in raising C-reactive protein level through studying the relationship between the concentration of C-reactive protein and antioxidants (reduced glutathione, reduced vitamin C).The study has been conducted on 87 patients with diabetes mellitus: 27 of them with type 1 DM (10 males and 17 females) and 60 patients with type 2 DM (27 males and 33 females). Thirty-nine healthy subjects are considered a Control Group. The results show that both types 1 and 2 diabetic patients have significant decrease in their antioxidants levels (reduced glutathione, reduced vitamin C) and significant increase in their glucose level in comparison with that of Control Group. Also, it has been revealed that C-reactive protein levels above the normal value constitute (13.79%) of the total number of diabetic patients, and they have been found to be either have diabetic complications, infection, or be newly diagnosed diabetes, and not all diabetic with hyperglycaemia have raised C-reactive protein level. It has been found also that raised C-reactive protein levels are higher in female sex than male.

الملخص :

تم في هذا البحث دراسة علاقة جديدة بين (البروتين -C-) وفيتامين(ج) والكلوتاثايون لدى مرضى السكري نوع I و II وقد دلت النتائج على أن مرضى النوعين ظهر لديهم تناقص معنوي في مستويات المضادات الاكسدة (الكلوتاثايون و فيتامين c) وزيادة في مستويات الاكسدة مقارنة بمجموعة السيطرة وبينت بان هنالك زيادة بحدود 13.79% بمستويات البروتين c في معظم المرضى ولكن الملاحظة المهمة هي أنه ليس جميع مرضى السكري يعانون من هذا الارتفاع اضافة انه الارتفاع يكون في الاناث أكثر من الذكور.

Introduction

Diabetes mellitus is the most common disorder associated with pancreatic islet dysfunction of the endocrine portion (Bullock, 1996). The pancreas consists of very different organs contained within one structure. The acinar portion of the pancreas has an exocrine function. The endocrine portion consists of islets of Langerhans (Murray *et al*, 1996). The hyperglycaemia of diabetes develops because of an absolute (type 1 diabetes) or a relative (type 2 diabetes) deficiency of insulin, resulting in decreased anabolic and increased catabolic effects. In both type 1 and type 2 diabetes, insulin's actions are also impaired by the insensitivity of target tissues. While this is a fundamental defect in type 2 diabetes, hyperglycaemia can also induce insulin resistance through glucose toxicity.

Decreased anabolism results in hyperglycaemia, which leads to glycosuria, osmotic diuresis and consequent salt and water depletion. Increased catabolism is represented by increased glycogenolysis, gluconeogenesis and lipolysis, which will result in hyperketonaemia and consequently acidosis occurs and the patient gets diabetic ketoacidosis (Haslett *et al*, 1999).

The counter - regulator hormones secretion will also increase, which includes glucagons. cortisol, growth hormone, and catecholamines. These hormones are catabolic and increase hepatic glucose production

initially by enhancing the breakdown of glycogen to glucose (glycogenolysis) and later by stimulating the synthesis of glucose (gluconeogenesis) (Burtis and Ashwood., 1996).

Qxidative Stress :-

Oxidative stress has been defined as an imbalance between pro-oxidants and antioxidants. Either an increase in the production of oxidants or a deficiency in the antioxidant defense system could disturb this balance, causing oxidative stress (Langseth, 1995). However, it is closely associated with aging and a number of diseases including cancer, cardiovascular disease, diabetes and diabetic complications (Atalay and Laaksonen, 2002). Oxidative stress may be localized, for instance in the joints in arthritis or in the vascular wall in atherosclerosis, or can be systemic e.g. in systemic lupus erythematosus (SLE), and possibly diabetes (Baynes and Dominiczak, 2005). Figure (1) shows oxidative stress which results from an imbalance between pro-oxidant and antioxidant forces (Baynes and Dominiczak, 2005).

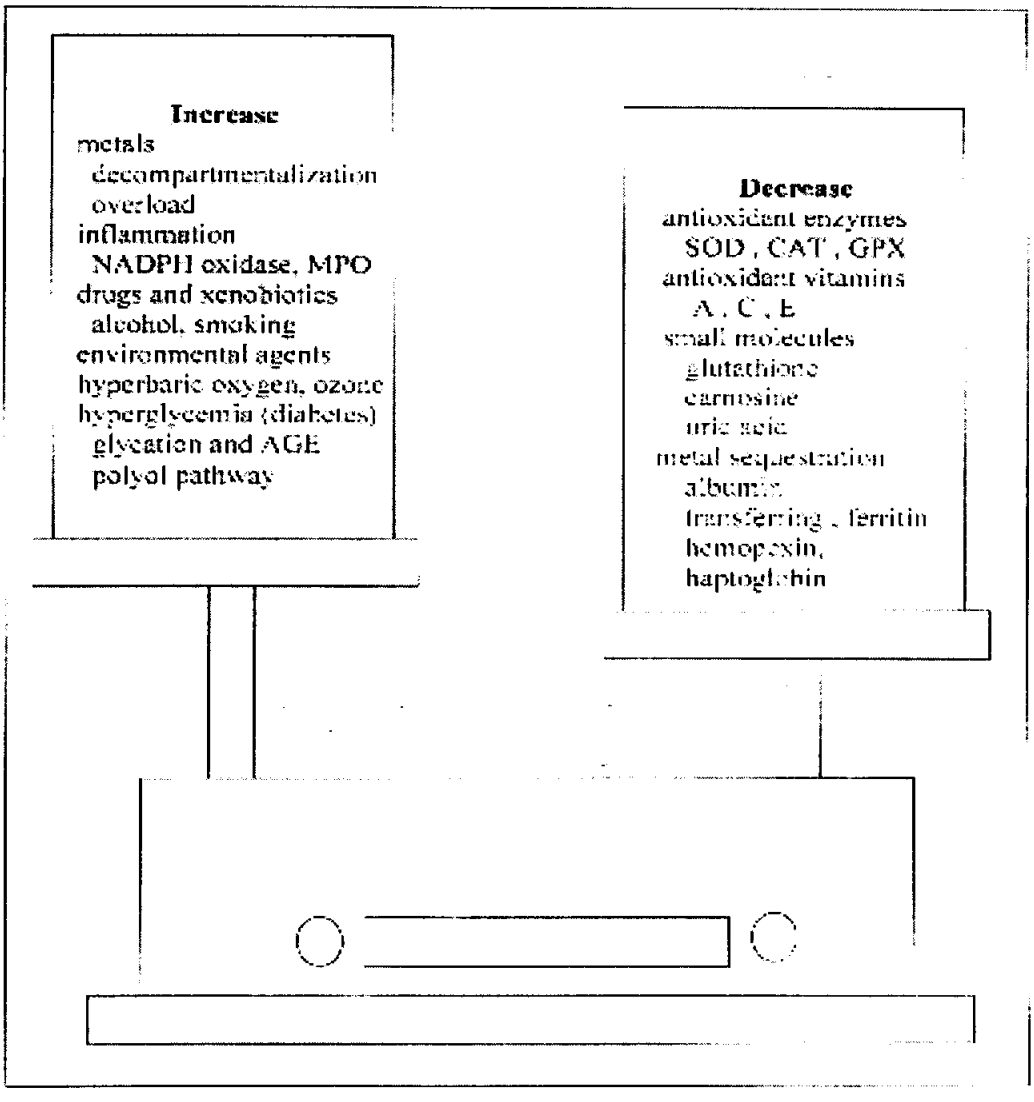


Figure (1) :Oxidative stress which results from an imbalance between pro-oxidant and antioxidant forces.

