X- Ray effects on free radical levels in diabetics & healthy controls

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ABSTRACT

The study was designed to show the effect of X-Ray on the level of antioxidants in normal healthy subjects and those with a habits mellitus. It was done in the department of physiology and medical physics in the Medical College of Al-Mustansiriyah University. The study was Design as Prospective, randomized, case control study. Antioxidant measurements include the estimation of erythrocyte glutathione and malandialdehyde levels.

Data showed that erythrocyte glutathione (GSH) is lower in diabetic subjects them in healthy individuals while the mulondialdehyde (MDA) is significantly higher in diabetics them in normal subjects. X-Ray irradiation caused significant increase in GSH level in both groups while it dose significantly lowered the level of MDA in diabetics and healthy subjects.

We concluded that Hyperglycemia is the major source of producing free radicals in diabetic subjects and ionizing radiations have great effects in the production of free radicals in both, healthy subject and diabetic individuals.

Key Words: Free radicals, Anti-oxidants, Diabetes mellitus and X-Ray radiation.

INTRODUCTION

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) "free radicals" and antioxidant defenses (enzymatic and non enzymatic) which is responsible for the protection against ROSs, this imbalance may be either due to overproduction of ROSs or a deficiency in the antioxidant system.(1)

Oxidative stress in human system comes from a variety of sources including normal physiological event such as mitochondrial respiration and exposure to ionizing radiation, an increase in oxygen supply (hyperoxia) increase the natural formation of ROSs(2). Cellular responses to oxidative stress up pear to extremely drive and may result in functional alteration of various biological processes. (3)

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Cell survival, cell cycle, metabolism and gene regulation have all been shown to be affected by exposure to oxidative stress. [4] Oxidative stress activity has been implicated during inflammation, aging, malignant changes and the development of microvascular complication in certain metabolic disorders like diabetes mellitus. [5]

One of the specific alterations in oxidative stress syndrome is lipid peroxidation. The lipid peroxidation which was formed of free radicals with polyunsaturated fatty acids (within the cell membrane or lipoprotein) as most fatty acid are highly susceptible to oxidation by oxygen free radicals, which result in the formation of lipid peroxides. [6] The intermediate lipid peroxy radicals (Loo^{o}) which may attack fatty acid side chains yielding a lipid hyperoxide (LooH) and a new polyunsaturated fatty acid radicals, thus initiating a free radical chain reaction [7] the measurement of putative elevated end-products of oxidation of polyunsaturated fatty acid is by the measurement of malondialdehyde (MDA) which is regarded as indicator of lipid peroxidation and oxidative stress [8].

The antioxidants are compounds protecting other components of the organism or cell (proteins, nucleic acid, and lipid). They act together as an integral system and are classified in to three groups:

1. Primary Antioxidants: Acts by preventing the initiation of peroxidation by scavenging species capable of abstracting hydrogen atoms such as hydroxyle radical (OH^{o}) e.g. albumin, ferritin, Trans ferrin and cerulplasmin loplasmin[9].
2. Secondary antioxidants: Act by scavenging the already formed ROS. So called chain breaker e.g. superoxide dismutase (SoD), catalase (CAT), Glutathione peroxidase (GSH) and Glutathione reductase (GSH-R), vit. C, vit. E and uric acid [10].
3. Tertiary Antioxidants: Act by repairing or removing the damaged tissues such as methionine, sulfoxide redactase and proteases [11]

The Glutathione (GSH) is a simple tripeptide forms a major cellular antioxidant and found in high concentration in most mammalian cells and reacts non-enzymatically with ROS so elevated levels of GSH augments intracellular protection against reactive intermediates [12].

Low levels of GSH have been implicated in numerous pathological conditions including diabetes [13] and it protects the cell membrane against oxidative stress & prevents endothelial dysfunction. [14]

Diabetic patients are stated to be under oxidative stress which the later is frequently proposed to be related to hyperglycemia,[15] increased generation of ROS is a feature of hyperglycemia in type II D.M [16] and the increased free radicals damage during hyperglycemia because of the ability of glucose for auto-oxidation which release different oxygen free radicals. [17]

People with DM have an early impairment of plasma antioxidant capacity and increased oxidative stress even in early stages of the disease which could cause tissue damage in diabetes which may be responsible for initiation and progression of diabetic complications. [18]

The last two decades studies had confirmed the association between hyperglycemia and enhanced plasma free radicals concentration. [19]

Aims of the study: this study was designed to:

1. Estimate the level of free radicals indirectly through the estimation of antioxidant system in healthy & diabetic subjects.
2. Investigate the effects of diagnostic X-Rays on free radical production in normal & diabetic blood samples in vitro.

MATERIALS & METHODS

Forty two healthy individuals (27 males & 15 females) with a mean age of 35.2 $\pm$ 8.5 years were included as a control group, all were normal an routine laboratory tests and free of glucose intolerance ensured by carbohydrate challenge test (WHO 1985) [20].

A fifty six diabetic subjects (17 males & 39 females) with a mean age of 47.07 $\pm$ 10 years having type II D.M who was on diet alone and or oral hypoglycemic agents. The obtained venous blood was divided into two groups, the first group was kept without irradiation and served as a control group and the second group was irradiated. The serum levels of MDA & erythrocyte glutathione were estimated for all blood samples.

The estimation of MDA is based on the reaction of thiabarbiturate acid (TBA) using the modified shah and Walker methods [21] while erythrocyte glutathione is calculated according to Beutler method. [22]

The X-Ray machine used for irradiation of blood samples is of diagnostic X-Ray machine.

