Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5, 2301-2309
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i5.1984

Evaluation the serum levels of some non-enzymatic antioxidant factors among diabetic patients with and without nephropathy

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Abstract: Hyperglycemia, an elevated concentration of blood glucose, is the characterization of diabetes mellitus caused by insufficiencies in insulin synthesis or insulin action. The global health threat posed by the diabetes mellitus epidemic and its sequelae is significant According to the International Diabetes Federation (IDF), 415 million persons (ranging from 20 to 79 years old) were diagnosed with diabetes in 2015. mellitus worldwide, or 1 in 11 adults. Objective was to examine the changes in blood levels of glutathione, melatonin, and homocysteine in patients with nephropathy from diabetes, those without nephropathy from diabetes, add to controls. One hundred thirty-five participants were split into three categories for the study, The initial cohort consists of forty-five individuals diagnosed with diabetes nephropathy. 45 individuals with diabetes who do not have nephropathy make up the second group. From December 2023 until the end of March 2024, 45 healthy individuals served as controls in the third group. A venous blood sample of five milliliters was obtained from the case and control groups. Centrifugation was used for 10 minutes at 2000 rpm to separate the serum from the blood samples, which were transported to the laboratory under standard circumstances. Serum results were frozen at -80°C before glutathione, homocysteine, and melatonin were evaluated using colorimetric and enzyme-linked immunosorbent assays, respectively. The present study enrolled 135 participants (45 diabetic patients with diabetes nephropathy 45 healthy controls and 45 diabetic patients without nephropathy (participated in this study. According to the current investigation, the mean serum homocysteine levels of the healthy control group were higher than those of the type II diabetic (T2DM) patients with nephropathy. Also show the mean levels of serum Glutathione levels were significantly lower in T2DM with nephropathy compare to healthy control and T2DM without nephropathy. Our results demonstrated that decreased serum levels of melatonin and glutathione levels are associated with nephropathy in T2DM.

Keywords: *Diabetes mellitus, Glutathione, Homocysteine, Melatonin, Nephropathy,*

1. Introduction

Hyperglycemia, an elevated concentration of blood glucose, is the characterization of diabetes mellitus caused by insufficiencies in insulin synthesis or insulin action [1]. Pancreatic beta cells produce the protein (hormone) insulin in response to a number of stimuli, including glucose, sulfonylureas, and arginine, but glucose is the primary determinant [2]. In addition to storing glucose, insulin also lowers serum fatty acid levels, inhibits the release of glucagon, and decreases the amount of glucose produced by the liver. Reduced glucose uptake by tissues as a result of insufficient insulin or insulin resistance in the body causes intracellular hypoglycemia and extracellular hyperglycemia [3]. The number of people with diabetes mellitus has doubled over the last thirty years, and it is currently the tenth most common cause of death [4]. When hyperglycemia results in end-stage renal failure, a kind of chronic kidney disease called diabetic nephropathy (DN) appears. DN is categorized as diabetic microangiopathy; however, tubules, glomeruli, and tubular stroma are all affected by the pathological alterations [5]. Apoptosis and differentiation in the development of DN can be impacted by excessive glucose damage.