In general, an antioxidant has been defined as any substance that, when present in low concentrations compared with that of an oxidized substrate,can delay or inhibit the oxidation of that substrate (Al-Ameri. 2002). whereas Wahlqvist and Wattanapenpaiboon have defined antioxidants as substances that reduce oxidation and so counteract the reactive species (Wahlqvist and Wattanapenpaiboon, 1999). The human body has several mechanisms for defense against the radicals and other ROS. The various defenses are complementan' to one another because they act on different oxidants or in different cellular compartments.

Reduced Glutathione (GSH) in Diabetes Mellitus :-

Reduced glutathione (GSH) is a non - specific reduction agent and plays an important role in oxidation mechanisms. It participates in antioxidative defense system as a free radical inactivator (Atamer *et al.*, 1998). The depletion of GSH has been speculated to be an important contributing factor to some serious human diseases, such as chronic renal failure, diabetes, Parkinson's disease and cataract formation (Lomaestro and Malone, 1995). Tessier and associates have observed that oxidized glutathione (GSSG) increases in the serum of diabetic patients and GSH / GSSG ratio decreases (Tessier *et al.*, 1999). Al-Ameri has observed in his study that type 2 DM patients have lower antioxidants levels, GSH in between them , as compared with control subjects (Al-Ameri. 2002). Atamer and associates have found that erythrocyte GSH levels of type 2 diabetes are sisnificantly lower as compared with control subjects (Atamer *et al.*, 1998).

C-reactive protein in Diabetes Mellitus :-

Diabetes mellitus (DM) is a chronic disorder, and comprises a group of syndromes with abnormal carbohydrate metabolism, all characterized at some point by hyperglycaemia. Changes in serum protein levels in diabetes are related to inflammation, secondary complications, and to the metabolic effects of abnormal insulin and glucose level (Craig *et al.*, 2002; Pickup, 2004). CRP is moderately elevated in DM (Craig *et al.*, 2002). Its concentrations are lowest among those with newly or previously diagnosed diabetics (Ford, 1999).
Previous studies have suggested that CRP is involved in the development of type 1 DM Chase and associates have found that type 1 DM is an immuno inflammatory disorder and elevated CRP levels is associated in the development of type 1 DM (Chase *et al,* 2004). Amrani and associates have reported that cytokines including IL-1, IL-6, and TNF-Ct are present in the inflammatory infiltrate of pancreatic islets, and have a potential role in beta cell destruction particularly when present in combination (Amrani *et al.*, 2000).Schram and associates have found that inflammatory activity, measured by inflammatory markers (CRP, IL-6, and TNF-CX), is increased in type 1 diabetes and may predispose to vascular disease (Schram *et al.*, 2003). Many studies have approved that CRP also plays a role in the development of type 2 DM (Pickup, 2004; Thorand *et al,* 2003; Pradhan *et al,* 2001). Schulze and associates have found that high plasma levels of CRP are associated with an increased risk of incident cardiovascular events among men with type 2 diabetes (Schulze *et al,* 2004). Although the mechanism of the CRP elevation is unknown, they suggest that it might be related to the activation of macrophages, increased oxidative stress, or induction of cytokines (Chase *et al,* 2004).

Materials :-

Patients and Conditions of The Study :-

The period covering the practical side of the study was from the 27th of November 2004 to the 17th of May 2005. It was performed at the Laboratory of Biochemistry Department, College of Medicine, University of Babylon. The study was conducted on 87 patients from the diabetic clinic in Mirjan Teaching Hospital, as well as from Babylon Hospital of Pediatric and Maternity. 27 patients were with type 1 DM (10 males and 17 females) whose ages ranged from 10-74 years old, and 60 patients-were with type 2 DM (27 males and 33 females) whose ages ranged from 30-85 years old.The medical history of each patient was taken regarding age,

gender,
type of DM, duration of DM, type of treatment, family history of DM, history ofischaemic heart disease. history of any other illness, and smoking status.Measurements of their height and weight were done to calculate their body massindex (BMI) and blood samples were also drawn. All patients underwent full physical examinations, and investigations: fasting serum glucose (FSG), randomserum glucose (RSG), complete blood film (CBF). general urine examination(GUE), blood urea, serum creatinine, and electrocardiograph (ECG). Otherinvestigations were done according to the condition for which the diabetic patient was admitted to hospital.

Healthy Subjects ;-

Thirty-nine healthy subjects were taken as a control group (16 males and 23 females). All were non smokers, did not have any history of chronic disease and did not take any treatment for chronic disease, and their ages ranged from 11—55 years old. Measurements of their height and weight were also done to calculate their body mass index (BMI) and blood samples were drawn.

Blood Sampling:-

Venous blood samples were drawn from healthy control subjects and diabetic patients using disposable syringes in the sitting position. Five ml of blood was obtained from each subject, blood was polled into plain disposable tube without anticoagulant blood was allowed to clot for 10-15 minutes, the clot shrinks and serum

Chemicals :-

was obtained by centrifuging 2500 xg for approximately 10-15 minutes.
:hemicals used highly punfed and used without any further punfication .

:thods:-

dy Mass Index (BMP) :-

I has been proposed as an alternative to the traditionally used height-weight tables in assessing obesity. BMI sures weight corrected for height and is significantly correlated with total body fat content. Body mass index (BMI) calculated as weight (in kilograms) divided by height (in meters) squared. (Grodner *et al.*,2000).

termination of Serum Glucose :-

he determination of glucose in the sera of tested individuals, enzymatic colorimetric test kit (Biocon GLU, nany) was used.

:asurement of Serum C-reactive protein (CRP):-

presence of CRP in the sera of the tasted individuals was evaluated by using CRP latex reagent kit (Standard, nany), which is a rapid test for the qualitative and semi quantitative measurement of CRP in serum by agglutination of particles on the slide.

