

Profile study of some oxidant and antioxidant levels in leukemic patients

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ABSTRACT

Objective: The aim of this study is to evaluate the oxidant and antioxidant level in cancer patients in Basrah during the period from the first of October 2002 till the end of April of the 2003. **Material and methods:** A total of 64 patients were investigated, who were admitted to the Basrah General Hospital, Teaching Hospital and Basrah Hospital for Maternity and Pediatric in Basrah City. 125 sex and age- matched persons without malignancy served as controls. They were 64 persons as control for leukemic patients (32 male and 32 female). The parameters measured were glutathione (GSH) level, uric acid, malondialdehyde (MDA) and phagocytic functional activity. The leukemic patients were sub classified into two groups according to their age (30 patients ≤ 16 years and 16 patients > 16 years). **Results:** The biochemical investigation showed a high significant depletion in GSH levels in leukemic patients ($P < 0.001$). The phagocytic activity was high significantly decreased in all leukemic patients ($P < 0.001$). There was no significant difference between male and female leukemic patients in phagocytic activity. The MDA level was highly significantly increased in ≤ 16 years old leukemic patients ($P < 0.01$) and extremely high significant in > 16 years old leukemic patients ($P < 0.001$). The investigation of uric acid concentration shows extremely high significant increase in all leukemic patients ($P < 0.001$). **Conclusion:** cancer patients suffer a high degree of ROS formation causing considerable oxidative stress which associated with decrease glutathione level and significant degree of lipid peroxidation. (MJBU,30,2: 2012, Page 115-121)

دراسة مستوى بعض العوامل المؤكسدة والمضادة للتأكسد في مرضى اللوكيميا

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الهدف: أجريت هذه الدراسة لتقييم مستوى العوامل المؤكسدة والمضادة للتأكسد عند مرضى السرطان في البصرة خلال الفترة من الأول من تشرين الأول 2002 إلى نهاية نيسان 2003. **طريقة الدراسة:** تمت متابعة ما مجموعه 98 مريضاً ويمثلون المرضى الذين ادخلوا إلى مستشفى البصرة العام والمستشفى التعليمي ومستشفى البصرة للولادة والطفل. من هؤلاء المرضى 46 مريضاً مصاباً ببيضاض الدم (24 ذكراً و 22 أنثى). إضافة إلى 125 شخصاً غير مصاب بالسرطان تم اختيارهم بوصفهم عينة ضابطة مع مراعاة توافق العمر والجنس. شملت لفحوصات الكيمياء الحياتية مستوى الغلوتاثيون المختزل وحمض اليوريك والديهيد المألون الثنائي (MDA) والفعالية البلعمية. قسم مرضى ابيضاض الدم الى مجموعتين اعتماداً على أعمارهم (30 مريضاً بعمر 16 سنة او اقل و 16 مريضاً بعمر أكثر من 16 سنة). **النتائج:** أظهرت الاختبارات الكيميائية الحياتية نقصاً معتداً عال إحصائياً في مستوى الغلوتاثيون المختزل عند مرضى ابيضاض ($P < 0.001$). وقد قلت الفعالية البلعمية لخلايا الدم البيضاء بشكل معتد عال إحصائياً عند كل مرضى ابيضاض الدم ($P < 0.001$). ولم يكن هناك فرق معتد إحصائياً بين الذكور والإناث في مرضى ابيضاض الدم بما يخص الفعالية البلعمية لخلايا الدم البيضاء. إن مستوى الدهيد المألون الثنائي (MDA) كان أكثر بشكل معتد عال إحصائياً عند مرضى ابيضاض الدم للفتة العمرية 16 سنة فاقل ($P < 0.01$). وأظهر اختبار حمض اليوريك زيادة باعداد عال جداً إحصائياً في كل مرضى ابيضاض الدم ($P < 0.001$). **الاستنتاج:** إن الدراسة أظهرت أن مرضى السرطان يعانون بدرجة عالية من تكون جذور الأوكسجين النشطة التي تسبب جهداً مؤكسداً مهماً مرتبطاً مع قلة مستوى الغلوتاثيون ومع درجة مهمة من أكسدة الدهون.

