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Diabetes Subjectsan Assessment of Interleukin 18, Copper, Lead and Chromium in Type Ii Diabetes Subjects

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Abstract

Aim: It has been observed that patients with type 2 diabetes mellitus have impaired balance between pro inflammatory and anti infalammatory markers as well as an imbalance between toxic and essential trace metals. The purpose of this research therefore is to assess the levels lead, chromium and serum interleukin- 18 levels in type 2 diabetics.

Methods: Thus, a total of 110 patients were investigated — 40 were either newly diagnosed with type 2 diabetes mellitus or had been previously diagnosed but had stopped treatment for at least 10weeks and currently not on drugs, 40 with type 2 diabetes mellitus and on treatment for at least six weeks, and 30 control subjects with normal plasma glucose level. Plasma glucose level, blood lead level, chromium and copper levels and serum interleukin 18 levels were estimated in all groups.

Results: Plasma glucose and serum interleukin 18 levels was significantly high in treated diabetics than in control ($P<0.05$), blood chromium was significantly higher in control than in treated diabetics ($P<0.05$), BMI and blood lead levels were insignificantly higher in treated diabetics than in control ($P<0.05$). In untreated diabetics, plasma glucose, blood lead, serum interleukin-18 and BMI were significantly higher while blood chromium and copper was significantly lower than control ($P<0.05$). When treated and untreated diabetics were compared, plasma glucose, blood lead and copper, serum interleukin 18 and BMI was significantly higher in the untreated group while blood chromium level was significantly higher in treated diabetics ($P<0.05$).

Conclusion: It appears that serum interleukin 18- a pro inflammatory cytokine is higher in diabetes subjects and can be significantly lowered through the intake of antidiabetic drugs, essential metal supplementation and lifestyle modifications over a period of time . This is because , in this research , serum

interleukin 18, Lead and Copper levels nosedived with a reduction in plasma glucose levels. This area of study requires further investigation with other antidiabetic drugs. This, on the long run might be economically helpful in designing better and economic treatment protocols with a possibility of better clinical outcomes.

Keywords: Diabetes mellitus, Essential metals, Toxic metals, Interleukin 18

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases that is characterized by hyperglycaemia resulting either from a deficiency in insulin secretion or impaired action of insulin or even both. Its classical charascteristic is a high blood sugar level over a prolonged period of time with symptoms which includes frequent urination, increased thirst and increased hunger. If left untreated for a long period of time, diabetes can lead to severe complications [1]. When there is chronic hyperglycaemia, there happens to be a long-term damage, dysfunction and failure in various organs such as the kidneys, nerves, heart and blood vessels [2]. Some acute complications can include diabetic ketoacidosis, non ketotic hyperosmolar coma, or death while some serious long term complications can include heart disease, stroke, chronic kidney failure, foot ulcers and damage to the eyes [3]. Diabetes Mellitus could be idiopathic or secondary. Idiopathic diabetes i.e that to which no cause can be adduced is categorised into different types which includes: Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus, Maturity onset diabetes mellitus and Neonatal diabetes mellitus [4].

Interleukin- 18 (IL-18) is also known as interferon gamma inducing factor is a protein present in humans which is encoded with the interleukin-18 gene, and this gene is known as proinflammatory cytokine [5]. Interleukin-18 is described as a member of the superfamily of interleukin-1. It is known to regulate both the innate and acquired immune response. Interleukin-18 shows itself at sites having chronic inflammation, cancers, varieties of autoimmune diseases and some infections [6]. Interleukin-18 was cloned from the murine liver cells cDNA which was gotten from animals primed with heat killed Propionibacterium [6]. Interleukin-18 matures via capase-1 and this is regulated by a large amount of protein complex which is called inflammasome [7]. Lead is an established toxic substance [8]. Lead is known to promote oxidative stress and it induces renal dysfunction[9] . Its inclusion in this research is predicated on its ability to substitute for essential metals in antioxidant enzymes, hence aggravating a diabetic condition. Chromium (Cr) is an essential micronutrient which is required for the normal functioning of insulin and regulation of blood sugar levels. It acts as a vital antioxidant for maintaining insulin homeostasis. In diabetes mellitus, the free radical production is increased and levels of antioxidants like chromium, vanadium, selenium and manganese are reduced [10]. In a hypoglycaemic condition, the insulin receptor kinase is activated by chromium [11]. It increases insulin sensitivity, glucose utilization and beta cells sensitivity because of the role it plays in insulin activity[11].