:rmination of Serum Total Vitamin C(Ascorbic Acid):

:rmination done by using the method described by(Lleyd *et al.*, 1945 : Burtis and Ash wood . 1999).

Calculation of Serum Reduced Vitamin C (Ascorbic Acid):-

In the present study, reduced ascorbic acid was calculated from a correlation student) between the 2.4-DNPH method (used to measure total vitamin C concentration) and 2,6-dichloroindophenol (2.6-DCIP) method (used to measure reduced vitamin C concentration) conducted on 611 patients samples, and having show good agreement (Lleyd *et al.*, 1945).

DNPH - 1.088 (DCIP) + 0.12 (r=0.95)

$$\text{Reduced Ascorbic Acid} = \frac{\text{Total ascorbic acid (DNPH)} - 0.12}{1.088}$$

Determination of Serum Reduced Glutathione (GSH):-

The determination done by using method described by (Ellman,1959; Alta'ee, 2003).

Statistical Analyses :-

Statistical analyses were performed using SPSS program version 10.0, all values were expressed as mean ± standard deviation (SD) or percentage (%).

Multiple comparisons between diabetic and control groups and between the diabetic groups (type 1 and 2 DM) were made, using one way-analysis of variance (ANOVA).The person rank correlation test and simple linear regression analysis were used to assess the relationship between 'variables. Results were considered significant when the probability (p) was less than 0.05 ($p<0.05$).

Results and discussion

In the present study, the demographic (sex , age) clinical, and biochemical characteristics of healthy control subjects, type 1 DM, and type 2 DM are shown in (Table 3.1) expressed by mean ± SD or percentage (%).

The prevalence rate of type 2 DM is more than the prevalence rate of type 1 DM, in which (69%) of total number of diabetic patients have type 2 DM, whereas (31%) of them have type 1 DM. (Figure 3.1). This can be due to the increasing obesity and reduced activity levels which are considered diabetogenic factors of developing type 2 DM (Kasper et al, 2005).

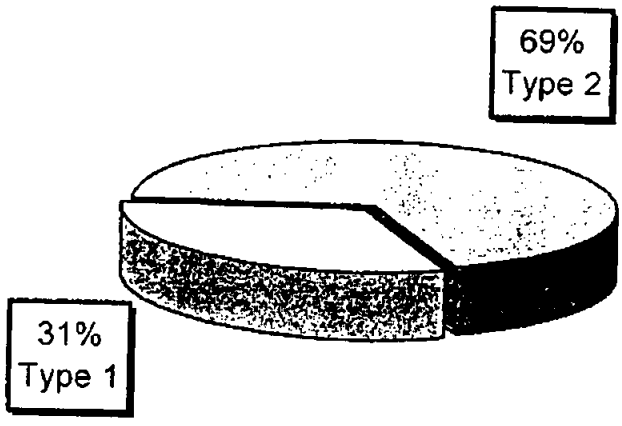


Figure (1): The prevalence rate of type 1 and type 2 DM.

Regarding the sex factor of the patients with DM, females more than males have diabetes for both types 1 and 2(17 females , 10 males) for type 1 DM and (33 females, 27 males) for type 2 DM. This is due to geographical variations because in Japan, Malaysia, and India diabetes is more common in men. In the West Indies, the sex distribution is equal , but the reason for increased occurrence in women may be related to the late effects of high parity, which may not be manifested until after 45 years of age (Bullock , 1996).

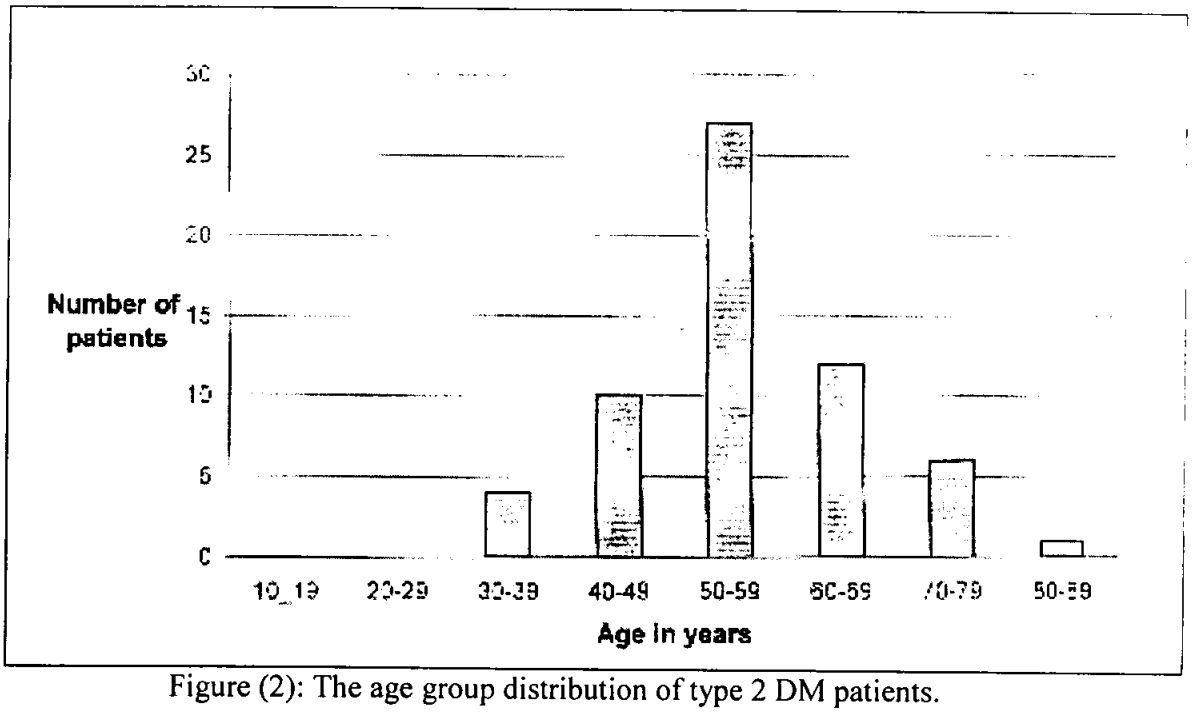


Figure (2): The age group distribution of type 2 DM patients.

