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THE ASSOCIATION OF PLASMA CERULOPLASMIN AND C-REACTIVE PROTEIN WITH TYPE2 DIABETES MELLITUS IN SUDANESE PATIENTS IN KHARTOUM STATE

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ABSTRACT

Background: The inflammatory markers cytokine C- reactive protein (CRP) and Ceruloplasmin are involved in multiple metabolic pathways, including insulin regulation, reactive oxygen species, lipoprotein lipase action and adipocytes function. **Objectives:** The study intended to examine the association of plasma C-reactive protein and Ceruloplasmin with type2 diabetes mellitus in Sudanese patients in Khartoum State. **Study design:** It was a descriptive, case control study, included fifty (50) participants, thirty (30) of them were patients with type2 diabetes mellitus and twenty (20) were healthy control group. It had been conducted in Khartoum State from March to July 2018. **Results:** The study found that the means of random blood glucose, plasma Ceruloplasmin and C-reactive protein were (284.97±74.87, 76.30±8.56, 35.76±29.92) and (118.35±14.96, 35.80±7.74, 2.80±0.755) in case and control group respectively with highly significant *P* value of 0.000. **Conclusion:** Plasma C-reactive protein and Ceruloplasmin levels were high in type2 diabetes mellitus patients. There was significant effect of disease duration on plasma Ceruloplasmin level where as no significant effect of disease duration on plasma CRP levels was observed.

KEYWORDS: Type2 diabetes mellitus, C-reactive protein , Ceruloplasmin.

Introduction and literature review

Diabetes mellitus is a major health challenge of this century. WHO projects that, by the year 2025 about 300 million people will have diabetes worldwide. In Sudan there were over 2.247.000 cases of diabetes in 2017[1]

The acute phase reactant or response (APR) proteins such as Ceruloplasmin, and C-reactive protein (CRP) are nonspecific response to inflammation or tissue damage. Plasma concentrations of the individual APR proteins change at different rates after the initial insult. In practice these changes are helpful in detection of inflammation, as well as monitoring of the progress of the inflammation or its response to treatment.[2,3]

Diabetes mellitus is a complex metabolic disease that affects between six to twenty percent of the population in western industrialized societies.[4] It is a group of metabolic disorder of carbohydrate metabolism in which underutilization of glucose producing hyperglycaemia.[56]

It is commonly accepted the existence of two types of diabetes; the type1 diabetes mellitus, where the basic defect is an absolute deficiency of insulin due to an autoimmune destruction of the β cells in the pancreas and the type2 diabetes mellitus where there is decreased secretion of insulin or an increased resistance to its action by the insulin sensitive tissue such as muscle and adipose tissue. This simple classification was complicated by the emergence of a spectrum of overlapping patient characteristics and the disease proper. Thus sub classifications like maturity onset diabetes of the young (MODY), latent autoimmune diabetes in adults (LADA) and Gestational diabetes mellitus (GDM)[7,8, 9,10]

The diagnostic criteria for diabetes mellitus are the following

- Random plasma glucose > = 200 mg/dl (11.1 mmol/L)+ symptoms of diabetes
- Fasting plasma glucose > = 126mg/dl (7.0mmol/L)
- Two-hour plasma glucose > = 200 mg/dl (11.1mmo/L) during an OGTT (75-g glucose load)
- Haemoglobin A1c is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test

plays a critical role in the management of the patient with diabetes.[11]

Acute phase reactants (APRs)

Ceruloplasmin and CRP are acute phase reactants (APRs) and markers of inflammation, since they are synthesized in response to tissue damage and inflammation. During inflammation, their concentrations increase than the normal levels. They are mainly produced by hepatocytes, but they can also be synthesized by a dipocytes, fibroblasts and endothelial cells.[12]

Ceruloplasmin

Ceruloplasmin is acute phase reactant, copper containing alpha (2) glycoprotein. It is synthesized in the liver parenchymal cells. Copper is added to the peptide chain by an intracellular ATPase, and it is essential for the normal folding of the polypeptide chain.

The functions of Ceruloplasmin include

1. Plasma reduction and oxidation (redox) reaction.
2. Act as oxidant or antioxidant, depending on factors such as the presence of free ferric ions and ferritin binding sites.
3. Regulating the ionic state of iron in particular oxidizing Fe^{2+} to Fe^{3+}.

Ceruloplasmin is weak, late-reacting acute phase protein. It increases due to pregnancy, inflammatory process, malignancies, oral estrogen, and contraceptive. It decreases due to severe liver disease, malnutrition, malabsorption and nephritic syndrome [12]

C-reactive protein

C-reactive protein (CRP) was so named because it precipitated with C substance, polysaccharides on the cell wall of streptococcus pneumonia first described in 1930. In 1941 it was proved to be a protein and given the name CRP. It is the first acute phase proteins (APPs) to become elevated in inflammatory disease also the one exhibiting the most dramatically increase in concentration. It is synthesized in the liver and appears in the blood of patients with diverse inflammatory disease. It is acute phase reactant and motivates phagocytoses in inflammatory disease.

