International Journal of Family Medicine and Primary Care

Research Article
Published: 06 Dec, 2021

Evaluation of C-Reactive Protein, Zinc and Copper Concentrations in Type 2 Diabetic Patients

Johnson JT and Agoro ES*

Department of Biochemistry, Federal University Otuoke, Nigeria

Abstract

The serum concentrations of C-Reactive Protein (CRP) zinc and copper in type 2 diabetic mellitus patients was assessed in this study. Serum obtained from blood samples of 50 non-diabetic and 50 diabetic patients was used in determining serum CRP, zinc and copper concentrations. Results showed that the mean concentration of CRP was significantly ($P<0.05$) higher in the diabetic group (11.76 ± 1.56 mg/l) compared with the non-diabetic (normal control) group (4.302 ± 1.80 mg/l). The mean serum glucose of diabetic patients (12.44 ± 1.64 mmol/l) was higher ($P<0.05$) compared to the non-diabetic group (4.51 ± 0.30 mmol/l) confirming the diabetic and the non-diabetic status of the patients. More so, further investigations showed that the mean concentrations of these micronutrients (zinc and copper) were significantly reduced in the diabetic group compared to the non-diabetic control group. Serum copper (13.09 ± 4.95) and zinc (0.49 ± 0.35) concentrations of the diabetic group were lower ($P<0.05$) when compared to the non-diabetic group (132.00 ± 9.61 and 0.90 ± 0.004 respectively). This study further affirms an association of insulin resistance and hyperglycemic states with inflammatory mechanisms in type 2 diabetes mellitus as demonstrated by a significant increase in CRP alongside it associated depletion of two micronutrients; copper and zinc in the type 2 diabetic group which are essential to various physio-biochemical processes.

Keywords: Diabetes Mellitus; C-Reactive protein; Zinc; Copper

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization (2013) has predicted that by 2030 the number of adults with diabetes would have almost tripled worldwide, from 177 million in 2000 to over 700 million [1]. Experts project that the incidence of diabetes is set to soar by 64% by 2025, meaning that a staggering 53.1 million citizens will be affected by the disease [2]. The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rise to around 439 million (7.7%) by 2030 [2-4].

There are two main types of diabetes mellitus

Type 1 diabetes, also called Insulin Dependent Diabetes Mellitus (IDDM), is caused by lack of insulin secretion by beta cells of the pancreas. Type 2 diabetes, also called Non-Insulin Dependent Diabetes Mellitus (NIDDM), is caused by decreased sensitivity of target tissues to insulin. In both types of diabetes mellitus, metabolism of carbohydrates, proteins, and lipids are altered. The basic consequences of insulin deficiency or insulin resistance on glucose metabolism are the inefficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result of this, blood glucose concentration increases, cell utilization of glucose falls increasingly lower and utilization of fats and proteins increases [5].

Type 2 diabetes mellitus (T2DM) is a chronic disease that is considered to be a foremost global health problem, the incidence of which quadrupled within the past 35 years from 108 million in 1980 to 422 million in 2014 and is expected to increase to 552 million by 2030 [6,7]. Chronic low-grade inflammation with production of high levels of inflammatory proteins has been implicated in the development of T2DM [1]. C-Reactive Protein (CRP) is considered to be a prime inflammatory marker of T2DM, which is produced by liver cells, and its expression is regulated by Interleukin 6 (IL-6) and TNF-α, which are produced by adipocytes [1,8]. A vast number of cohort studies, having noted elevated levels of CRP in male and female participants Nakanishi et al. [9], Torand [10] suggested that CRP is a risk factor for development of T2DM. According to Suganya et al. [1] many studies have elucidated the role of CRP in the development of T2DM, reporting that

OPEN ACCESS

Correspondence:
Johnson JT, Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria, E-mail: johnsonjt@fuotuoke.edu.ng

Received Date: *02 Nov 2021*
Accepted Date: *29 Nov 2021*
Published Date: *06 Dec 2021*

Citation:
Johnson JT, Agoro ES. Evaluation of C-Reactive Protein, Zinc and Copper Concentrations in Type 2 Diabetic Patients. Int J Fam Med Prim Care. 2021; 2(5): 1050.

even after adjustment for BMI, the relationship between CRP level and incidence of T2DM remained statistically significant. Moreover, observed gender-specific differences have suggested that women have a higher risk of diabetes than men.