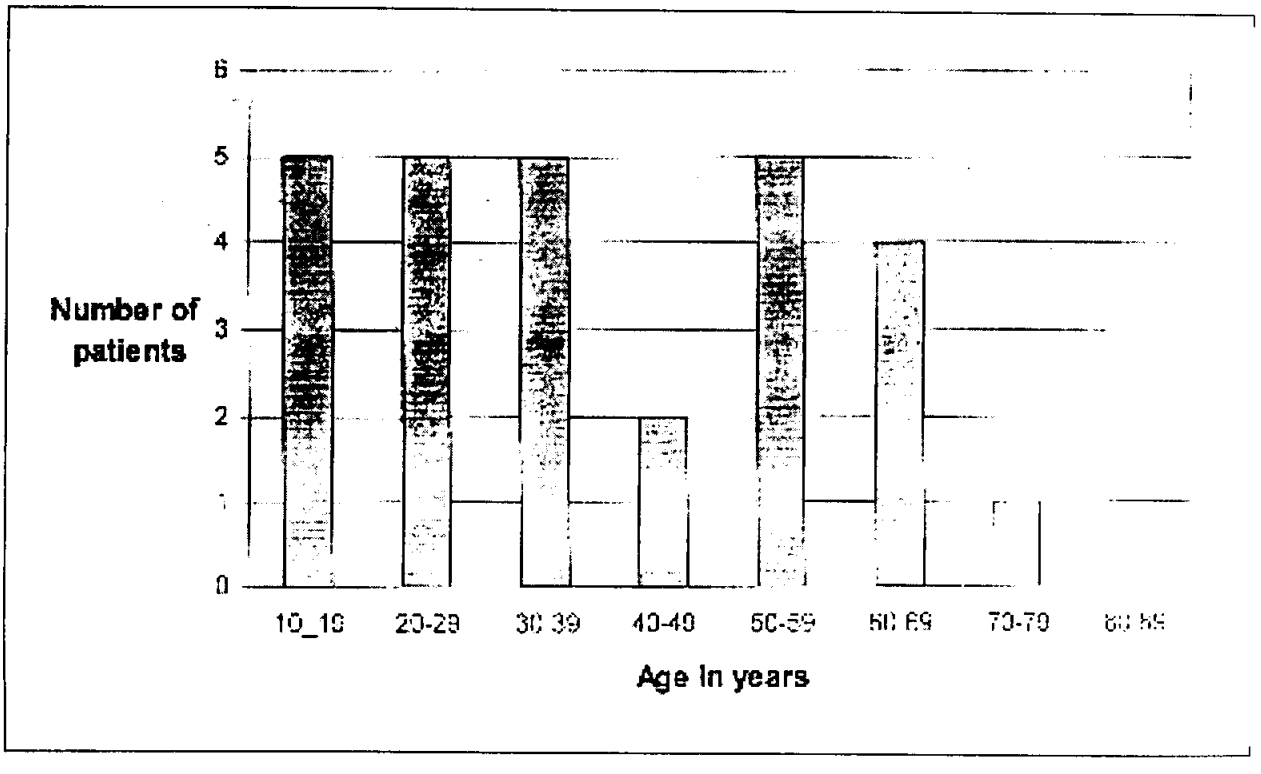


Figure (3) : The age group distribution of type 1 DM patients.

Figures (2) and (3), excluding the duration of DM. also show that in type 1 DM the distribution of age group starts from (10) years, i.e. early age group, whereas in type 2 DM the distribution starts from (30) years, i.e. middle age group. The body mass index (BMI kg/m2) of diabetic patients is classified as: underweight (<18.5), normal (18.5 - 24.9), overweight (25.0 - 29.9), obesity (30.0 - 39.9). and extreme obesity (≥ 40.0) (Grodner *et al*, 2000). (Figure 4 and 5)

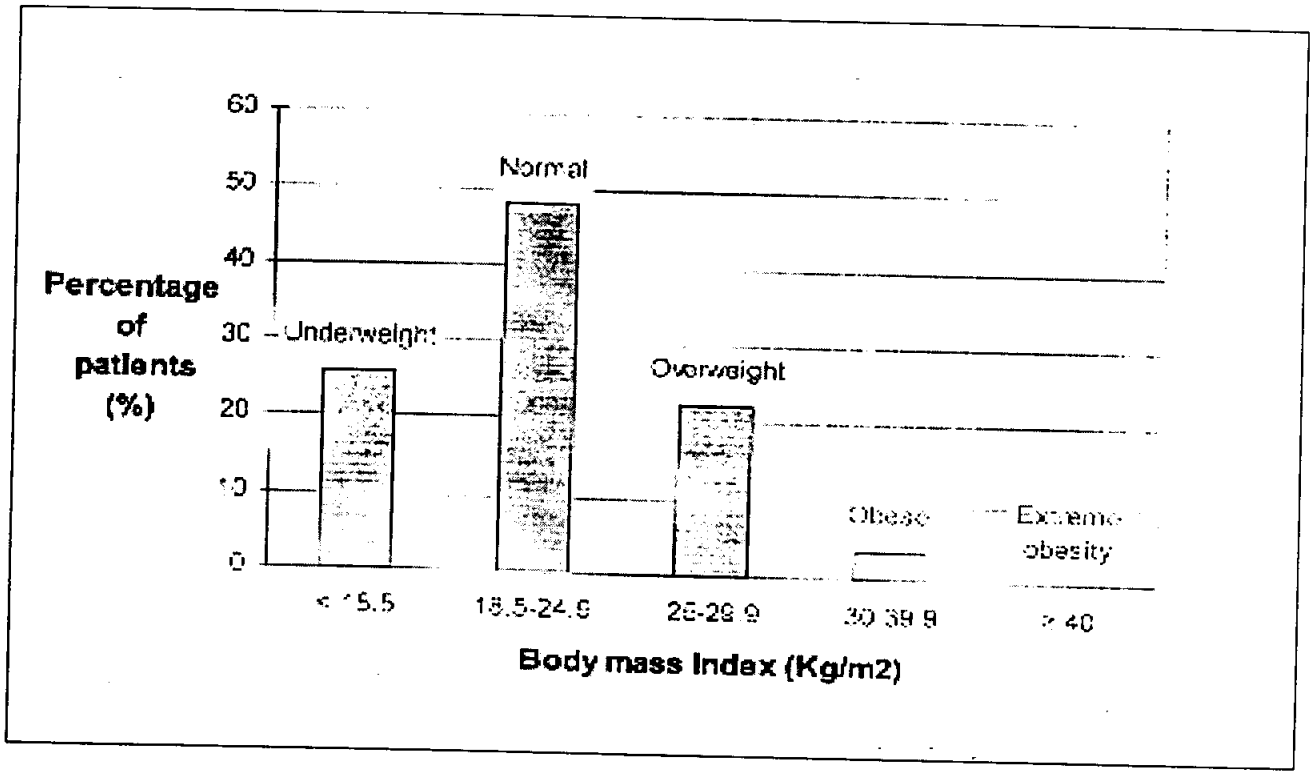


Figure (4): Classification of body mass index in type 1 DM patient .

