

Study of Some Antioxidant Levels in Blood Serum of Patients with Hodgkin disease

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Abstract: This research has been accomplished to determine the antioxidant levels of Hodgkin disease in thirty blood serum samples of patients in the ages that rang from (15-70) years attending hospitals in Mosul town and twenty serum samples as control group of the same age. The investigated antioxidants were Glutathione , Uric acid, Ceruloplasmin , Vitamin E, Vitamin C, Vitamin A, Bilirubin , Albumin ,Superoxide dismutase and Malondialdehyde.

The analysis of results showed that there were a considerable decrease in Glutathione, Vitamin E ,Vitamin A ,Vitamin C and Albumin levels and a significant increase in level of Malondialdehyde , Superoxide dismutase , Uric acid , bilirubin and Ceruloplasmin for Hodgkin disease patients when compared with control group.

Keywords: Glutathione (GSH), Uric acid, Ceruloplasmin (Cp.), Vitamin E, Vitamin C, Vitamin A, Bilirubin ,Albumin, Superoxide dismutase (SOD) and Malondialdehyde (MDA).

دراسة مستويات بعض مضادات الأكسدة في مصل دم المصابين بمرض الهوجكن

الملخص: تم إجراء هذا البحث لتقدير مستوى مضادات الأكسدة للمرضى المصابين بمرض الهوجكن حيث تم أخذ 30 عينة من مصل الدم للمصابين تراوحت أعمارهم من (15-70) سنة من المراجعين لمستشفيات مدينة الموصل و 20 عينة كمجموعة سيطرة اختيرت من ذات الفئة العمرية. اذ تضمنت هذه المتغيرات: كلوتاثاين، حامض اليوريك، السليروبلازمين، فيتامين E، فيتامين C، فيتامين A، البيلروبين، الألبومين، انزيم السوبراوكسايد دسيموتيز و المالوندايالديهايد وقد أظهرت النتائج وجود انخفاض معنوي في مستوى الكلوتاثاين، فيتامين E، فيتامين A، فيتامين C والألبومين وارتفاع معنوي في مستوى المالوندايالديهايد ، انزيم السوبراوكسايد دسيموتيز، حامض اليوريك، البيلروبين والسليروبلازمين في مصل دم المرضى المصابين بمرض الهوجكن عند مقارنتهم مع مجموعة السيطرة.

الكلمات الدالة: كلوتاثاين، البيلروبين، الألبومين، انزيم السوبراوكسايد دسيموتيز، حامض اليوريك، السليروبلازمين، فيتامين A، C، E، والمالوندايالديهايد .

INTRODUCTION

Hodgkin lymphoma (HL) is a kind of lymphoma in which cancer found from a special type of leukocytes called lymphocytes (Bower, 2011). Hodgkin disease is subdivided into a classical form and a nodular lymphocyte predominant form(Ansell, 2018). The age, gender and Epstein-Baw infection can affect the risk of Hodgkin lymphoma. Signs of Hodgkin lymphoma include fever ,night sweat, swollen lymph nodes and weight loss . The growing lymph nodes are exist in the neck, beneath arm and in groin. the lymph nodes tests are used as a sign to detect and diagnose Hodgkin lymphoma. The disease may be managed with radiation therapy, chemotherapy and stem cell transplant (Bonadonna *et al.*, 1975; Chen *et al.*, 2017).

The cells can be damaged by unstable molecules known as free radicals, the antioxidant can protect the cell from this damage, it is the main defense line versus the free radical.

Human cells are controlled with both enzymatic and non-enzymatic antioxidant defense mechanisms to

reduce the cellular damage resulting from the reaction between the cellular constituents and the reactive oxygen species (Mila *et al.*, 2004; Ibrahim, 2013).

oxidative stress by opposing the reverse effects of free radicals which are produced by metabolic activities in the body , furthermore, they retard or inhibit the oxidation of molecules like carbohydrate, protein, fat and DNA(Kerr *et al.*, 1999 ;Al-hassany ,2010).

The synthesis of reactive species involving free radicals, is a whole part of human metabolism. Because of the elevated possibility to damage vital biological systems, reactive species have now been accused in aging and in many others disease states (Halliwell *et al.*, 1992 ; Ames *et al.*, 1993) .

A complicated system of neutralizing antioxidants founds in plasma, internal and external cellular fluids, but an imbalance (oxidant stress) between free radical production and use can give rise to the damage of DNA lipids, proteins, and other biomolecules (Aboutwerat *et al.*, 2003).