2. Materials and Methods

Study Design: The study design is a cross sectional study which involved 100 subjects, assessing serum interleukin-18, blood lead chromium and copper and type 2 diabetes subjects. It involved the use of a stratified random sampling method, stratification would be by age, gender and therapy

Inclusion Criteria/ Exclusion Criteria: Men and women who are confirmed to be type 2 diabetes mellitus were included in this study. Pregnant or breastfeeding mothers, Adolescents with respiratory, cardiovascular, rheumatic, musculoskeletal and orthopaedic diseases were excluded. In addition, subjects who had renal diseases, hepatic disorders, those who use alcohol, those on nonselective β blockers and presence of malignancy were excluded. Those patients who could not avoid taking hypoglycaemic drugs or insulin sensitivity altering drugs for 12 hours before blood sampling were also barred from participating in the study.

Sample Collection: Blood samples were collected from brachial vein after an overnight fast for the measurement of serum interleukin-18, plasma glucose, and blood lead, copper and chromium levels. About 6ml of blood was collected, 2ml was collected in fluoride oxalate bottle, mixed well centrifuged and the plasma separated. Another 2ml was collected in a plain bottle allowed to clot, centrifuged, separated and the serum freezed at -20 ° C, used for serum interleukin 18 estimation. The other 2ml was collected in lithium heparin bottle and it would be used for the estimation of blood lead, copper and chromium levels.

Method of Determination of Parameters: Glucose was estimated using the glucose oxidase-Peroxidase method.

Interleukin 18 was estimated using ELISA based Genway Biotech patented kits while the determination of blood lead, copper and chromium levels was done by using Atomic absorption spectrophotometry (AAS) as explained by Varma [13]

Statistical analysis

Results obtained were subjected to statistical analysis using Statistical Package for Social Sciences version 23 (SPSS 23). All parameters were expressed as mean ± SD. Values were statistically significant at $p< 0.05$. Results were also illustrated with the aid of tables and charts wherever they are necessary.

3. Results

Table 1, Chart 1: Plasma glucose, serum Interleukin-18 and copper in diabetes on treatment was significantly higher than control (P=0.04, P<0.001 and P<0.001 respectively), also blood lead level and BMI for diabetes on treatment was insignificantly higher than that of control. Chromium was significantly lower(P=0.01) in treated diabetics relative to control

Table 4.2, Fig. 2: Plasma glucose(P< 0.001), blood lead level(P=0.024), interleukin-18 (P<0.001)and BMI(P<0.013) for diabetes not on treatment was significantly higher than control, however blood chromium level for control was significantly lower than diabetes not on treatment(P<0.001).

Table 4.3, Fig. 1 and 2: Plasma glucose(P=0.04), and copper levels(P<0.001), interleukin-18(P< 0.001) and BMI(P=0.011) for diabetes not on treatment was significantly higher than that seen in diabetics on treatment (p<0.05) while blood chromium (P=0.01) level for diabetics on treatment was significantly higher than diabetics not on treatment.