It has been reported that serum CRP levels are elevated in patients with Impaired Glucose Tolerance (IGT) Temelkova-Kurktschiev et al. [11], or diabetes Ford [12]. According to Yasufumi et al. [13], a few prospective studies have shown that increased CRP levels are an independent risk factor for future diabetes. Most studies have been conducted with Western populations, and a relatively small number of studies on Asian populations. More so, it has been stated that although some micronutrients are known to be involved in the pathogenesis and progression of diabetes mellitus, reports of studies are most times contradictory. At times, serum or tissue contents of certain elements, such as copper, manganese, iron and selenium, zinc may be reportedly higher in diabetic patients than in non-diabetic controls. At the other hand, although the majority of diabetic patients do not have micronutrient deficiencies, zinc, chromium, and magnesium deficiencies have been identified in a subgroup of patients. No study has been carried out to investigate the relationship between CRP and type 2 diabetes in diabetic patients alongside their serum zinc and copper concentrations in Nigeria which may help to inform health care providers of the need (if any) for possible supplementation of these micronutrients as part of diabetic management strategies for positive outcome in diabetic care as well as manage associated inflammations.

The aim of the study was to determine the concentration of C reactive protein, zinc and copper in serum samples of type 2 diabetes mellitus patients receiving treatment in Niger Delta University Teaching Hospital Bayelsa State.

Materials and Methods

Samples from non-diabetic and diabetic patients who submitted their blood for clinical chemistry investigations in the medical laboratory unit of the hospital were used in determining serum CRP, zinc and copper concentrations. Forty samples, twenty each from non-diabetic and diabetic patients were tested and data generated in two groups respectively.

Human serum samples obtained from the hospital laboratory were divided into two groups. Group A was for the normal non-diabetic patients which served as the control group while group B was for diabetic patients. Serum CPR, zinc and copper determinations were carried out on both groups of samples. A total of 40 samples were used for this study.

Serum CRP levels were measured by immunoturbidimetric method using Roche Cardiac C-Reactive Protein High Sensitive (Roche Diagnostics Penzberg, Germany). The particle-enhanced immunoturbidimetric method was used to measure CRP, with the possibility of measuring CRP within the limits of 0.3 mg/l to 350 mg/l. This method quantifies C-Reactive Protein (CRP) by latex-enhanced nephelometry. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell are used in order to link anti-CRP antibodies covalently. A dilute solution of test sample was mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample forms an antigen-antibody complex with the latex particles. Light scattering, measured by a nephelometric procedure after 6 min, is proportional to the concentration of the analyte present in the sample. An automatic blank subtraction was performed. CRP concentrations were calculated by using a calibration curve. Data reduction of the signals was performed by using a storable logit-log function for the calibration curve. The assay was performed on a Behring Nephelometer for quantitative CRP determination [14-16].

The serum copper and zinc measurement was performed using an air/acetylene flame Atomic Absorption Spectrometer (AAS) Varian model Spectr AA - 20 Victoria, Australia according to the method reported by Gnogbo et al. [17]. The blood tubes used were immersed in a nitric acid solution (HNO3) at 10% (v/v), following a previous day (12 h) washing in a solution of Hydrochloric acid (HCl) at 10% (v/v). They were then rinsed twice with distilled water and dried. Protein precipitation was made by diluting 1 mL of serum in 4 mL of a solution of hydrochloric acid (2 M). After homogenization, each sample was allowed to settle. The clear supernatant obtained was sucked directly into the flame atomic absorption spectrophotometer at the wavelength of 324.8 nm; 213.9 nm for the copper and zinc, respectively. A multi element-standard solution 1000 ppm (Merck, USA) previously diluted at 1/500 with nitric acid-deionized water (0.03 M) was used to prepare the calibration range (0; 0.5; 1.5; 2.0; 4 ppm). The concentration measurements were performed in triplicate and adjusted against the white (HCl solution 2 M).

Statistical analysis

The analysis was done in triplicate. Mean results of serum CRP, zinc and copper levels obtained were subjected to statistical analysis using Student's t-test at $P<0.05$, with the aid of Statistical Package for Social Science version 21.

Results

The result of serum glucose and CRP, zinc and copper levels obtained in this study is presented on the Table below.

The mean serum glucose concentration of diabetic patients (12.44 ± 1.64) was higher ($P<0.05$) compared to the non-diabetic group (4.51 ± 0.30) confirming their hyperglycermic and normoglycermic status respectively. From the result obtained, the mean serum CRP concentration of diabetic patients (11.76 ± 1.56 mg/l) was significantly higher ($P<0.05$) compared to the non-diabetic group (4.302 ± 1.80 mg/l). More so, Serum copper (13.09 ± 4.95) and zinc (0.49 ± 0.35) concentrations of the diabetic group were significantly ($P<0.05$) lower when compared to the non-diabetic group (132.00 ± 9.61 and 0.90 ± 0.004 respectively).