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INTRODUCTION

All living cells are prone to oxygen toxicity. This toxicity has been related to the intermediates of oxygen reduction referred to as reactive oxygen species (ROS) which include the super oxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot).^[1] These may lead to many human degenerative disease, such as atherosclerosis, certain types of cancer and cataract.^[2-4] ROS are also formed during non enzymatic processes, for example, exposure to sunlight and ionizing radiation.^[1] The MDA – modified proteins are potentially as deleterious as free MDA, and could be involved in aging as well as in degenerative complications of diseases with increased oxidative stress such as diabetes mellitus, atherosclerosis and cancer.^[5] Serum MDA levels are increased in leukaemia and are higher in the active phase of disease as compared to those in remission, hence, serum MDA estimation in leukaemia can be of help in diagnosis and to predict the chances of relapse.^[5,6]

The present study aims to determine oxidant and antioxidant level of patients with leukemia.

1. Measurement of phagocytic activity by as oxidant by chemiluminescence (CL) in blood of leukemic patients to compare with those of their control.
2. Measurement of malondialdehyde as a biomarker of lipid peroxidation in blood of patients in comparison with control.
3. Measurement of uric acid and reduced glutathione as antioxidants in blood of patients in comparison with control.

PATIENTS AND METHODS

Patients

Forty-six patients with leukemia, they were 22 females and 24 males; their age ranged from 1.5 to 50 years old. Control group for leukemic patients group; they were 64 persons include 32 females and 32 males; their age ranged from 2 to 45 years old having 32 with +ve and 32 with -ve family history for leukemia.

Data collections:

From each subject full information were obtained using a questionnaire list that included name, age, sex, occupation, as well as the following questions about:

1. Medical history for any previous and recent illness and their type of treatment.
2. Family history for any malignant condition.
3. Social history for smoking and drinking habits.

Physical and radiological examinations were done including U/S and X-ray.

Methods

Blood samples:

Blood samples (5ml) were collected by venopuncture using a sterile disposable syringe in a heparinized tubes, (4ml) of this blood was centrifuged; the plasma was transferred to sample tube and stored at (-4c) until analyzed. While the rest fresh (1ml) of whole blood used for determination of the glutathione concentration and phagocytic activity.

Biochemical parameters:

Determination of glutathione (GSH):

The GSH determined according to method^[7] The GSH concentration was obtained by using the following equation:

$$\text{GSH mg/dl of erythrocytes} = \frac{\text{GSH conc. (from standard curve)}}{\text{Heamatocrit}}$$

Estimation of malondialdehyde (MDA):

MDA, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. The thiobarbituric acid assay of Buege and Aust (1978) was used to measure the (MDA).^[8]

Principle: MDA has been identified as the product of lipid peroxidation by its reaction with thiobarbituric acid to give a red species absorbing at 535nm. The absorbance of the sample was determined at 535nm against a

blank that contains all the reagents except the serum. The MDA concentration of the sample was calculated using an extinction coefficient of $(1.56 \times 10^5 \mu^{-1} \text{ cm}^{-1})$.

Calculation:

$$\text{MDA} = (\Delta A / 1.56) \times 10 \mu\text{mol/L}$$

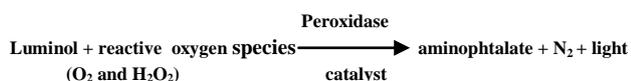
For accuracy and precision, the intra-coefficient of variation were estimated to be 1.6% and 6.0% respectively.

Chemiluminescence (CL):

CL was taken to indicate that phagocytes were generating singlet oxygen during the respiratory burst.^[9] So the CL was taken to determine myeloperoxidase activities an indicator for leucocytes phagocytic activity.