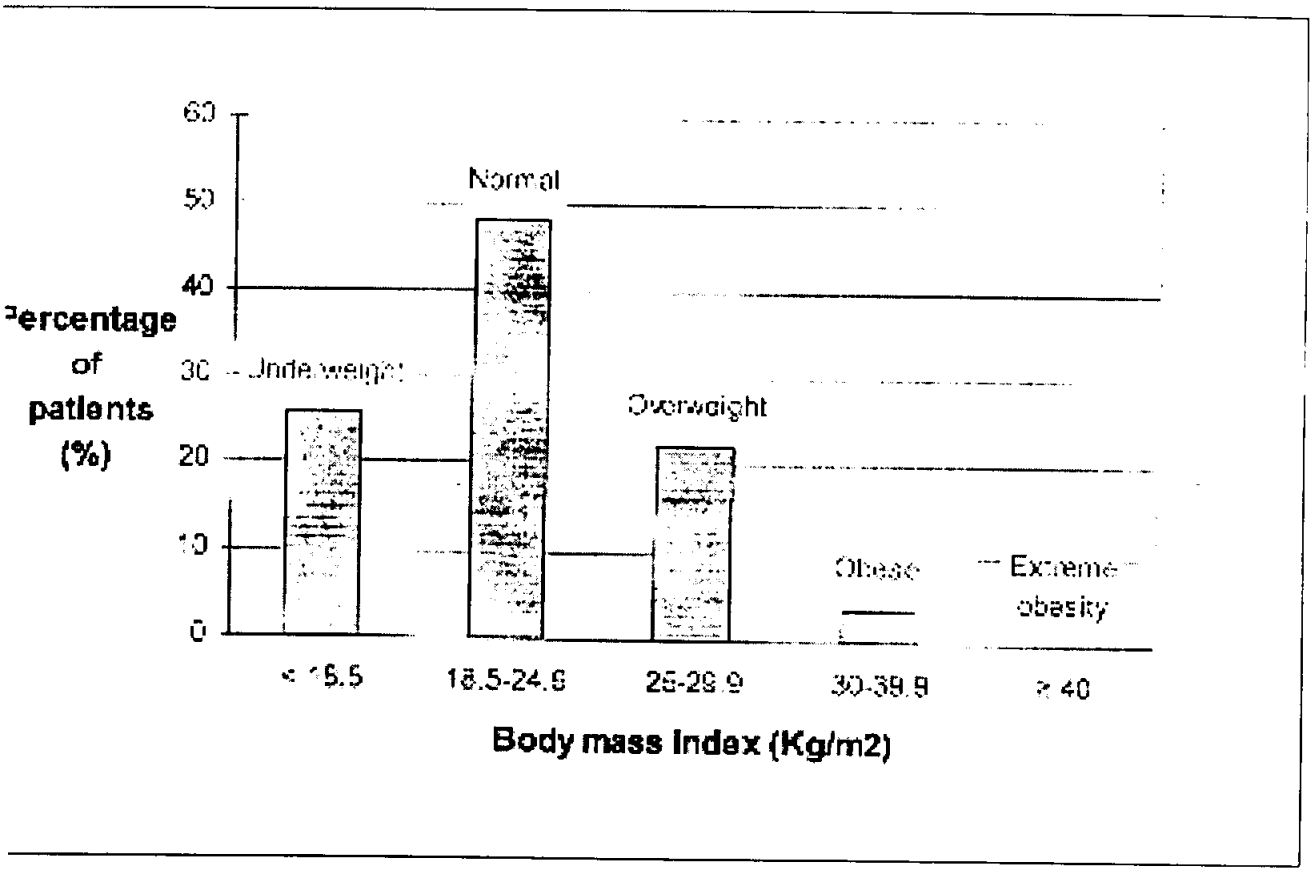


Figure (5): Classification of body mass index in type 2 DM patients

tients of type 1 DM who have BMI < 25.0 kg/m2 (i.e. underweight and normal weight) constitute 74% of the al number of type 1 DM patients (20:27) (Figure 6). On the other hand, in type 2 DM patients who have MI > 25.0 kg/m2 (i.e. overweight, obese, and extreme obesity) constitute 72% of the total number of type 2 M patients (43 : 60) .

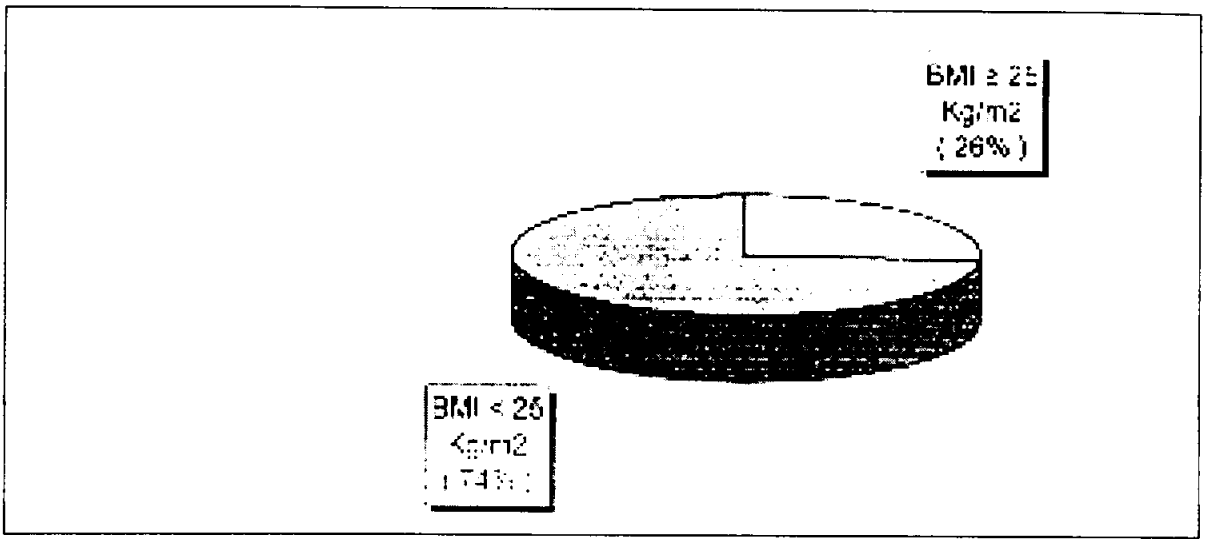


Figure (6) : The percentage of obese and non obses patients in type 1 DM.