Epidemiological index regularly relates decreased antioxidants intake or low blood levels of antioxidants with an increased cancer danger. Examples of antioxidants include Glutathione, Ceruloplasmin, Uric acid, Malondialdehyde, Vitamin C & E and other substances (Kasai , 1997)

Objectives: the study aims to determine the extent of changes in antioxidant levels of patients with Hodgkin's disease.

MATERIALS AND METHODS

From each patient Five milliliters of blood was taken then kept for (10) minutes to complete coagulation then separation of serum by centrifuge for (10) minutes was done, then frozen at (-20°C). 20 blood sample from healthy control group was taken, ages ranging from (15-70) years for both patients and control group, living in Mosul city.

Glutathione was evaluated using the modified method used by the researchers(Sedlak and Lindsay , 1968). The method is based on the use of (DTNB Ellman's reagent) [5,5-Dithio-

bis(2-Nitrobenzoic acid)], which reacts quickly with glutathione and is reduced by (SH group) to the glutathione. This lead to form a colored product whose absorption is red at 412 (nm).

The method of measurement of the ceruloplasmin is depends on the (Cp) effectiveness to oxidized para-phenyldiamine (PPD) to a blue violet solution measured at 530 (nm)and the speed of product formation depends on the concentration of ceruloplasmin in the serum (Sunderman and Nomato , 1970).

The level of malondialdehyde in the serum was estimated using the modified method. Based on this method, Serum lipid peroxide was estimated by measuring the amount of malondialdehyde. The method was based on the interaction between lipid peroxide and chiefly malondialdehyde and thiobarbituric acid (TBA) is acidic medium and has a colored product whose absorption strength is measured at 532 (nm) (Lunec , 1990)

The method for measuring of vitamin E in serum is depend on oxidation reduction reaction. Relying on specific elution techniques, fractions are

commonly undergone to the Emmerie-Engel producer, in this process tocopherol is oxidized to tocopherolquinone with FeCl_3 , and the Fe^{++} in the end FeCl_2 is mixed with α, α -dipyridyle to produce a red color with absorbance at 520 (nm) (Emmerie and nomato,1970).

The ascorbic acid (Vitamin C) is oxidized by copper to be dehydroascorbaic acid and diketogluonic acid. These products interact with 2,4-diphenylhydrazine to be a derivative of 2,4-dinitriphenylhydrazine and the latter compound with the addition of concentrated sulfuric acid it gets a rearrangement to be a product that gives an absorption package measured at 520 (nm) (Roe and Kuther, 1943).

Uric acid was measured using the phosphotungstic acid method. Uric acid is a reduced agent that reduces the phosphotungstic acid solution in the basal environment to the blue phosphotone blue color solution known as tungsten blue, which is measured at 700 (nm).(Varley , 1967)

Vitamin A has an intense absorption in the ultraviolet space with a

max level at 327 (nm) . It is damaged by ultraviolet light . The optical density varies among irradiated and non-irradiated serum extracts for this reason it can be used to measure vitamin A concentration (Wootton , 1974).

SOD activity in the serum was determined using photochemical methods which rely on an indirect access to mark the SOD activity over the change in formazene absorbance composed from the reduction of O_2^- , that produced by radiating the specimen of serum with light for nitroblue tetrazolum (NBT) dye . Decreased difference in formazen absorbance purpose that SOD activity is increased. (Brown , 1983).

The level of serum total bilirubin measured by Diazo method which is depended on the interaction of bilirubin both direct and indirect with diazotized sulfanilic acid (diazo method) (Toro and Ackermann,1975)

Serum Albumin level was measured by bromocresol green dye (BCG) binding method (Doumas B.T. *et al.*,1971).

Statistical analysis

The result of this method are expressed as mean \pm SD the data were analyzed using paired t-test to compare the subjects result for various bioparameters through the various groups tested in the work. The difference is considered significant at ($P \leq 0.05$) (Indrayan and Sarmukaddam , 2001).

RESULTS AND DISCUSSION

The results in table (1) showed a significant decrease in glutathione, vitamin E, Vitamin A, vitamin C and Albumin levels. And the results showed a significant increase in malondialdehyde, SOD, uric acid, Bilirubin and ceruloplasmin in the serum of Hodgkin disease patients when we compared it with the control group .

Table (1): The measured biochemical parameters in normal control and Hodgkin patients.

