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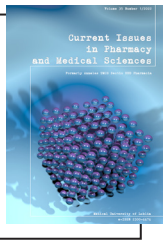

Investigation and assessment of oxidant – antioxidant status of some enzymes and vitamins in Iraqi patients with chronic myeloid leukemia

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ABSTRACT

Aim. To investigate and assess the concentrations of glutathione peroxidase, superoxide dismutase, catalase, vitamin A and vitamin C in Iraqi patients infected by chronic myeloid leukemia (CML).

Material and methods. Forty-eight Iraqi patients of ages ranged between (15-75) years took part in the current study. Enzymatic antioxidants (Gpx, SOD and CAT) and antioxidant vitamins (A and C) were estimated spectrophotometrically in blood sera of all patients according to age and sex variables, and then all clinical parameters were compared with healthy individuals (control groups).

Results. Evaluation of oxidant-antioxidant status. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes concentrations recorded high significant decreasing (***p <0.0001) in blood serum of CML patients according to age and sex factors. Moreover, vitamins A and C showed a high significant decreasing (***p <0.0001) in their concentrations as measured in blood serum of CML patients with diverse ages and sexes. The GPx, SOD, CAT, Vitamin A and Vitamin C in the current study, are considered excellent biochemical markers for following the acuity of CML disease depending on age and sex variables.

Conclusion. The clinical significance of oxidative stress represented by lack of enzymes and vitamins in the current study was proven. Statistically significant differences were recorded and observed between patients and control groups depending on age and sex factors.

INTRODUCTION

Chronic myeloid leukemia (CML) can affect individuals of assorted ages, blood groups, marital statuses and family histories, as well as both sexes. CML disease is a dysfunction of hematopoietic malignancy. A chemical abundance of free radicals, especially reactive oxygen species (ROS), is essential for the proliferation of CML [1,2]. The biochemical activity of ROS increases with decrease of antioxidants, the presence of mitochoridrial disorders, inflammatory conditions, exogenous stress, myeloperoxidase potential, as well as exposure to ultra-violet radiation, hyperoxides, some chemical substances and endotoxins [3,4]. Variables such as age, sex, blood group, use of tobacco products or chronic exposure to atmospheric toxins affect the severity of chronic myeloid leukemia, and lead to alteration in the concentrations of diverse biochemical parameters (*i.e.* enzymes, vitamins, lipids profile, trace elements, as well as blood proteins, malondialdehyde, creatine, creatinine and uric acid levels).

Published studies have investigated and evaluated the clinical biochemical roles belonging to various chemical markers in different CML patients in many countries [5-7]. Given the importance of leukemia disease, different researches were carried out to discern the real reasons for this disease coming about. To follow the severity of leukemia, in the present work, a number of biochemical markers were established. Among others, these include differences in the presence of enzymes (catales, superixode dismutase and glutathione peroxidase) and vitamins (A, C and E). The study confirmed the role of enzymes and vitamins as necessary antioxidants, their biochemical correlation with Leukemia disease [8] and that this varied in relation to age and sex variables.

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MATERIALS AND METHODS

A total patients number represented by forty-eight (25 males and 23 females) afflicted with chronic myeloid leukemia (CML), but no other disease or illness, were enrolled in this study. The CML patients were divided into four groups according to their ages, the first group (15-30 years), the second (31-45 years), the third (46-60 years), and the fourth (61-75 years). Forty healthy individuals served as control (Table 1). The blood samples were obtained from enrollees at the Al-Sadr Teaching Hospital Carcinoma centre, Basrah Governorate, Iraq from July 2022 to November 2022. All who took part in the study were not users of tobacco products. In the current study, ethical approval was confirmed and obtained from the University of Basrah – college of education for pure sciences, by official Abdel Sattar Jaber Ali Al-Saif (case number 2331/7/3, dated 23/6/2022).

Clinical collection of blood samples

Five milliliters of venous blood was drawn from the CML patients and corresponding healthy individuals. All blood samples were clotted and centrifuged at 4500 rpm for twelve minutes, then total sera were kept at 20°C until the day of estimation of assorted biochemical markers. The remaining blood was put in no-heparin special tubes, then blood plasma was separated and centrifuged at 3000 rpm for seven minutes. The erythrocytes were subsequently washed carefully in sodium chloride solution with concentration equal to 9% w/v, after which the blood solution was lysed using deionized water 1:1 v/v [9,10].