Principle:^[10]

The luminol-amplified chemiluminescence activity can be simplified by the following formula



Chemiluminescence inducer and measurement:^[11-13]

Calculations

The area under CL kinetic curve represented the granulocytes functional activity. The peak high of CL kinetic curve represent the functional activity. The granulocytes functional activity yield were estimated using the formula below:

Peak heights

$$\text{Functional activity yield} = \frac{\text{Peak heights}}{\text{Number of granulocytes}}$$

Enzymatic determination of uric acid:^[14, 15]

Calculation:

$$\text{Serum uric acid (mg/dl)} = \frac{\text{(O.D serum)}}{\text{O.D standard}} \times 8$$

O.D (Optical density) measured at 530nm

Statistical analysis:

The results were expressed as mean±SD. The comparisons between groups were performed with analysis of variance (ANOVA). While correlation was assessed by the Pearson correlation coefficient (r) using computerized SPSS program (Statistical Program for Social Sciences). P<0.05 was considered to be the lowest limit of significance.

RESULTS

Table-1, shows the characteristics of the leukemic patients and their controls there were forty six patients (24 male and 22 female), twenty four patients of them were with positive family history for leukemia. Forty subjects from sixty four subjects (32 males and 32 females) served as control group were with positive family history for leukemia.

Table 1. Characteristics of the Leukemic patients and their controls

| | Leukemic patients | | Control subjects | |
|----------------|-------------------|-------|------------------|-------|
| | X±SD (n) | % | X±SD (n) | % |
| No. | 46 | 41.8 | 64 | 58.18 |
| Age (years) | | | | |
| ≤ 16 | 7.88±3.49 (30) | 65.21 | 30.47±8.05 (37) | 57.8 |
| > 16 | 37.06±12.28 (16) | 34.78 | 7.96±4.36 (27) | 42.2 |
| Family history | | | | |
| +ve | 24 | 52.17 | 40 | 63.75 |
| -ve | 22 | 47.83 | 24 | 36.25 |
| Sex | | | | |
| Male | 24 | 52.17 | 32 | 50 |
| Female | 22 | 47.83 | 32 | 50 |

Glutathione:

GSH levels in different age groups and each sex in leukemic and their control group are shown in (Table-2). The GSH concentration in blood of leukemic patients was lower than their control group with significant difference (P<0.001).

Table 2. GSH levels in different age groups and sex in leukemic and control subjects.

| | Age groups | | Sex | |
|---|-------------------|------------------|-------------------|------------------|
| | ≤16 | >16 | Male | Female |
| Leukemic patients (mg/dl of erythrocytes) | 63.5±6.3 (30) | 68.4±5.9 (16) | 65.9±5.8 (24) | 64.6±7.3 (22) |
| Control (mg/dl of erythrocytes) | 82.8±10.4 (35) | 87.7±9.5 (29) | 84.2±11.4 (32) | 85.2±8.8 (32) |
| Significant diff. | E.S | E.S | E.S | E.S |

Values expressed as X±SD

E.S: Extremely significant (P<0.001)

Malondialdehyde:

The MDA levels in leukemic and their control group with respect to age group and sex are shown in (Table-3). MDA levels in leukemic patients are higher than those of control subjects with highly significant difference in age group ≤16 (P<0.01) and with extremely significant difference in age group >16 (P<0.001). The MDA levels in females patients are significantly higher than their controls (P<0.05), but significantly higher in males leukemics than their controls (P<0.01).

Table 3. MDA levels in different age groups and sex in leukemic and control subjects.

| | Age groups | | Sex | |
|--------------------------------------|------------------|-----------------|-------------------|-------------------|
| | ≤16 | >16 | Male | Female |
| Leukemic patients (µmol/l) | 0.8±0.08 (30) | 1.3±0.2 (16) | 0.99±0.32 (24) | 0.96±0.27 (22) |
| Control (µmol/l) | 0.7±0.3 (35) | 0.9±0.1 (29) | 0.74±0.22 (32) | 0.79±0.3 (32) |
| Significant diff. | H.S | E.S | H.S | S |

Values expressed as X±SD

S: Significant (P<0.05)

H.S: Highly significant (P<0.01)

E.S: Extremely significant (P<0.001)

Uric acid:

Uric acid levels of leukemics and their control group with respect to their age group and sex are presented in (Table-4). The uric acid levels are higher in leukemic patients in both age groups and both sex as compared with their control with a significant difference (P<0.001), but still within normal range.