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Different kinds of kidney cells are affected, and ultimately, it results in pathological damage that is characteristic, including renal tubular atrophy, renal glomerulosclerosis, and renal interstitial fibrosis [6]. The body now uses a variety of processes to manufacture antioxidants in order to counteract elevated levels of free radicals, shield cells from their damaging effects, and prevent the negative impacts of these free radicals. It's crucial to strike a balance between the rate at which free radicals are formed and eliminated. Overproduction of cellular radicals can be detrimental. Nonetheless, A cell experiences oxidative stress when its radical synthesis increases significantly while its radical removal decreases noticeably [7]. Strong experimental and clinical evidence implicates oxidative stress as a critical component in the aetiology of diabetes, and both types of the disease are characterized by increased production of reactive oxygen species (ROS) [8], that caused by many processes including the reaction of lowering glutathione levels and increase ROS even in other conditions associated with increase Cu levels [9]. The exact function that oxidative stress may have in the development of diabetes complications is not yet known [10]. Many mechanisms, such as excessive production of oxygen radicals by autoxidation of glucose, glycated proteins, and saccharification of antioxidant enzymes that limit the ability to detoxify oxygen radicals, contribute to the etiology of oxidative stress in diabetes [11]. An amino acid contains sulfur, homocysteine created from methionine demethylated have the possibility to increase oxidative stress. Methionine and cystathionin are produced through the methylation and trans-sulfuration of homocysteine, respectively, which folic acid and B12 are needed for the conversion of homocysteine to methionine [12,13]. Antioxidants can reduce the production of ROS by either upregulating the expression or activity of the enzymes that generate ROS (xanthine oxidase and NADPH oxidase) or by upregulating the expression and activity of the enzymes that function as antioxidants (catalase, glutathione peroxidase, and superoxide dismutase) that body uses these enzymes to protect itself from free radical damage by give or take electrons from them [14]. Certain antioxidants can occasionally produce new free radicals that are less harmful to the body [15]. Antioxidants halt the damage that radicals do by breaking the chains of events that free radicals start [16]. Enzymes such as catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase neutralise ROS [17]. An objective of the study was to examine the variation in of glutathione, melatonin, and homocysteine blood levels in patients with who have diabetes-related nephropathy and those who do not, in addition to controls.

2. Materials and Methods

2.1. The Study Designs

One hundred thirty-five participants were split into three groups for this case-control research. Fourty-five patients with DN are the first group. Fourty-five individuals with diabetes who do not have nephropathy are the second group. Fourty-five individuals serving as healthy controls are the third group. Each group was selected from a pool of patients chosen by the treating physician, and appropriate testing was conducted. The collection of blood samples was done using gel tubes and sodium citrate tubes, centrifuged, and frozen at −20°C. All patients gave their informed consent to participate in the trial. The selection of samples based on the clinical diagnosis of DN or undiabetic nephrology from the period between December 2023 to March 2024. All patients were over 18 years old. The exclusion criteria included all individuals with a history of heart diseases, chronic inflammatory disorders like rheumatological diseases, and liver failure. Pregnant and lactating women were also excluded.

3. Methods

The equipment utilized in this investigation is Roche Cobas e411 (Roche Diagnostics, Mannheim, Germany) instrument in a number of laboratory tests, including total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL), high density lipoproteins (HDL), and very low-density lipoproteins (VLDL) as a lipid profile. In addition to renal function urea and creatinine. Glutathione, homocysteine and melatonin were measured by sandwich ELISA method. Glutathione (Cat. No.: MBS265674; MyBioSource, USA), homocysteine (Cat. No.: CSB-E13814h; Biocompare, USA),

Edelweiss Applied Science and Technology
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Melatonin (Cat. No.: E-EL-H2016; Elabscience, USA), which uses a purified antibody to capture antigen, increasing the sensitivity and specificity.

3.1. Statistical analysis

The analysis of data done using the Statistical Package for Social Sciences version 26.0 (SPSS v26). Mean±standard deviation (SD) used to display the results. ANOVA test were utilized to compare the data. To determine the accuracy of adipocytokines between patients and controls, Significant value set at $P < 0.05$.

4. Results

The demographic details of the control participants and patients are shown in table 1. According to age, the mean age of diabetic patients with nephropathy was 63.06± 9.91 years old, 56.2± 8.93 years old for diabetic patients without nephropathy, and that of control subjects was 50.93± 6.83 years old additionally, several groups differed significantly (P = 0.011). Regarding to gender, in overall, 50 (37.0%) male and 85 (63.0%) female were included. Diabetic patients with nephropathy included 17 (37.8%) cases were male gender and 28 (62.2%) patients with diabetes who were female and did not have nephropathy There were 33 (73.3%) female cases and 12 (26.7%) male cases as control individuals. Gender did not play a role in the frequency distribution of either the patients or the control group (P = 0.509), with 21 cases (46.7%) being male and 24 cases (53.3%) being female. Serum levels of HbA1C, FBS, Creatinine, and Urea varied significantly between the three groups.