Discussion

Assessment of serum concentration of CRP, zinc and copper in non-diabetic and type 2 diabetic patients was carried out in this study. Serum obtained from blood samples of 20 non-diabetic and 20 diabetic patients was used. Results indicated that the mean concentrations of serum glucose and CRP were higher ($P<0.05$) in the diabetic group compared to the non-diabetic control group.

Findings from this present study is in line with the report of Barbara et al. [18], who observed a positive association between the levels of CRP and incident diabetes mellitus among middle-aged men. The study suggested the role of inflammation in the etiology of diabetes mellitus in their study and similar association between DM and CRP has also been reported recently by [16]. It was determined from their study that elevated plasma CRP level was significantly

associated with the increase in the prevalence of dyslipidemia, diabetes and metabolic syndrome.

The underlying mechanism leading to increased levels of the acute-phase inflammatory protein (CRP) as seen in the present study might be linked to increased visceral fat associated with obese conditions in type 2 DM. This association, according to Barbara et al. [18], might be explained by the fact that adipose tissue produces significant amounts of different inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α) and Interleukin 6 (IL-6), which in turn regulates the production of CRP by the liver. Increase cytokines from the adipose tissue might therefore stimulate increased production of CRP as observed in this study. These cytokines might provide a link between obesity and insulin resistance observed in DM. Although plasma CRP levels closely correlated with mean BMI at a population level across ethnic groups, Jeong et al. [16] noted that obesity does not, however, completely explain the plasma CRP levels.

Increased serum CRP could also be an immune response to tissue damage due to increased oxidative stress which is reportedly associated with diabetes mellitus [18]. Oxidative stress induced vascular damage may trigger the activation of inflammatory cells such as macrophages to produce cytokines which are mediators of inflammatory processes, with the attendant increase in the production of CRP.

Plasma CRP level was positively associated with serum glucose level in this study which agrees with the findings of [16]. Earlier study revealed that plasma CRP level was elevated in participants with diabetes compared with those with normal fasting glucose in the US adult population ford [12]; elevated plasma CRP concentration was positively associated with glycated hemoglobin A1c levels in patients with diabetes; subjects with elevated acute-phase proteins were more likely to develop type 2 diabetes in the insulin resistance atherosclerosis study [19-20]. Hyperglycaemia may contribute to inflammation by inducing endothelial dysfunction and stimulating reactive oxygen species generation [21,22]. Elevated plasma CRP was significantly associated with increased mean fasting insulin concentration according to Gelaye et al. [23]. Plasma CRP induces endothelial insulin resistance and dysfunction through the spleen tyrosine kinase and RhoA-activation signaling pathway [23].

The mean concentration of zinc and copper (micronutrients) were significantly reduced in the diabetic group compared to the non-diabetic control group. This finding is in agreement with studies earlier conducted by Manal et al. [24]. Who reported lower levels of zinc and higher levels of glycated hemoglobin HbA1c in diabetic groups, and also supported by earlier findings [25-27]. Deficiency of trace elements have been noted to possibly be directly or indirectly associated with oxidative stress which proceeds to insulin resistance or diabetes [28]. Zinc is an essential micronutrient for metabolism that regulates more than 100 enzymes for protein folding, gene expression, as well as in the production and neutralization of ROS. Disturbances in zinc homeostasis are reportedly associated with diabetes and insulin resistance [29]. Zinc partially functions as an antioxidant and Zn supplementation results in reduction in reactive oxygen species production, which is beneficial in ageing and diabetes mellitus [30]. Zinc is vital for the appropriate processing, storage, secretion and action of insulin in mammalian pancreatic cells, and its deficiency augments the cytokine-induced damage in the autoimmune attack, resulting in the destruction of the islet cell in T1DM [31]. From the foregoing, it is apparent that diabetes mellitus type 2 may affect zinc homeostasis. The decrease in serum zinc concentration might largely be due to increased urinary loss associated with diabetic nephropathies as reported [32-34]. Decrease in zinc levels in diabetic subjects in these previous studies were attributed to the adverse effects of high glucose concentration on the renal tissues leading to a decrease in the reabsorption of zinc in the kidneys, hence increased urinary loss and decreased total body zinc. In a similar study, the researchers concluded that diabetes disrupts Zinc metabolism [35]. Direct association of minerals, trace elements and vitamins in the pathogenesis and natural course of both type 1 and 2 diabetes mellitus has been observed in many research studies. An alteration in the metabolism of these minerals has been demonstrated. Diabetes mellitus is regarded as a heterogeneous disease associated with an absolute or relative deficiency of minerals. It is generally accepted that disturbed concentration of Zinc (Zn) and Magnesium (Mg) in the body are often found in patients of diabetes mellitus Thiyam et al. [33], and this study further demonstrates that assertion.