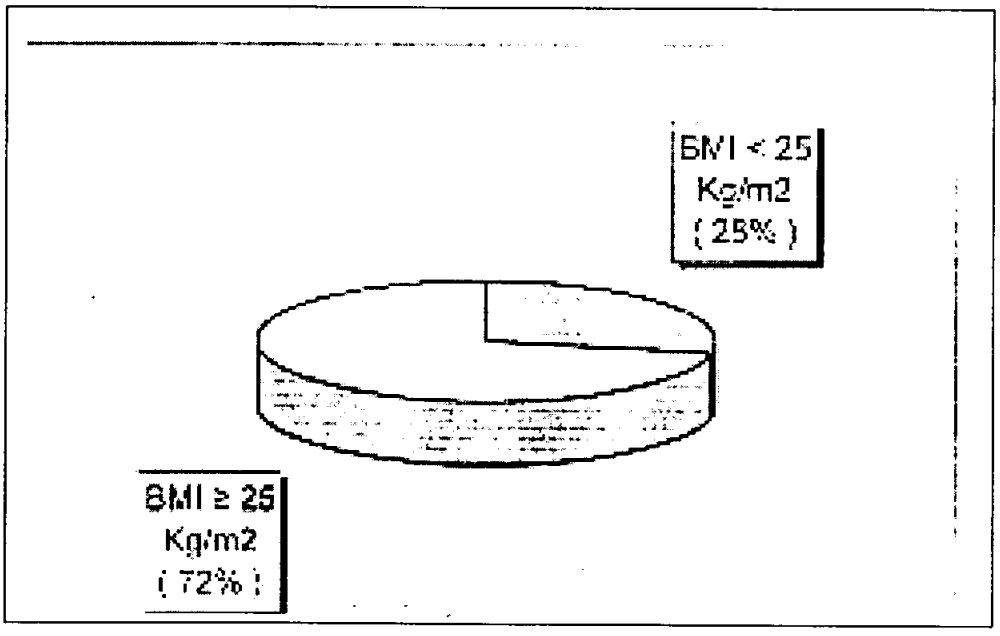


Figure (7):The percentage of obese and non obese patients in type 2 DM.

Regarding the history of ischaemic heart disease, (14.81%) of type 1 DM patients have a positive history of ischaemic heart disease, whereas in type 2 DM patients, the percentage is (21.66%). This shows that positive history of ischaemic heart disease is more in type 2 DM patients than in type 1 DM patients, but in both types, patients with negative history of ischaemic heart disease are more than those with positive history of ischaemic heart disease.

Biochemical parameters random serum glucose (RSG), total vitamin C (TvitC), reduced vitamin C (RvitC), reduced glutathione (GSH), and C-reactive protein (CRP) expressed by mean ± SD are measured in the sera of healthy control subjects, type 1 DM, and type 2 DM patients (Table 1).

Table (1): Demographic , clinical, and biochemical characteristics of healthy control subjects and diabetic patients.

Variables	Healthy	Type 1 DM	Type 2 DM
-No	39	27	60
-sex (M/F)	16/23	10/17	27/33
-Age (year)	33.20±12.33	38.48±18.77	54.93±10.95
-Family history %	76.92 %	62.96 %	60%
-Smoking status :			
Never	100%	81.48%	71.66%
Past	-	3.70%	16.66%
Current	-	14.81 %	11.66%
-BMI (Kg/m2)	26.04±3.76	22.19±4.28	27.53±5.75
-Classification of BMI:			
Underweight%	0%	25.92 %	0%
Normal%	41.02%	48.14%	28.33 %
Overweight%	43.58 %	22.22 %	43.33 %
Obesity%	15.38%	3.70%	25%
Extreme Obesity%	0%	0%	3.33 %
-Duration of DM (year)	-	9.68±10.25	8.08±8.28
-Ischaemic heart disease%	-	14.81 %	21.66%
-RSG (mmol/L)	4.84±0.77	16.02±6.10	13.27±5.32
-TvitC (mg/L)	14.19±4.56	7.29±3.35	7.26±3.34
-RvitC (mg/L)	12.93±4.19	6.59±3.08	6.57±3.07
-GSH (n M)	29.16±14.74	7.96±4.75	10.62±4.99
-CRP:			
Positive test%	-	18.51 %	11.66%
Cone, of CRP in positive test (mg/L)	-	35.20±13.38	32.00±16.00

The mean of reduced glutathione (GSH μM) shows a decrease in its level in type 1 DM patients in comparison with that of the Control Group and the difference is significant between them. In type 2 DM patients the mean of GSH shows a decrease in its level in comparison with that of control group and the difference is significant between them. But no significant difference is found between the mean of GSH of type 1 DM patients and that of type 2 DM patients.

In diabetes, the decrease in the antioxidant GSH level may be an outcome of greater GSH consumption by ROS. It is also largely consumed, mainly because of the regeneration of vitamin C, which is largely oxidized in diabetic patients. Lower amounts of GSH may also be explained by the glycation of the enzyme γ-glutamyl cysteine synthetase (an enzyme catalyze GSH synthesis), which generates decreased amounts of GSH synthesis in diabetes (Shurtz-Swirski ***etal.*** ,2001).

In addition, under hyperglycaemic conditions, as much as 30% of the glucose is channeled into the polyol pathway, causing a substantial depletion of NADPH, in which the latter is used as a co-factor by aldose reductase (AR) to reduce glucose to sorbitol. and by that it competes with glutathione reductase (GR) for their co-factor NADPH which is also required to regenerate GSH. The depletion of NADPH consequently leads to a significant decrease in the GSH level (Figure 8) (Chung ***et al.***, 2003).

Patients with raised CRP level in both types 1 and 2 DM are found to be either having:

1-Diabetic complications [diabetic ketoacidosis DKA 8.33 (1:12), diabetic foot ulcer DFU 25% (3:12) or acute myocardial infarction AMI 8.33% (1:12)], or

2-Infection [urinary tract infection UTI 16.66% (2:12), respiratory tract infection RTI 25% (3:12)], or being

3-Newly diagnosed diabetes 16.66% (2:12), (2weeks) for typel and 2 DM patients respectively (Table2).

These durations are the shortest ones noticed in diabetic patients whether of type 1 or 2 DM. This proves that CRP is involved in the development of type 1 and 2 DM. All of the above conditions show to have raised CRP level in many other studies (Crais ***et al.***, 2002: Jain ***et al.*** 2003; Ford , 1999; Pagana and Pagana, 2001; Fischbach , 2000: Chase ***et al.***. 2004; Thorand ***et al.***, 2003).

Chronic hyperglycaemia is not sufficient to induce inflammation. This result is supported by other studies (Pickup. 2004; Chase ***et al.***. 2004). can be clarified by two points:

a- Not all diabetic patients with hyperglycaemia whether of type 1 or 2 DM have a raised CRP level.

b- There is a non significant correlation between the raised CRP concentration and serum glucose concentration in diabetic patients of both types 1 and 2 ($p > 0.05$).