CRP initiates opsonization, phagocytosis and recognized potentially toxic, autogenously substance release from damaged tissue, bind them and the then detoxify them or clear them from the blood. The level of CRP increase as result of tissue necrosis, regardless the damage originates from a pneumococcal infection or other source. Also it increases dramatically in case of myocardial infarction, stress, trauma, surgery, neoplastic proliferation. The increasing of CRP begins within six to twelve hours of the infections. In addition the level of CRP decreases as result of smoking cessation, exercise, and weight loss. It is marker of inflammation and coagulation disorder.[13]

CRP is used for

1. Screening for organic disease.
2. Assessment of the activity of inflammatory disease.
3. Detection of inter-current infections such as systemic lupus erythematous (SLE), Leukemia and after surgery.
4. Management of neonatal septicemia and meningitis

Relationship between Ceruloplasmin, C-reactive protein and Diabetes mellitus

Low grade inflammation is associated with the risk of developing type 2, so it has been accepted that chronic and sub chronic inflammation is the part of the insulin resistance syndrome.[14]

The mechanisms by which chronic inflammation can evoke type2 diabetes mellitus are not clear. However it is known the adipose tissue can synthesize and release the pro-inflammatory cytokines, such as tumors necrosis factors – alpha, IL-1, IL-6.[15]

The pro-inflammatory cytokine and Ceruloplasmin and CRP are involved in multiple metabolic pathways which are relevant to insulin resistance, including insulin regulation, reactive oxygen species, lipoprotein lipase action and adipocytes function.[14]

Therefore the activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes, with convincing data that type 2 diabetes includes an inflammatory component[16]

Recent research revealed that high CRP might be associated with colon cancer, onset of diabetes complications, obesity, and the risk of developing type 2 diabetes.[17]

Rational of study

Since there was a little information about the interaction between diabetes mellitus type2 and ceruloplasmin and C-reactive protein level in plasma. The result of this study will increase existing knowledge and hence enable clinicians to provide better management for patients with type2 diabetes mellitus.

OBJECTIVES

The main objective of this study was to examine the association of Ceruloplasmin and C-reactive protein and random blood glucose plasma level among type2 diabetic mellitus Sudanese patients.

Specific objectives

1. To examine the effect of age on Ceruloplasmin and C-reactive protein levels in type 2 diabetes mellitus patients.
2. To evaluate whether there was gender variation as regard to Ceruloplasmin and C-reactive protein levels in type 2 diabetes mellitus patients.
3. To demonstrate whether there was effect of disease duration on Ceruloplasmin and C-reactive protein plasma levels in type 2 diabetes mellitus patients.

MATERIALS AND METHODS

It was a descriptive, case control study, included fifty (50) participants, thirty (30) of them were patients with type2 diabetes mellitus and twenty (20) were healthy control group. It had been conducted in Khartoum State from March to July 2018.

Patients with type 1 diabetes mellitus, pregnant women, and those who had liver diseases or acute infections are excluded from the study.

After informed verbal consent data were collected from Sudanese with type 2 diabetic patients using a preformed questionnaire. Approximately five (5) ml of venous blood was collected from each participant. The blood samples were drawn to lithium heparin containers then centrifuged at (3000) rpm for (5) minutes and serum was separated, and stored at (-20°C) until analyzed**,** serum samples were tested by Mispa i2 and Biosystem 350 BTS technique (Mispa i2) India.

Blood glucose level was determined by the semi-Automatic-analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain).

The reagent containing polyclonal goat anti-human ceruloplasmin antibodies when mixed with serum sample containing ceruloplasmin causes changes in absorbance, due to the development of turbidity, which is directly proportional to the concentration of ceruloplasmin in the sample.

CRP samples binds to specific anti- CRP antibodies, which have been adsorbed to latex particles and agglutinates. The agglutination is direct proportional to the quantity of CRP in the sample.

All collected data was analyzed using SPSS for windows, version 21, Pearson Chi-Square test was used for categorical data with p value ≥ 0.05 as significant. Analysis of variance (ANOVA) was used for continuous data and the statistical results were presented as means ± SD.

Ethical clearance

Ethical approval for the study was obtained from the Board of the Faculty of Graduates Studies and Scientific Research in Shendi University. Verbal informed consent for participation in the study was obtained from each participant before recruitment into the study.

RESULTS

Table (1): The means of random blood glucose, plasma Ceruloplasmin and C-reactive protein in the control and case group.