Copper (Cu) is also an important trace element involved in oxidative stress, which is associated with the onset and progression of Diabetes Mellitus (DM). However, clinical studies comparing plasma or serum Cu levels in patients with DM and in healthy individuals report conflicting findings. A study by Thiyam et al. [33] and Priyanka et al. [36], showed increased level of serum copper in diabetics. Studies involving a total of 1079 DM patients and 561 healthy controls showed higher Cu levels than the healthy controls [37]. In this present study, results showed a decrease in copper concentration in diabetic patients compared to non-diabetic group. Copper in its free form is a potent cytotoxic element because of its redox chemistry as it causes redox imbalance due to it's highly redox active nature to generate ROS [38]. Ceruloplasmin and serum albumin are the main Cu binding proteins in plasma and there is some evidence that chronic hyperglycemia can damage the Cu binding properties of both transport proteins, resulting possibly in urinary loss of copper coupled with nephropathies associated with this condition. This might contribute to the decrease in copper concentrations observed in this study.

Conclusion

Assessment of serum concentration of CRP, zinc and copper in non-diabetic and type 2 diabetic patients was investigated. Type 2 diabetic mellitus patients had higher levels of serum glucose and C - reactive protein ($P<0.05$) compared to the non-diabetics while the concentration of both zinc and copper were reduced in type 2 diabetic patients as compared to the non-diabetic group.

In conclusion, this study suggests the role of insulin resistance and hyperglycemic states in inflammatory process in type 2 diabetes mellitus which is also associated with micronutrient deficiencies that may be linked to urinary loss due to nephropathies associated with this condition.

References

1. Suganya K, Minji K, Moon-Kyung S, Mi Kyung K. Association of C-reactive protein with risk of developing type 2 diabetes mellitus, and role of obesity and hypertension: A large population-based Korean cohort study. Sci Rep. 2019;9:4573.

2. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus: Present and future perspectives. Nat Rev Endocrinol. 2012;8(4):228-36.

3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;33(Suppl 1):S62-69.