Assessment of biochemical paramaters in CML patients

Concentrations of glutathione peroxidase and superoxide dismutase were established in CML patients and corresponding healthy individuals by using the enzyme-linked Immunosorbent assay (ELISA) method, while the catalase concentrations were assessed spectrophotometrically depending on the formation of coloured complex and measurement of the absorbance [11,12].

Statistical Analysis

All values of results were presented and indicated as mean ± standard deviation (SD) depending on the applied variance univariate programme. All parameters were statistically analyzed for all CML patients and healthy subjects by using the Statistical Program for Social Sciences (SPSS) (IBM, USA, version 23). Statistical relationships were established by measuring the regression coefficient to distinguish between all the means in relation to CML patients and control groups. Significance difference between CML patients and healthy individuals (control group) was ascertained according to the SPSS program. P value was established at <0.05.

RESULTS

Table 1 shows the concentrations belonging to antioxidant enzymes (GPx, SOD and CAT) in blood serum of CML patients in relation to age, as compared with healthy individuals (control groups).

Table 1. Concentration of antioxidant enzymes in blood serum of CML patients in relation to age

Age Category (year)	Subjects Groups	GPx (Pmol/ml)	SOD (Pg/ml)	CAT (K/ml)
First 15-30	Control (no.=6)	45.833±0.05	3.050±4.72	3.225±2.13
	CML patient (no.=10)	13.564±0.14	0.5186±8.62	0.1173±3.12
Second 31-45	Control (no.=18)	63.129±0.04	4.783±4.22	2.505±2.15
	CML patient (no.=10)	8.4955±0.02	0.4328±7.86	0.1059±4.37
Third 46-60	Control (no.=11)	86.775±0.06	4.900±3.92	2.963±2.26
	CML patient (no.=20)	14.936±0.023	0.3544±8.67	0.0704±5.63
Fourth 61-75	Control (no.=5)	48.857±0.072	3.502±4.23	3.560±2.46
	CML patient (no.=8)	10.961±0.034	0.4762±9.87	0.0742±7.28

Values are represented as mean ± SD; ***p<0.0001, **p<0.001, *p<0.05; SOD – Superoxide dismutase; CAT – Catalase; GPx – Glutathione Peroxidase

Glutathione Peroxidase (GPX) concentrations were recorded at 13.564, 8.4955, 14.936 and 10.961 Pmol/ml in the blood serum of CML patients of first, second, third and fourth age categories, respectively. SOD enzyme also showed alteration in concentration, with values equal to 0.5186, 0.4328, 0.3544 and 0.4762 Pg/ml, respectively, in the CML groups, while the concentration of CAT enzyme concentration were recorded at 0.1173, 0.1059, 0.0704 and 0.0742 K/ml, respectively. Maximum concentrations of GPx, were noticed at the first, third and fourth age categories, while that of SOD was seen at the first, fourth and second age categories, and that for CAT was evidenced at first, second and fourth categories.

Vitamins A and C are considered to be important biochemical antioxidants with regard to indication of the severity of CML. Concentrations of these vitamins in relation to age factor are illustrated in Table 2.

Table 2. Concentration of antioxidant vitamins in the blood serum of CML patients in relation to age

Age Category (year)	Subjects Groups	Vitamin A (Pg/ml)	Vitamin C (mg/ml)
First 15-30	Control (no.=6)	216.088±22.13	24650.5±2.34
	CML Patients (no.=10)	114.116±18.45	2401.23±2.79
Second 31-45	Control (no.=18)	256.106±21.72	20103.8±2.56
	CML Patients (no.=10)	146.644±17.92	4489.55±2.34
Third 46-60	Control (no.=11)	304.294±20.34	18687.8±2.43
	CML Patients (no.=20)	122.126±15.78	2902.71±1.77
Fourth 61-75	Control (no.=5)	174.757±19.36	13277.4±2.32
	CML Patients (no.=8)	174.036±16.24	2797.123±1.89

Values are represented as mean ± SD, ***p<0.0001, **p<0.001, *p<0.05

Vitamin A concentrations were 114.166, 146.644, 122.126 and 174.036 Pg/ml in the blood serum of the CML groups of, respectively, first, second, third and fourth age categories, and that of Vitamin C was: 2401.236, 4489.55, 2902.71 and 2797.12 mg/ml, respectively. The highest values of Vitamin A concentration were noted in the fourth, second and third age categories, while that of Vitamin C were seen in the second, third and fourth categories.

The sex variable is considered as a substantial and remarkable clinical factor for GPx, SOD and CAT levels (Table 3).