Contribution of Hyperglycaemia in Raising CRT Level through Oxidative Stress :-

Hyperglycaemia may contribute to raise CRP level indirectly by increasing reactive oxygen species (ROS), in which glucose present in excess undergoes auto-oxidation, yielding ROS and intracellular precursors of advanced glycation end products (AGE), in which the latter stimulates proinflammatory cytokine production. Hyperglycaemia also alters the cellular redox state by increasing the NADH / NAD^+ ratio and decreasing NADPH / $NADP^+$. This causes the flux of substrate , through the polyol pathway. The ROS creates oxidative stress, which damages molecules and activates a number of signaling molecules such as protein kinase C or the transcription factor NFKB (NFidB is a key determinant of inflammatory-response), and thus CRP elevation occurs (Baynes and Dominiczak, 2005; Bloomgarden. 2004; King ***etal***, 2003).

In type 1 DM patients, there is an inverse significant correlation between the concentration of CRP (mg/L) and GSH (μM), r (correlation coefficient) = -0.960, p=0.009 (p significant when < 0.05) (Figure 8).

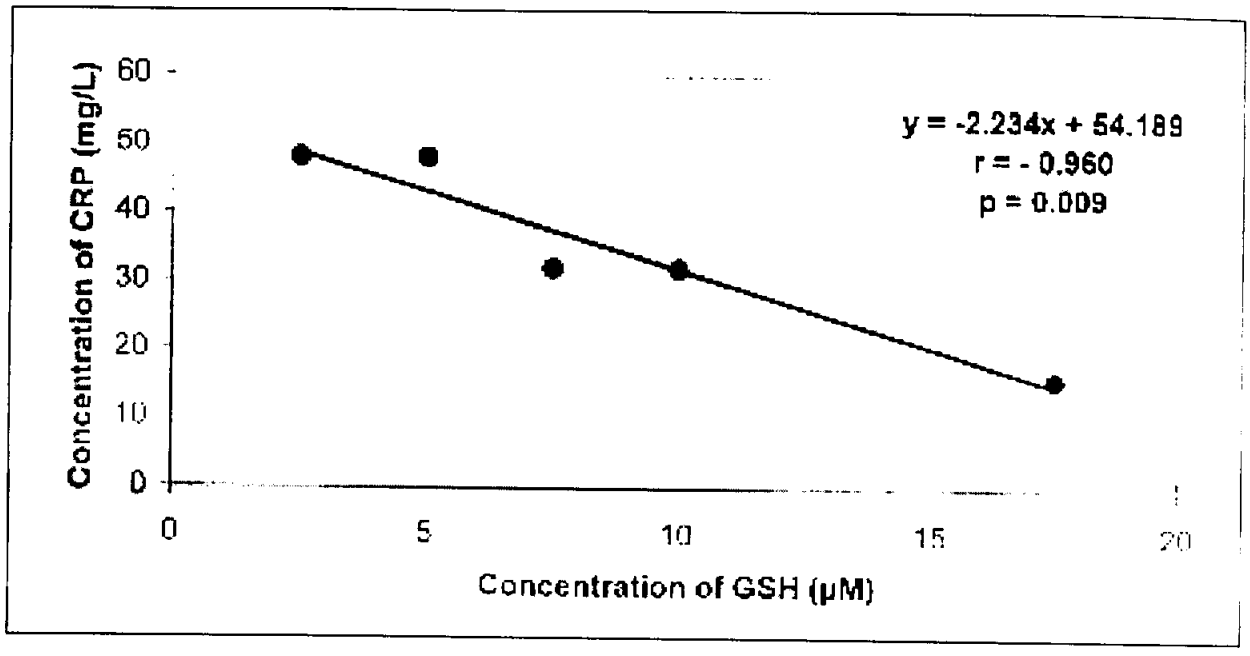


Figure (8): The relationship between the concentration of CRP and GSH in type 1 DM patients.

Considering RvitC, there is an inverse significant correlation between the concentration of CRP (mg/L) and RvitC (mg/L) , r = -0.932 , p = 0.021 (Figure 9).

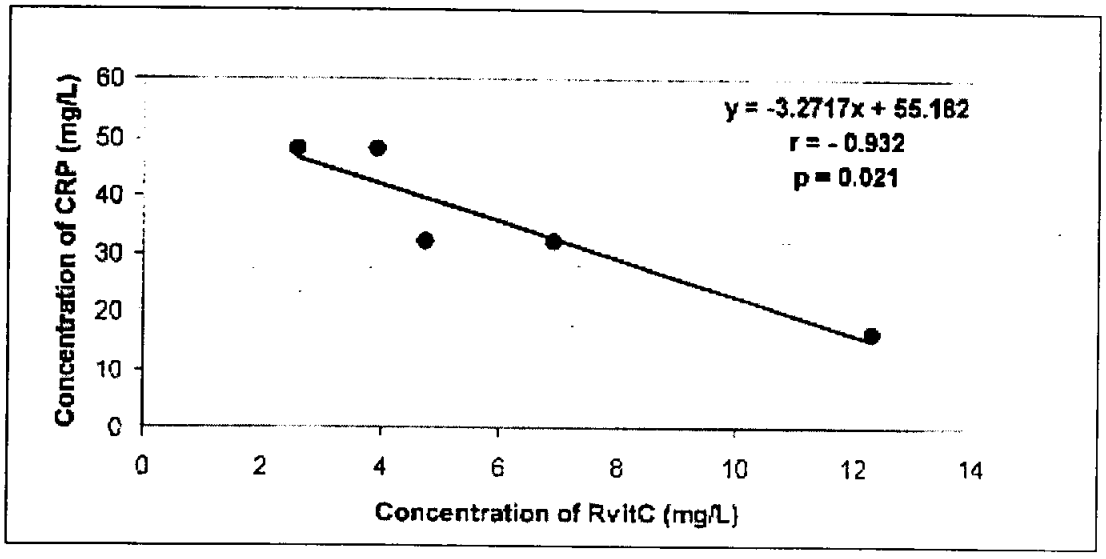


Figure (9): The relationship between the concentration of CRP and RvitC in type 1 DM patients.

In type 2 DM patients, there is an inverse significant correlation between the concentration of CRP (mg/L) and GSH (µM), r = -0.852 . p = 0.015 (Figure 10).