Table 1: Mean, Standard Deviation (S.D) and p.value when all estimated parameters in diabetics on treatment were compared with control

Variables	DM on treatment	Control	P value
Plasma glucose(mmol/l)	4.405±0.717	4.065±0.403	0.04
Blood lead(µmol/L)	0.854±0.267	0.839±0.254	0.835
Blood copper (µmol/L	19.50±6.59	16.56±4.41	<0.001
Blood chromium (µmol/L)	0.103±0709	0.174±0.099	0.01
BMI (kg/m2)	25.380±4.030	23.170±2.784	0.068
Interleukin 18 (pg/ml)	205.885±101.029	28.91±11.193	<0.001

Table 2: Mean, Standard Deviation (S.D) and p.value when all parameters in diabetics on treatment was compared with that of diabetics not on treatment

Variables	DM not on treatment	Control	p.value
Plasma glucose(mmol/l)	10.385±2.762	4.065±0.403	<0.001
Blood lead(µmol/L)	1.428±1.027	0.839±0.254	0.024
Blood copper (µmol/L	24.91±5.72	16.56±4.41	<0.001
Blood chromium (µmol/L)	0.100±0.107	0.174±0.099	<0.001
BMI (kg/m2)	27.280±5.795	23.170±2.784	0.013
Interleukin 18 (pg/ml)	420.560±161.503	28.91±11.193	<0.001

Table 3: Mean, Standard Deviation (S.D) and p.value when all parameters in diabetics on treatment was compared with that of diabetics not on treatment

Variables	DM on treatment	DM not on treatment	P value
Plasma glucose(mmol/l)	4.405±0.717	10.385±2.762	0.04
Blood lead(µmol/L)	0.854±0.267	1.428±1.027	0.835
Blood copper (µmol/L	19.50±6.59	24.91±5.72	<0.001
Blood chromium (µmol/L)	0.103±0709	0.100±0.107	0.01
BMI (kg/m2)	25.380±4.030	27.280±5.795	0.011
Interleukin 18 (pg/ml)	205.885±101.029	420.560±161.503	<0.001

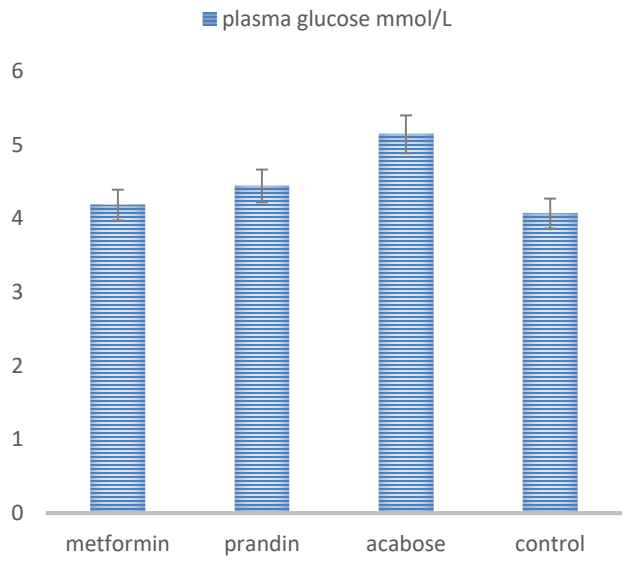

Figure 1: Plasma glucose level in treated diabetics in response to antidiabetes drugs