Parameters	Groups	No	Mean± Std. Deviation	P value
RBG (mg/dL)	Control	20	118.35±14.96	0.000*
	Case	30	284.97±74.87	
Cer (mg/dL)	Control	20	35.80±7.74	0.000*
	Case	30	76.30±8.56	
CRP (mg/L)	Control	20	2.80±0.755	0.000*
	Case	30	35.76±29.92	

Values expressed as mean ± St.D, * Significant (P≤0.05)

Table (2): The means of random blood glucose, plasma Ceruloplasmin and C-reactive protein according to age in the case group.

Parameters	Age (years)	No	Mean± Std. Deviation	P-value
RBG (mg/dL)	35-60	20	287.65±78.07	0.78
	61-90	10	279.60 ± 71.73	
Cer (mg/dL)	35-60	20	76.80 ± 8.87	0.65
	61-90	10	75.30 ± 8.29	
CRP (mg/L)	35-60	20	32.33 ± 31.12	0.36
	61-90	10	42.62 ± 27.60	

Table (3): The means of random blood glucose, plasma Ceruloplasmin and C-reactive protein according to gender in the case group.

Parameters	Gender	No	Mean± Std. Deviation	P-value
RBG (mg/dL)	Males	15	304.67 ± 84.23	0.781
	Females	15	265.27 ± 60.71	
Cer (mg/dL)	Males	15	78.53 ± 8.72	0.653
	Females	15	74.07 ± 8.07	
CRP (mg/L)	Males	15	31.37 ± 21.22	0.367
	Females	15	40.15 ± 36.92	

Table (4): The means of random blood glucose, plasma Ceruloplasmin and C-reactive protein according to duration of disease in the case group.

Parameters	Duration/years	No	Mean± Std. Deviation	P-value
RBG (mg/dl)	1-10	27	283.44±73.17	0.830
	11-20	3	298.67±106.61	
Cer (mg/dl)	1-10	27	74.44±6.66	0.008*
	11-20	3	93.00±4.58	
CRP (mg/L)	1-10	27	33.46±28.15	0.470
	11-20	3	56.40±44.38	

Table (5): Correlation between random blood glucose with plasma Ceruloplasmin in the case group.

Parameters	Number	Pearson Correlation	Sig. (2-tailed)
RBG (mg/dL)	30	0.789	0.000**
Cer (mg/dL)	30		

** Correlation is significant at the 0.01 level (2-tailed). ** High Significant. (P≤0.05)

Table (6): Correlation between Random Blood Glucose with C- reactive protein in case group.

Parameters	No	Pearson Correlation	Sig. (2-tailed)
RBG(mg/dL)	30	0.586	0.000**
CRP(mg/L)	30		

** High Significant at the 0.01 level (2-tailed).

Table (7): Correlation between plasma Ceruloplasmin with C-Reactive Protein in the case group.

Parameters	No	Pearson Correlation	Sig. (2-tailed)
Cer (mg/dL)	30	0.660	0.000**
CRP (mg/L)	30		

** High Significant at the 0.01 level (2-tailed).

DISCUSSION

Acute phase reactants proteins such as ceruloplasmin and CRP are the markers of inflammation and they were significantly higher in case group compared with control group, as depicted in table (1). The findings of this study also go well with those of Vishakha V Mahajan, *et al*., who demonstrated significant positive correlation between ceruloplasmin and CRP and the RBG level in type2 diabetics.[19] These findings point to the slow chronic inflammation associated with obesity as pathogenic cause for type 2 diabetes mellitus[31], and it is important to be taken into consideration in the prevention and management of the disease. The findings also consolidated the link between obesity and type 2 diabetes mellitus, where adipose tissue is infiltrated by macrophage secreting cytokines.

The results of the current study was different from those obtained by Ashok Kumar Jeppu, et al., as regard to Ceruloplasmin level in Malaysian diabetic patients.[22] Because the decrease in the serum Ceruloplasmin in the type 2 diabetes mellitus patients observed might be due to the increased utilization of the antioxidants, to neutralize the reactive oxygen species produced in excess in these. The current study illustrated significant positive correlation between blood glucose levels and CRP and Ceruloplasmin levels as appeared in table (5) & (6).

There was statistically significant effect of disease duration on serum Ceruloplasmin levels, as demonstrated in table (4) but there was no significant effect of age and gender on estimated parameters, that highlighted in table (2) & (3).

CONCLUSION

This study concluded that the levels of Ceruloplasmin and CRP were significantly high in type 2 diabetes mellitus patients. There was also significant effect of disease duration on serum ceruloplasmin level, but insignificant effect of disease duration on serum CRP level. The gender and age of patients have insignificant effect of on the three estimated parameters.

Recommendations

This study recommended that the estimation of acute phase proteins such as CRP, Cer can be used as risk, diagnostic and prognostic predictors of type2 diabetes mellitus.[20, 21, and 22]

The role played by other acute phase reactant proteins in the pathogenesis of type2 diabetes mellitus need to be further studied.

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