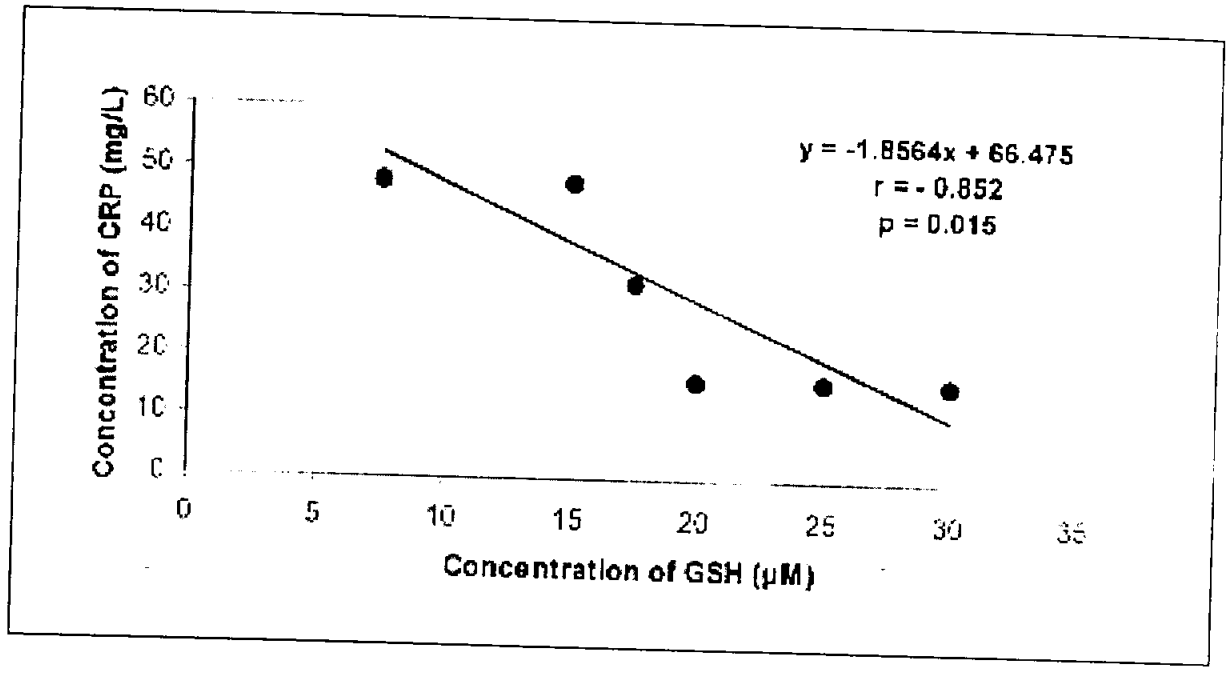
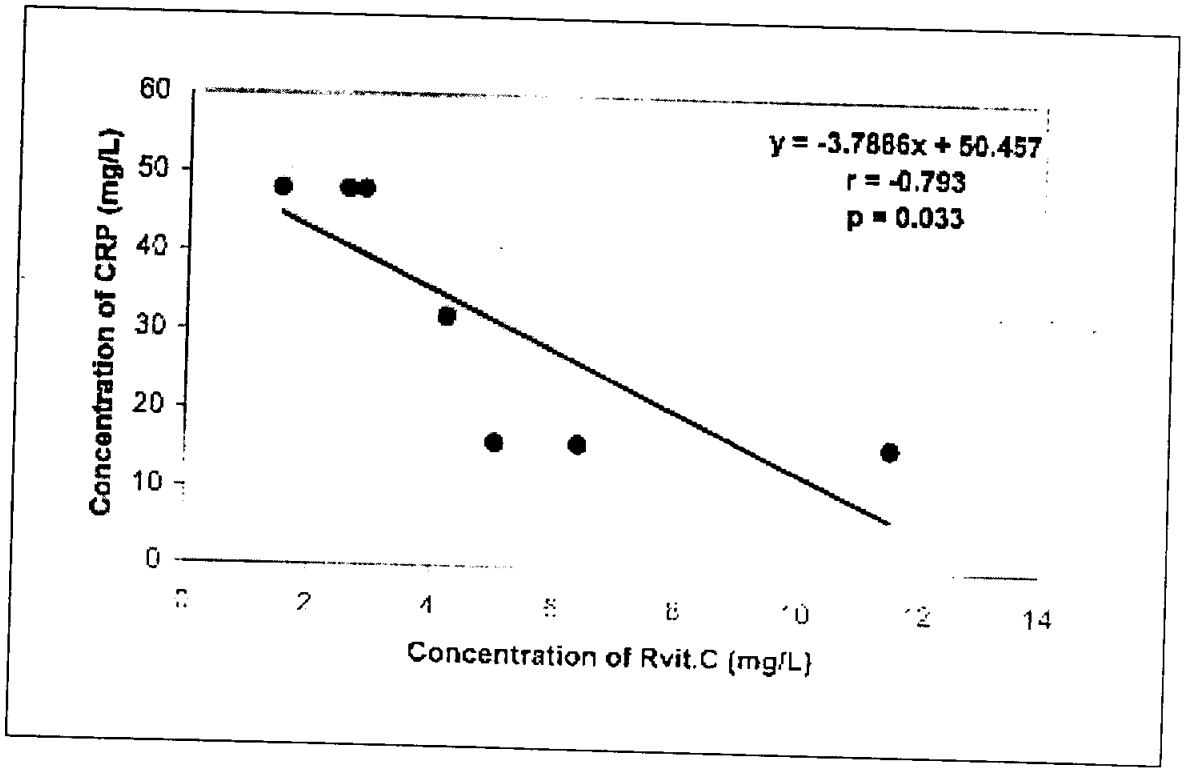


Figure (10) :The relationship between the concentration of CRP and GSH in type 2 DM patients.

Considering RvitC, there is an inverse significant correlation between the concentration of CRP (mg/L) and RvitC (mg/L), r = -0.793, p = 0.033 (Figure 11).



The measurement of the antioxidants (GSH, RvitC) in both types 1 and 2 DM patients with raised CRP level are used as indicators of oxidative status and by using statistical analyses, they reveal the existence of oxidative stress, in which a significant decrease of the antioxidants (GSH. RvitC) in both types 1 and 2 DM patients with raised CRP level in comparison with that of the healthy Control Group. Although the mean of GSH level in type 2 DM patients with raised CRP level shows no significant difference in comparison with the mean of GSH level of the healthy Control Group, it is close to a significant level p = 0.06 ($p<0.05$ consider significant) (Table 2).

Table (2) :The comparison between type 1 and type 2 DM patients' antioxidants (GSH, RvitC) and healthy control group' antioxidants.

Variables	Mean Difference (I-J)	Sig.	Mean Difference (I-K)	Sig.
GSH (μ M)	20.666	S	10.595	NS
RvitC (mg/L)	6.827	S	8.063	S

(1= mean of healthy control group, J= mean of type 1 DM patients with raised CRP levels, K> mean of ype 2 DM patients with raised CRP levels. S = significant (when $p<0.05$), NS= not significant).

From the above analyses done on the relationship between hyperglycaemia, oxidative stress and raised CRP level, all these results demonstrate the indirect contribution of hyperglycaemia in raising CRP level through oxidative stress in people with established diabetes of both types land 2.

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