Table 1.
Characteristics of patients with Diabetes mellitus and healthy control.

Characteristic	DM without nephropathy (n=45)	DM with nephropathy (n=45)	Healthy control (n=45)	*P*
Age (Years)	56.2± 8.93	63.06± 9.91	50.93± 6.83	0.011
Sex				
Male	12 (26.7%)	17 (37.8%)	21(46.7%)	0.509
Female	33 (73.3%)	28 (62.2%)	24 (53.3%)	
Urea (mg/dl)	36.55± 5.23^{A}	110.04± 51.96^{B}	33.80± 5.31^{A}	> 0.001
Creatinine (mg/dl)	1.03± 0.18^{A}	2.79± 1.05^{B}	1.028± 0.14^{A}	> 0.001
FBS (mg/ dl)	150.53± 21.58^{A}	165.80± 30.79^{A}	88. 88± 9.42^{B}	> 0.001
HbA1c%	7.08± 0.64^{A}	7.54± 0.86^{A}	5.34± 0.32^{B}	> 0.001
Homocysteine nmol/mL	11.19± 1.27^{A}	10.55± 1.01^{A}	12.16± 1.29^{B}	0.003
Glutathione mmol/L	0.39 ± 0.07^{A}	0.34± 0.054^{B}	0.40± 0.046^{A}	> 0.001
Melatonin ng/L	187.92± 35.5^{A}	184.22± 60.21^{A}	196.67± 38.54^{B}	> 0.001

Serum Homocysteine levels in DM patients with Nephropathy and DM patients without nephropathy were significantly lower than healthy control subjects (10.55± 1.01 mcmol/L, 11.19± 1.27 mcmol/L and 12.16± 1.29 mcmol/L, respectively, P= 0.003), figure 1.

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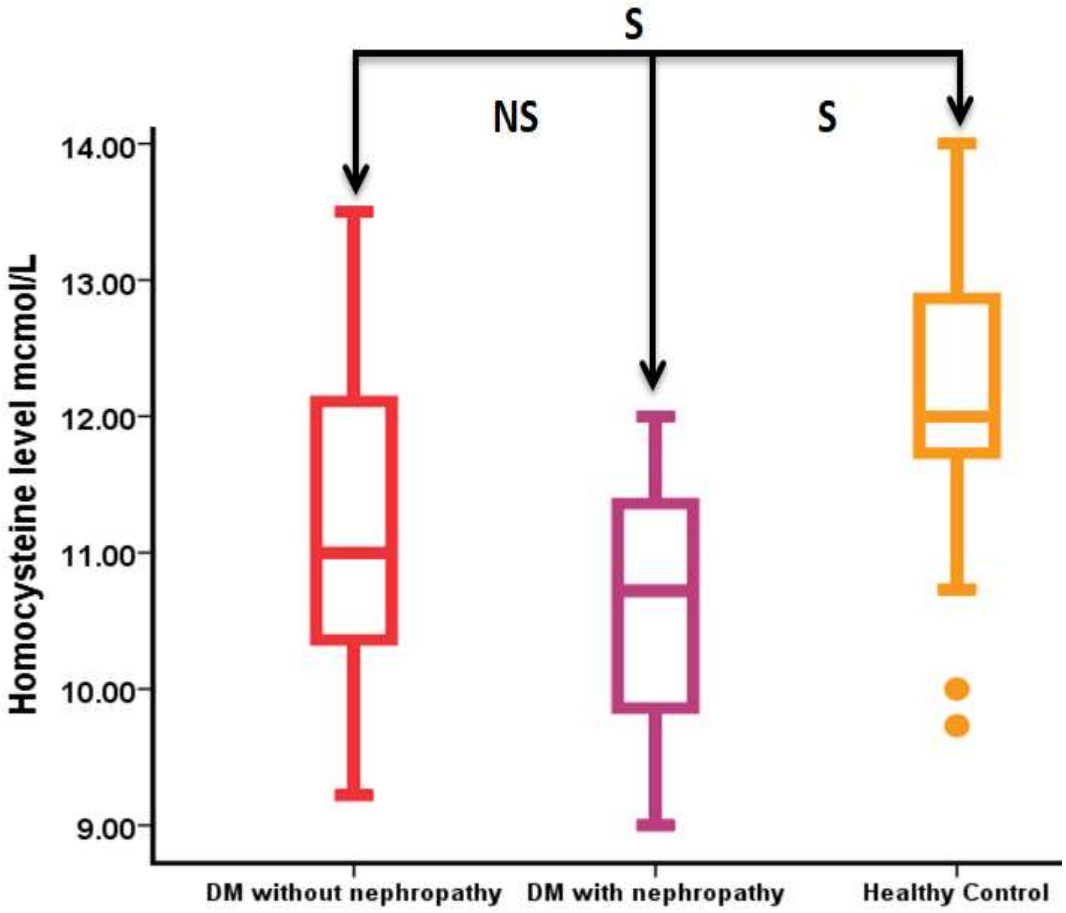