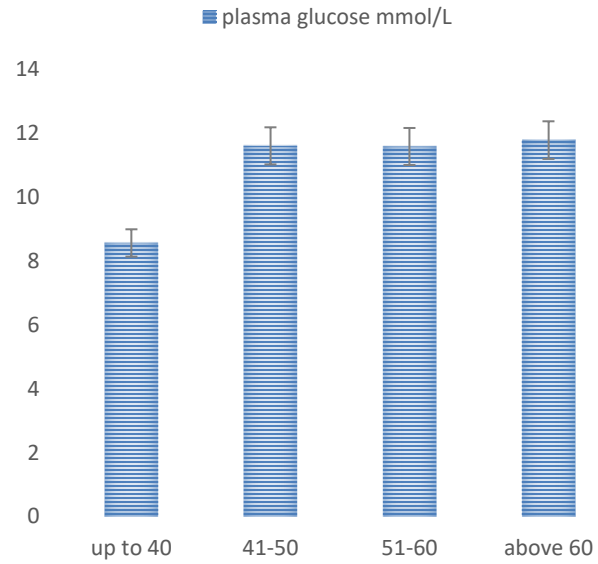

Figure 2: plasma glucose levels in different age groups

4. Discussion, Conclusion and Recommendation

4.1 Discussion

Diabetes mellitus (DM) is a chronic endocrine disorder, characterized by hyperglycaemia resulting from absolute or relative deficiency in insulin quantity or action [14]. DM has a varied aetiology but by far the majority of cases are classified as either type 1 or type 2 diabetes[14] type 1 being an immune-mediated disorder that involves the depletion of beta-cells which results in lifelong dependence on exogenous insulin [15] while type 2 is a condition in which the body does not use insulin properly [16]. As DM and its complications have been described to have or be a result of, both toxicological and immunological factors [17] and undertones, this research was therefore primarily set to assess the levels of serum interleukin 18, blood lead, copper and chromium in both treated and untreated diabetes patients relative to apparently healthy individuals with normal glucose homeostasis. It was also designed to assess the effect of treatment or otherwise on all estimated parameters.

Glucose is a monosaccharide with the molecular formula of C6H12O6 and is the major metabolic fuel in living cells [18]. It is distributed to parts of the body by the blood in every animal as it is known to be the fuel for many tissues [18]. In this research, plasma glucose levels in both treated (P= 0.040) and untreated diabetics (P= 0.000) was found to be significantly higher than in control. This goes further to support the notion that a sustained higher-than-normal blood glucose is a characteristic of diabetes mellitus. This agrees with the works of Blessing et al. [19]; Toma et al. [20], Yassa and Tohamy [21] where glucose levels was found to be significantly higher in diabetes patients when compared to control. Furthermore, a significant increase in plasma glucose was observed in untreated diabetics when compared with treated diabetics. The reason need not be farfetched; the rationale being that treatment was up to the task in effecting a significant reduction in blood glucose in diabetics. This agrees with the work of Sunali et al., [22] where both nutrition and chemotherapy was found to effect a significant decrease in blood glucose. Three antidiabetic drugs were included in this study. They are metformin, Prandin and acabose. Treatment was found to be most effective in treated diabetics on metformin while acabose was found to be the least effective in bringing the blood glucose level nearer to that seen in control subjects. As glucose is the classical analyte in the assessment of the severity of diabetes in this study, DM was most severe in the 61 and above age group, followed by 41-60 group while DM is least severe at below 40 years of age. This agrees with the works of Otsuki et al. [23] and Silink, [24] where diabetes severity progresses with age. In short, the pattern of severity of type 2 DM in this research is consistent with advancement in age.

Body Mass Index (BMI) is the quantification of body fat thereby categorising if an individual is underweight, normal weight, overweight or obese depending on the value gotten [25]. In this research, there was an insignificant increase in BMI when treated diabetics (p=0.068) was compared with control but a significantly higher level was observed when untreated diabetics (p=0.013) was compared with control. Lastly, when treated diabetics were compared with untreated diabetics, BMI was significantly higher. This finding agrees with the works of Bakari et al., [26] Denko and Malemud [27] and Colditz et al. [28] where diabetes was associated with increased BMI supporting the widely accepted notion that obesity is a risk factor for diabetes.

Lead (Pb) is an established toxic and carcinogenic metal[8]. It has also been proved that lead can be toxic at levels well below the current safety standards if the exposure is chronic [29]. In this research, there was an insignificant increase in blood lead level when treated diabetics were compared with control (p=0.835) but a significantly higher level was observed in untreated diabetics (p=0.024) when compared with control. Lastly, when treated diabetics were compared with untreated diabetics, blood lead level was significantly high (p<0.001). This findings agree with the works of Tyrrell et al.[30] and Agarwal et al.[31] where diabetes was associated with increased Pb levels. Other studies of the effects of Lead in humans which the investigators in this research are thinking could aggravate diabetes have focused on its association with increased oxidative stress [32], loss of endothelial function and promotion of inflammation [33] and down regulation of nitric oxide production [34]. As regards the deleterious effects of heavy metals i.e lead on the control of diabetes; metalloenzymes, upon contamination with any heavy metal, are likely to