Table 3. Concentrations values of antioxidant enzymes (GPx, SOD, and CAT) in the blood serum of CML patients in relation to sex factor

Sex type	Subjects groups	GPx (Pmol/ml)	SOD (Pg/ml)	CAT (K/ml)
Male	Control (no.=21)	60.475±3.62	4.773±12.23	3.0268±0.12
	CML Patients (no.=25)	12.321±6.82	0.467±28.45	0.0883±0.16
Female	Control (no.=19)	70.534±4.83	4.083±11.24	2.698±0.09
	CML Patients (no.=23)	12.999±9.22	0.379±30.31	0.0881±0.03

Value are represented as mean ± SD ***p<0.0001, **p<0.001, *p<0.05; Gpx – Glutathione peroxidase; SOD – Superoxide dismutase; CAT – Catalase

The results shown in Table 3 indicate that concentrations of GPx, SOD and CAT in the blood serum for both CML patients and healthy subjects varied according to sex. In males with CML, the concentrations of these enzymes were equal to 12.321 P mol/ml, 0.467 Pg/ml and 0.0883 K/ml, respectively. In females, the corresponding levels were 12.999 Pmol/ml, 0.379 Pg/ml and 0.0881 K/ml, respectively. The maximal concentration of GPx was noticed in female patients, whereas as regards SOD and CAT, the highest concentrations were recorded in male CML patients.

The sex factor has a biochemical role in alteration of vitamins concentrations of CML patients as illustrated in Table 4.

Table 4. Concentrations of antioxidant vitamins in blood serum of CML patients in relation to sex variable

Sex type	Subjects group	Vitamin A (Pg/ml)	Vitamin C (Pg/ml)
Male	Control (no.=21)	245.564±27.22	20443.02±2.08
	CML Patients (no.=25)	130.122±22.34	3050.49±2.13
Female	Control (no.=-19)	261.61±20.23	18611.6±1.24
	CML Patients (no.=23)	138.689±23.25	3177.24±3.21

Values were represented as mean ± SD, ***p<0.0001, **p<0.001, *p<0.05

Vitamin A and Vitamin C levels in CML male patients were 130.122 Pg/ml and 3050.49 Pg/ml, respectively, and that of females, 138.689 Pg/ml and 3177.24 Pg/ml, respectively. Vitamins A and C showed the maximal concentrations in blood serum of female patients.

DISCUSSION

The dangerous complications seen in Chronic myeloid leukemia (CML) are due to alterations in oxidative stress as represented by differences in the levels of the antioxidant enzymes and vitamins that have biochemical roles associated with the severity of this disease [13,14]. In the present study, the concentrations of antioxidant enzymes GPx, SOD and CAT demonstrated highly significant decreases (***p <0.001) in the blood serum of CML patients in relation to healthy individuals according to age, especially in the first, second and third age categories. These findings confirm the clinical significance of age as a substantial and serious factor in the progression of CML [15-17]. The levels of vitamins A and C also showed high significant decreases (***p <0.0001) in their concentrations in CML patients, as compared with healthy subjects and with the age factor. The outcome of our work, is the confirmation of previous studies of this aspect of the affliction [18,19]. In the present study, concentrations of vitamins A recorded high significant decreases (***p <0.0001) in the blood serum of male and female CML patients, whereas vitamin C showed significant increase (**p <0.001) in the blood concentrations of male patients. These outcomes reveal the biochemical role of antioxidant vitamins A and C as clinical markers for the severity of CML [20].

Clinically, sex is considered a substantial variable in the severity and progress of cancer diseases (especially CML) because the difference in the patient sex has a biochemical role in alteration of concentrations of chemical parameters, including antioxidant vitamins and enzymes [20]. The antioxidant enzymes GPx, SOD and CAT in our study showed high significant decrease (***p <0.0001) in their concentrations in the blood serum of both male and female CML patients, and especially so in male patients [21,22]. GPx enzyme levels, however, had higher significance as compared with CAT and SOD enzyme levels. The sex variable also is associated biochemically with diversity of the levels of antioxidant vitamins such as A and C, thus the clinical relationship between sex of CML patients and the concentrations of these vitamins is considered as an indicator for CML acuity and severity [23-28].

CONCLUSIONS

In the current research, differences in oxidant–antioxidant status were demonstrated by differences in the levels of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and the antioxidant vitamins A and C. The concentrations of these enzymes and vitamins recorded highly significant differences in the blood serum of CML patients, notably when the factors of age and sex were taken into account. Thus, the enzymes and vitamins of the current study can be considered as important clinical biochemical variables of CML disease.

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