Parameters	Mean \pm S.D.		p-value
	Control group	Patients	
Glutathione ($\mu\text{mol/L}$)	16.29 \pm 0.42	9.21 \pm 0.35	< 0.001
Ceruloplasmin ($\mu\text{mol/L}$)	372.8 \pm 42.90	465.2 \pm 18.61	< 0.001
malondialdehyde ($\mu\text{mol/L}$)	4.62 \pm 0.35	8.25 \pm 0.46	< 0.001
Vitamin E ($\mu\text{mol/L}$)	18.45 \pm 2.62	11.20 \pm 2.22	< 0.001
Vitamin C ($\mu\text{mol/L}$)	32.27 \pm 2.51	17.43 \pm 1.62	< 0.001
Vitamin A ($\mu\text{mol/L}$)	1.57 \pm 0.07	0.75 \pm 0.081	< 0.001

Uric acid ($\mu\text{mol/L}$)	244.6 \pm 31.8	315.5 \pm 20.51	< 0.001
SOD (U/ml)	8.97 \pm 0.28	12.22 \pm 0.43	< 0.05
Total Bilirubin ($\mu\text{mol/L}$)	7.15 \pm 0.14	12.51 \pm 0.54	<0.05
Albumin (gm/100ml)	4.09 \pm 0.02	3.56 \pm 0.05	< 0.001

The reduction in GSH level may be resulted in increasing the defense against oxidant damage in cancer GSH can play directly as an oxidant in many ways as a free radical scavenger or as a Co-substrate of some enzymes like glutathione peroxidase, lower levels of glutathione may aid an increased production of free radicals and lipid peroxidase which in turn may motivate damage to the DNA and the cell membrane (Droge , 2002 ; Kohen and Nyska , 2002 ; Suleyman *et al.*, 2003).

The increase in ceruloplasmin (Cp.) as a part of the defense case may be due to its use as an antioxidant, which is involved through the capture of ions that act as free radicals, there by increasing the Cp. To decrease the formation of free radicals and oxidation. The Cp. has an effective role in removing the oxidation because it is protein containing approximately 25% of the total copper existed in the serum which prevents oxidation (Surmen *et al.*, 2003).

The increase in MDA level might result from the elevated collecting of (ROS) due to the increased oxidative damage in Hodgkin patients generated. The production of oxygen radicals, which increase with the clinical progression of the diseases, includes increased lipid peroxidation as a result of the cellular membrane degeneration and DNA damage. The increase in the levels of MDA indicated the raise of lipid peroxidation as a result of the elevation in free radical generation these free radical may cause profound variation in the function of the cell membrane and also structural organization of the DNA leading to mutations therefore it can be said that lipid peroxidation product is

one of the probable reasons of Hodgkin disease progression (Marnett , 1999 ; Wagner , 1998).

The reason for decreasing of vitamin E concentration in Hodgkin disease patients might be attributed its ability to scavenge lipid peroxides and prevents the cellular damage by the free radicals (Ansell 2018). Vitamin E is the major antioxidant in cell membranes where it protects membrane structure from lipid peroxidation and severe oxidative damage. Which tends to be initiated at polyunsaturated fatty acids (Gillhan *et al.*, 2000 ; Maureen , 2004).

The decrease in vitamin C levels may be associated with its act as antioxidant directly react with O_2 and OH^- and resynthesis of vit.E from tocopherol radicals which is formed from lipid peroxidation, for that reason vit.C participates with vit.E in the protection of cells from injury (Koechlin , 1998 ; Stahl and sies , 1997).

Uric acid is also an internal antioxidant (which is built into the body) and is involved in the removal of oxidation either directly by its association with metal ions or by its

direct removal of the free radicals formed by oxidations (Banerjee , 2008). Many metabolic abnormality can be seen in patients with cancers like lymphomas received chemotherapy or can happen without chemotherapy spontaneously in patients with a high tumor cells burden, the cause is the rapid lysis of tumor cells resulting in the release of high quantities of intracellular components in the serum like uric acid, phosphate and potassium (Yiwei *et al.*, 2018).

Elevated bilirubin level and Jaundice according to Hodgkins lymphoma has been observed , the causes of abnormal liver function in these patients include hepatic infiltration by lymphoma cells, extrahepatic bile duct obstruction , complication of chemotherapy or viral hepatitis (Kiong – Ming , *et al.*, 2013).

Decrease albumin level in patients with hodgkins lymphomas was noted in this research . A defect in synthesis of albumin is the most accepted mechanism for hypoalbuminemia in hodgkins disease (Gobbi, *et al.*, 1985) .

Serum SOD activity has a significant increase in patients when

compare to control . which in agreement with a study investigating many cases with cancer showed elevated activity of SOD especially in advanced stages of disease. (Chan *et al.*,. 2007) .

Vitamin A has been involved as a biological agent in reducing the incidence with cancer, this research show reduce level of vitamin A, this result is conformable with several study which observe decreased levels in vitamin A in patients with brain , breast, lung and gastric tumors .(Chen *et al.*,.(2017).

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