replace essential metals as the metal constituents of enzymes [35]. When this happens the enzyme activity diminishes, summarily leading to a poorer glucose tolerance as an after effect of inflammation, oxidative stress and the down regulation of nitric oxide. There seems to be a vicious cycle where diabetics easily accumulate heavy metals which in turn will likely worsen the glucose intolerance.

In this research, there was a significant decrease when chromium levels in treated (p= 0.010) and untreated diabetics (p=0.000) were compared with control. The rationale for this finding need not be probed further as Chromium has been known to be involved in glucose and lipid metabolism and reduction in its blood level can cause impaired glucose tolerance and very rarely, chromium supplements may be needed in patients on prolonged parenteral nutrition [36]. This finding agrees with the works of Hellerstein [37]; Cefalu and Hu [38]; Volpe et al., [39] where blood chromium levels was said to be higher in treated diabetics than non-treated patients. There was also an insignificant increase when untreated diabetics (p=0.864) were compared with treated subjects. The reason could have been due to the fact that perhaps chromium supplementation may not have been part of the treatment plan or there might have been low levels of chromium in the environment under study.

Copper is an essential trace element that has both pro and antioxidant capabilities. Therefore its properties may be linked with the onset and development of atherosclerosis because of its known catalytic function in lipid peroxidation [40]. Because Copper is a cofactor for Cu-Zn SOD and ceruloplasmin, one an important antioxidant while the other a pro oxidant enzyme, some of its possible pro or antioxidant activity may be accounted for, at least in part, by its role in these enzymes [41]. In this research the blood Cu levels was found to be significantly higher when both treated and untreated diabetics were compared with the control. A possible implication of this is that copper containing enzymes pro oxidant activity gained the upper hand over its antioxidant counterparts hence a likely increase in reactive oxygen species, or possibly because ceruloplasmin, a major copper binding protein increases in concentration in plasma due to it being an acute phase protein. This finding agrees with the research of Ferdous and Mia [42] where blood copper levels was significantly higher in diabetes subjects compared to control. Furthermore there was a significant increase in blood copper levels when the untreated were compared with diabetics.

Interleukin 18 is described as a member of interleukin 1 cytokine superfamily, it regulates innate and acquired immune response [6] In a few words interleukin 18 is a common participant in the inflammation cascade and has been known to be a pro inflammatory interleukin. Serum interleukin 18 was significantly higher in treated diabetics (p=0.000) and untreated diabetics (p=0.000) when compared with control. Furthermore, there was also a significant increase in IL-18 in untreated diabetics (p=0.000) when compared with treated diabetics. This agrees with the works of Yudkin [43] and Esposito et al.,[44] where IL-18 and other pro inflammatory cytokines are increased in subjects with impaired glucose tolerance, giving a possible indication that diabetic hyperglycaemia and the immune activation are possibly positively correlated.

4.2 Conclusion

This research discovered that the efficacy of treatment on diabetes is incontrovertible. It seems there is a viscious cycle where the diabetes condition favours heavy metal accumulation via an unknown mechanism and, the activation of the inflammation cascades, which in turn brings about an aggravated diabetes condition. It is also confirmed that treatment could alleviate the inflammatory manifestations of diabetes.

Recommendation

Since metformin is the most effective drug in bringing tolerance under check in this research, it should be recommended for diabetics in this environment. This can be combined with chromium supplementation and regular exercising for obese patients. Further evaluation of drug efficacy on diabetes control should be done with new line of drugs. BMI and toxic metal levels should also be monitored. We also insinuate that control of inflammation, heavy metal chelation and essential trace metal supplementation could also be beneficial.

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