4. Mahler RJ, Adler ML. Clinical review 102: Type 2 diabetes mellitus: Update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab. 1999;84(4):1165-71.
5. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013;93(1):137-88.
6. World Health Organization. Global report on diabetes. 2016:1–84.
7. World Health Organization. Expert Committee on definition diagnosis and classification of diabetes mellitus and its complications, Geneva: 1999:1-59.
8. Freeman DJ, Norrie J, Caslake MJ. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland coronary prevention study. Diabetes. 2002;51(5):1596–600.
9. Nakanishi S, Okubo M, Yamane K, Kohno M, Kamei N. Elevated C-reactive protein is a risk factor for the development of type 2 diabetes in Japanese Americans. Diabetes Care. 2003;26:2754–7.
10. Torand B. Sex differences in the prediction of type 2 diabetes by inflammatory markers: Results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Diabetes Care. 2007;30(4):854-60.
11. Temelkova-Kurktschiev T, Henkel E, Koehler C, Karrei K, Hanefeld M. Subclinical inflammation in newly detected type II diabetes and impaired glucose tolerance. Diabetologia. 2002;45(1):151.
12. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care. 1999;22(12):1971–7.
13. Yasufumi D, Yutaka K, Michiaki K, Yumihiro T, Ken O, Toshiharu N, et al. Relationship between C-reactive protein and glucose levels in community dwelling subjects without diabetes. Diabetes Care. 2005;28 (5):1211-12.
14. Kind CRH, Pepys MB. The role of serum C- Reactive Protein (CRP) measurement in clinical practice. Int Med. 1984;5:112-51.
15. Pearson TA, Mensah GA, Hong Y, Smith SC. CDC/AHA workshop on markers of inflammation and cardiovascular disease: Application to clinical and public health practice: Overview. Circulation. 2004;110(25):e543–e544.
16. Jeong H, Sun-Young B, Seon WK, Eun-Jung P, Jaejoon L, Hyungjin K, et al. C reactive protein level as a marker for dyslipidaemia, diabetes and metabolic syndrome: Results from the Korea National Health and Nutrition Examination Survey. BMJ Open. 2019;9(8):e029861;1-11.
17. Alexis Bahi G, Boyvin L, Méité S, Melaine M'Boh G, Yeo K, N'Guessan KR, et al. Assessments of serum copper and zinc concentration, and the Cu/Zn ratio determination in patients with Multidrug Resistant pulmonary Tuberculosis (MDR-TB) in Côte d'Ivoire. BMC Infectious Diseases. 2017;17(1): 257.
18. Barbara T, Hanneclore L, Andrea S, Hubert K, Christa M, Margit F. C reactive protein as a predictor for incident diabetes mellitus among middle-aged men; results from the MONICA Augsburg cohort study 1984-1998. Arch Intern Med. 2003;163(1):93-9.
19. King DE, Mainous AG, Buchanan TA. C- reactive protein and glycemic control in adults with diabetes. Diabetes Care. 2003;26(5):1535–9.
20. Festa A, D'Agostino R Jr, Tracy RP. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: The insulin resistance atherosclerosis study. Diabetes. 2002;51(4):1131–7.
21. Mohanty P, Hamouda W, Garg R. Glucose challenge stimulates Reactive Oxygen Species (ROS) generation by leucocytes. Clin Endocrinol Metab. 2000;85(8):2970–3.
22. Hoffman RP. Hyperglycemic endothelial dysfunction: Does it happen and does it matter? J Thorac Dis. 2015;7(10):1693–5.
23. Gelaye B, Revilla L, Lopez T. Association between insulin resistance and C-reactive protein among Peruvian adults. Diabetol Metab Syndr. 2010;2(1):30.
24. Manal Kamal MS, Naglaa K, Khadega A. Evaluation of trace elements and malondialdehyde levels in type II diabetes mellitus. Diabetes Metab Syndr Clin Res Rev. 2009;3(4):214–8.
25. Maher M, Ahmed SRH. A Study of serum magnesium, zinc, copper and glycohemoglobin in children with Type 1 diabetes mellitus. Alex J Pediatr. 2002;16(2):285–9.
26. AbouSeif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. Clin Chim Acta. 2004;346(2):161–70.
27. Aly HF, Mantawy MM. Comparative effects of zinc, selenium and vitamin E or their combination on carbohydrate metabolizing enzymes and oxidative stress in streptozotocin induced-diabetic rats. Eur Rev Med Pharmacol Sci. 2012;16(1):66-78.
28. Dubey P, Thakur V, Chattopadhyay M. Role of minerals and trace elements in diabetes and insulin resistance. Nutrients. 2020;12(6):1864.
29. Foster M, Samman S. Zinc and regulation of inflammatory cytokines: Implications for cardio-metabolic disease. Nutrients. 2012;4(7):676-94.
30. Kloubert V, Rink L. Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct. 2015;6(10):3195-204.
31. Li YV. Zinc and insulin in pancreatic beta-cells. Endocrine. 2014;45(2):178–89.
32. Meenakshi PUG, Nayyar SB. Comparative study of serum zinc, magnesium and copper levels among patients of type 2 diabetes mellitus with and without microangiopathic complications. Innovative J Med Health Sci. 2013;3:274–8.
33. Romola Devi T, Hijam D, Dubey A, Debnath S, Oinam P, Taruni Devi NG, et al. Study of serum zinc and copper levels in type 2 diabetes mellitus. Int J Contemporary Med Res. 2016;3(4):50.43.
34. Bandeira VDS, Pires LV, Hashimoto LL, De Alencar LL, Almondes KGS, Lottenberg SA, et al. Association of reduced zinc status with poor glycemic control in individuals with type 2 diabetes mellitus. J Trace Elem Med Biol. 2017;44:132-136.
35. Anil Kumar VSPD, Jaiprabhu J, Krishnan R. Serum copper and zinc levels significance in type 2 diabetic patients. J Trace Elem Med Biol. 2017;43:46-51.
36. Priyanka Mor P, Anjana Kanwar Ra, Vanita S, Neha S, Ashish S. A study of serum copper, zinc and magnesium in Type 2 diabetes mellitus with complications and without complications. Biomed Pharm J. 2020;13(4):1927-30.
37. Qiu Q, Zhang F, Zhu W, Wu J, Liang M. Copper in diabetes mellitus: A meta-analysis and systematic review of plasma and serum studies. Biol Trace Elem Res. 2017;177(1):53-63.
38. Sarkar A, Dash S, Barik BK, Muttigi MS, Kedage V, Shetty JK, et al. Copper and ceruloplasmin levels in relation to total thiols and GST in type 2 diabetes mellitus patients. Indian J Clin Biochem. 2010;25(1):74-6.