Figure 1.
Serum Homocysteine level in DM patients with Nephropathy, DM patients without nephropathy and healthy control subjects. NS: Not statistically significant, S: statistically significant $P < 0.05$.

Serum Glutathione concentrations in DM patients have Nephropathy were significantly lower than in DM patients without nephropathy, or healthy control subjects (0.34± 0.054 mmol/L, 0.39 ± 0.07 mmol/L and 0.40± 0.046 mmol/L, respectively, P= 0.003). Furthermore, compared to healthy control persons, diabetic patients with and without nephropathy had substantially reduced serum melatonin levels (184.22± 60.21 ng/L, 187.92± 35.5 ng/L and 196.67± 38.54 ng/L, respectively, P>0.001), Figure 2.

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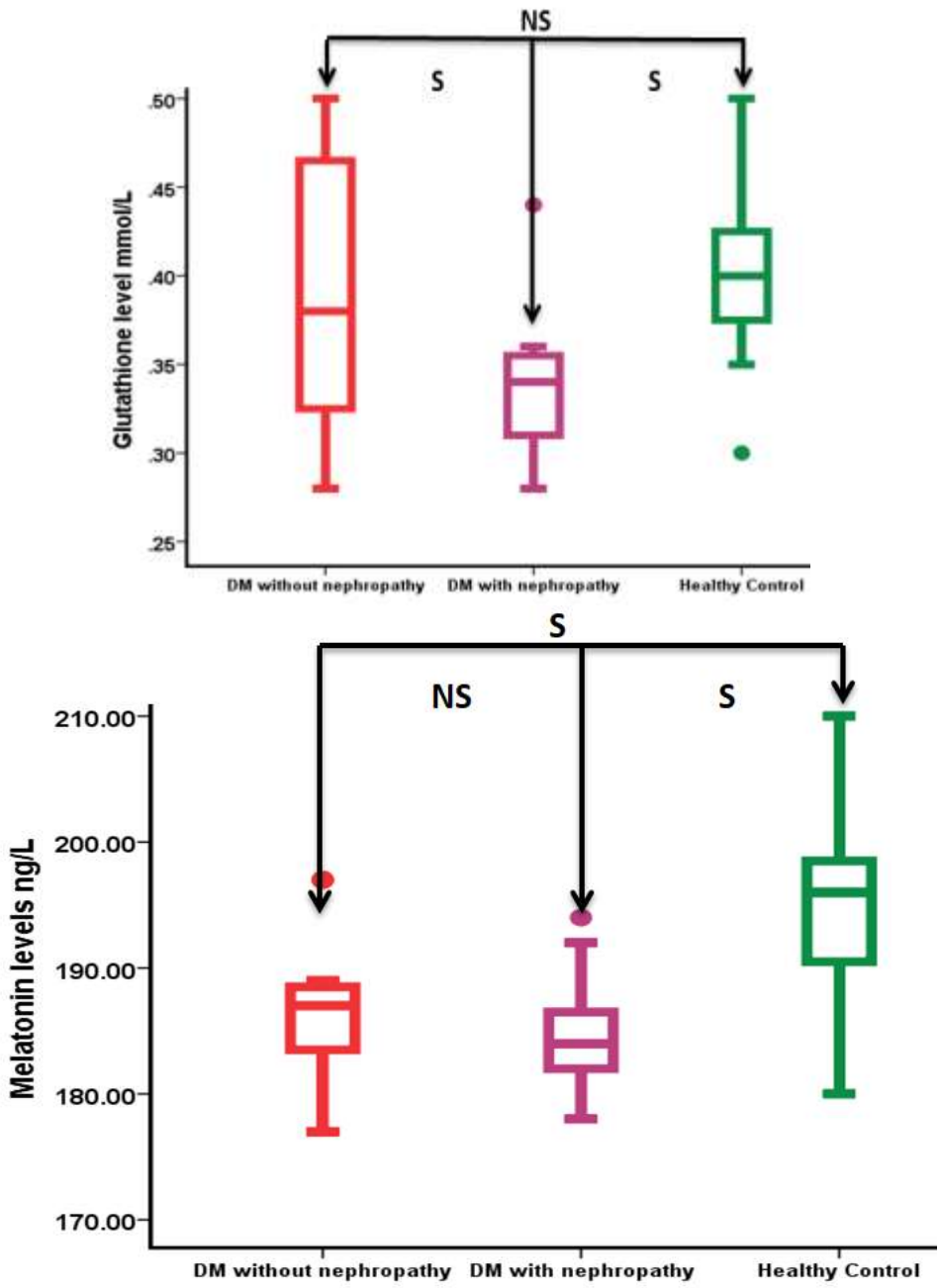