Table 4. Difference in uric acid levels between leukemics and their control group with respect to their age groups and sex.

| | Age groups | | Sex | |
|----------------------------------|-----------------|------------------|-----------------|-----------------|
| | ≤16 | >16 | Male | Female |
| Leukemic patients (mg/dl) | 3.7±0.4 (30) | 4.3±0.4 (16) | 4.0±0.5 (24) | 3.0±0.3 (22) |
| Control (mg/dl) | 2.8±0.3 (35) | 3.1±0.35 (29) | 3.8±0.4 (32) | 2.9±0.4 (32) |
| Significant diff. | E.S | E.S | E.S | E.S |

Values expressed as $\bar{X} \pm SD$

E.S: Extremely significant (P<0.001)

Phagocytic activity

Table-5, represented the difference in phagocytic activity with extremely significant phagocytic activity between leukemics and their controls in each age group and each sex. The are lower in patients than their controls in each age group and so in each sex group (P<0.001).

Table 5. Phagocytic activity in different age group and each sex in leukemic patients and their control group.

| | Age groups | | Sex | |
|---|------------------|--------------------|--------------------|--------------------|
| | ≤16 | >16 | Male | Female |
| Leukemic patients (Phagocytic activity/cell) x 10⁻⁴ | 135±30 (30) | 160.9±25.3 (16) | 144.6±27 (24) | 148.8±34.2 (22) |
| Control (Phagocytic activity/cell) x 10⁻⁴ | 169.3±11 (35) | 186.3±8 (29) | 175.7±11.7 (32) | 174.6±14.4 (32) |
| Significant diff. | E.S | E.S | E.S | E.S |

Values expressed as $\bar{X} \pm SD$

E.S: Extremely significant (P<0.001)

Heamatological parameters

DISCUSSION

The study was designed to measure the oxidant and antioxidant levels in leukemic patients. The study showed depletion of reduced glutathione concentration (the major endogenous antioxidant) in leukemic patients as shows in (Table-2), these results were in agreement with other studies.^[16,17] There can be two reasons for GSH depletion in cancer. Firstly; elevated glutathione peroxidase will use more GSH in an attempt to cope with the excessive production of oxyradicals as revealed by elevated lipid peroxidation. Secondly, if little replenishment of GSH occurred, the level of GSH would become lower.^[18,16,19,20] Uric acid with powerful antioxidant properties was proposed to play a key role in the antioxidant protection in humans;

it is a very effective scavenger such that it is as effective as ascorbic acid.^[21] The present study shows an increase in plasma urate concentration of leukemic patients. This results were in agreement with other studies,^[22-25] but in disagreement with observation of others.^[26] High plasma urate concentration may be caused by increased turnover of nucleic acids due to rapidly growing malignant tissue, especially in leukemia's, or may caused by increased tissue breakdown by the treatments of cancer.^[27] This study revealed a significant elevation of MDA among all groups of patients. This implies that cancer patients are exposed to a considerable degree of lipid peroxidation. This finding in consistent with observations of others.^{[5,6, 22, 28-}

^{30]} The cause of elevated MDA level in cancer patients may be the free radicals (which increased in cancer) are associated with an impaired antioxidant defense enzyme activity resulting in increased lipid peroxidation.^[31] The principle phagocytes are neutrophils and monocytes because the melonperoxidase (MPO) is more abundante in these cells. ^[32] In this study, phagocytic activity was reduced significantly in leukemic patients. This result in agreement with other studies^[33]and in disagreement with others. ^[34] This may be due to neutropenia, which is the most important deficiency phenomenon in acute leukemia.^[35] Cancer chemotherapy may also produce defects in neutrophil function, and combination of some drugs can produce a significant decrease in the phagocytic killing capability.^[36] Other study suggested that the H₂O₂ inhibits the ability of different antineoplastic chemotherapeutic drugs to induce the phagocytosis.^[37]

In conclusions, leukemic patients suffer from a significant degree of free radical formation as indicated by the significantly higher MDA level among those patients. As a result of continuous production of ROS, the level of GSH is depleted. Elevated urate concentration in cancer patients caused by increase of nucleic acid turnover. The WBC phagocytic activity was reduced in leukemic patients due to neutropenia. No significant difference in the level of GSH, uric acid, MDA and phagocytosis activity in patients with leukemia regarding to age and sex of those patients.

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