Figure 2.
Serum level of Glutathione and melatonin in DM patients with Nephropathy, DM patients without nephropathy and healthy control subjects. NS: Not statistically significant, S: statistically significant $P < 0.05$.

5. Discussion

Diabetes is considered as a chronic condition commonly referred to elevated blood glucose levels (hyperglycemia) which leads to a variety of complication [18]. Some studies have suggested that imbalance of oxidants and antioxidant status play a pivotal role in pathogenesis of diabetic nephropathy [19]. Homocysteine is able to enhance oxidative stress, so it's considered as a pro-oxidant factor by

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produce hydrogen peroxide and peroxynitrite [13]. On the other hand, homocysteine increases endothelial cells' ability to produce thrombin that catalyzed protease-activated receptors (PARs), which are linked to elevated ROS generation and NADPH oxidase expression in endothelial cells [20]. Furthermore, homocysteine reduces the function of inducible NOS and endothelial nitric oxide synthase (eNOS) by accumulating asymmetric dimethylarginine (NOS inhibitor). Thus, homocysteine contributes to the decrease of NO. Elevated homocysteine levels were linked to high-risk diabetes complications [21]. GSH is an antioxidant produced inside the cells. GSH can interact with reactive ROS, RNS and electrophiles or be a cofactor for various enzymes [22]. The diagnosis at an early stage of diabetic nephropathy properly allows the determination of targets for effective treatment. Considering that so far, no study has compared antioxidant factors such as glutathione and homocysteine pro-oxidant factor simultaneously in patients with DM nephropathy and without DM nephropathy. It is evidence that hyperglycemia augments oxidative stress through generation of ROS [23]. Excess ROS are able to attack macromolecules such as proteins, DNA and lipid, subsequently cause disorders such as nephropathy [24]. For this research, glutathione level was significantly lower in T2DMwith nephropathy compare to healthy control and T2DMwithout nephropathy, this finding agrees with the findings of an earlier study [25]. Jain *et al.*, [26] shown That, in comparison to a healthy group, diabetic individuals have lower GSH levels. They found that in patients with uncontrolled diabetes, reduced GSH levels play a critical part in cellular damage and poor insulin secretion. One of the most significant endogenous antioxidants for defending organs from oxidants, such as the kidney, is GSH. Two distinct approaches identify GSH as an oxidant and an electrophilic species scavenger, respectively. It acts as a direct antioxidant, neutralising reactive hydroxyl free radicals as well as other oxygen-centered free radicals and DNA radical centers. Additionally, GSH uses as a co-substrate to reduce peroxides, including lipid and hydrogen peroxides, and produce GSSG [27]. Taken together, current study suggested that decreasing of GSH level is associated with nephropathy diabetic. The finding of this study showed that the level of melatonin is decreasing T2DMwith nephropathy compared to healthy control. Consist with our results has shown reduced levels of melatonin in patients with chronic renal failure compared to controls [28]. Another study found that dialysis reduced serum melatonin levels by about 25% in patients with end-stage renal failure [29]. In 2022, Tang *et al.* demonstrated that melatonin treatment has protective effect in diabetes nephropathy via elevation of AMPK phosphorylation, action of mitophagy, reduction of oxidative stress and inhibition of inflammation [30]. When melatonin, folic acid, or both were administered to diabetic rats, uric acid, total protein, creatinine, and urea levels all showed improvement [31]. In this research, melatonin levels were lower in T2DM without nephropathy compared to healthy controls although no significant difference was observed. Reports on melatonin levels and T2DM that are linked to either elevated or decreased melatonin are contradictory. Hikichi et al. found no statistically significant difference in the levels of melatonin during the day between the groups of people without diabetes and those with diabetes, corroboration of our findings [32]. It was reported that the levels of melatonin were decreased in both Kakizaki rats and patients with T2DM in comparison with Wistar rats and healthy subjects, respectively [33]. Moreover, it was demonstrated that elevated melatonin signaling in insulin-secreting cells decrease secretion of insulin. It was linked to higher glucose levels (hyperglycemia) [34]. Melatonin has a circadian rhythm and sampling time; small sample size and different measurement methods can be the reason for these differences. The pineal gland secretes melatonin, a multipurpose hormone with significant effects on sleep patterns and cell death (apoptosis) [35]. Additionally, melatonin has antioxidant and anti-inflammatory characteristics, according to recent research. Besides, Melatonin, according to recent research, can reduce ROS by enhancing the production or activity of antioxidant enzymes including superoxide peroxidase and superoxide dismutase [36]. The results showed that compared to a healthy control group, patients with nephropathy and T2DM had significantly lower homocysteine levels. Also, that the level of homocysteine was lower in T2DMwithout nephropathy compared to healthy subjects although no significant difference was observed. There are contradictory results. Wijekoon *et al.*, [37] reported that homocysteine level in

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 2301-2309, 2024
DOI: 10.55214/25768484.v8i5.1984

T2DM rate was lower. Another study was demonstrated that serum levels of homocysteine were reduced in diabetic rats and it was negatively associated with urinary protein concentration (nephropathic symptoms) [38]. The researcher's Li et al. also found that early diabetic nephropathy was associated with elevated plasma total homocysteine levels compared to those without the condition [39]. it is clear that several factors, both enzymes (methyltransferases, cystathionine β-synthase (CBS) and cystathionine γ-lyase) and coenzymes (Folate and vit B12) can alter homocysteine level and interpret the results [40]. Wijekoon *et al.*, [37] claimed that expression and activity of CBS and betaine: Hcy methyltransferase (BHMT) enzymes in the liver has increased, which subsequently decreases the amount of serum homocysteine in diabetic rats. Surprisingly, it seems that imbalance hormones for example, insulin is able to change homocysteine concentration. Indeed, it was observed that insulin resistance results in decreasing of homocysteine level [41].

6. Conclusions

Our results demonstrated that decreased melatonin and glutathione levels are associated with nephropathy in T2DM. The results of this study lend credence to the idea that melatonin and glutathione could be used as therapeutic targets to address the metabolic imbalance at the root of DN. further research is needed to evaluate oxidized glutathione level (GSSG), other enzymes involved in homocysteine metabolism such as CBS and BHMT, and assay other endogenous pro- oxidant and antioxidant agent (Bilirubin, biliverdin, and Alpha-lipoic acid) in large sample size.

Ethics approval and consent to participate: Ethical approval was received from the ethical and research committee of Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq. Informed consent was obtained from all caregivers of participated.

Acknowledgements: This research is supported by the Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq.

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ISSN: 2576-8484
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DOI: 10.55214/25768484.v8i5.1984

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