The irradiated blood samples were put in plain tubes of 10ml and set up in phantom container which contains bolus grains, the container is considered as tissue equivalent material which was put under the window of X-ray machine, in limited field size of 8 x 6 cm at source to skin distance of 80 cm, the samples are irradiated at total doses between 0.5 to 6.5 cGy (Fig.1). The oxidative stress markers measurements were performed on the control and irradiated samples. The time of irradiation was determined depending on the total dose required.

RESULTS

The level of reduced glutathione in the control group was 54.89±10.70 mg/dL (before radiation) which was significantly higher ($P<0.01$) than that of the diabetic group which was 33.25±7.80 mg/dl. After irradiation at dose of 0.5 cGy the level of reduced glutathione in control group was increased insignificantly from 54.89±11.13 to 58.57±11.13.

While that of the diabetic group was increased from 33.25±7.80 to 36.86 which was also insignificant (Table 1).

For the control group, when the doses of radiation were gradually increased, the level of glutathione showed similar progressive increase in its level with the subsequent increase radiation.

The increase in the level of reduced glutathione was significant in its value ($P<0.01$) for each increase in the radiation doses (Table2) (Figure 2).

The diabetic subjects showed parallel increment in the level of reduced glutathione when exposed to gradual increasing doses of radiation.

The subsequent level of reduced glutathione was significantly higher than the previous reading before radiation ($P<0.01$) for all doses of radiation except the first dose (Table 2) (Figure 2).

Then when we compare the level of GSH between control and diabetic group for individual radiation doses the results showed that the increase in the radiation doses by 1 cGy had lead to gradual increase of the level of reduced glutathione with a significant difference between control and diabetic group for the corresponding dose ($P<0.01$) (Table 1).

Assessment of lipid peroxidation:

The by-product of lipid peroxidation, malondialdehyed (MDA) was determined in plasma tend to be significantly higher ($P<0.01$) in diabetic subjects 8.46 ± 1.35 μmol/L than the controls 5.56 ± 1.41 μmol/L (Table 3). After irradiation, in control group at dose of 0.5 cGy the level of MDA showed insignificant decrease from 5.56 ± 1.41 to 4.75 ± 1.11 (Table 4), (Fig. 3). In diabetic subjects, also after irradiation at

dose of 0.5 cGy there was insignificant decrease in MDA, While higher doses of radiation lead to gradual significant decrease ($P<0.01$) in the level of MDA (Table 4), (Fig. 3). In comparing the post irradiation value of MDA between the control and diabetic group for each individual dose of radiation, the control group showed significant lower levels than the diabetic subject ($P<0.01$), (Table 3).

Table (1): Erythrocytes glutathione values relative to dose radiation (cGy) in control and in diabetic sample and compare between two groups.

		Group I……. (Control)		Group II…..(Diabetes mellitus)	
		BeforeRadiation	AfterRadiation	BeforeRadiation	AfterRadiation
0.5 cGy	Mean	54.89	58.57	33.25	36.16
	± SD	10.70	11.13	7.80	5.86
	P1-value		NS		NS
	P2-value			P<0.01	P<0.01
1.5 cGy	Mean	54.89	66.28	33.25	42.85
	± SD	10.70	10.27	7.80	4.55
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
2.5 cGy	Mean	45.89	74.08	33.25	48.96
	± SD	10.70	8.03	7.80	6.16
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
3.5 cGy	Mean	45.89	82.11	33.25	54.92
	± SD	10.70	7.26	7.80	7.64
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P>0.01
4.5 cGy	Mean	54.89	88.31	33.25	59.88
	± SD	10.70	9.63	7.80	8.35
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
5.5 cGy	Mean	45.89	93.42	33.25	63.09
	± SD	10.70	4.69	7.80	8.17
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
6.5 cGy	Mean	54.89	98.54	33.25	65.20
	± SD	10.70	2.34	7.80	7.99
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01

Table (2): plasma malondialdehyde (MDA) values relative to radiation doses (c GY) in control and diabetic patients

	Before	Radiation Doses

		Radiation	0.5 cGY	i.5 cGY	2.5 cGY	3.5 cGY	4.5 cGY	5.5 cGY	6.5 cGY
Group 1 (Control)	Mean	5.56	4.75	3.90	3.72	3.52	3.15	2.88	2.50
	± SD	1.41	1.11	0.86	0.90	1.16	1.03	1.05	0.88
	P		NS	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01
Group 2 (Diabetes Mellitus)	Mean	8.46	7.76	7.42	6.97	6.65	6.34	5.98	5.69
	± SD	1.35	1.57	1.33	1.36	1.29	1.25	1.22	1.26
	P		NS	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01

- **NS: Not Significant, P-value: statistical differences before and after irradiation.**

Table (3): Erythrocytes glutathione values relative to radiation doses (cGy) in control and in diabetic Sample

		Before Radiation	Radiation doses						
			0.5 cGy	1.5 cGy	2.5 cGy	3.5 cGy	4.5 cGy	5.5 cGy	6.5 cGy
Group I (Control)	Mean	54.89	58.57	66.28	74.08	82.11	88.31	93.42	98.54
	± SD	10.70	11.13	10.27	8.03	7.26	9.63	4.69	2.34
	P		NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Group II (Diabetes mellitus)	Mean	33.25	36.16	42.85	48.96	54.92	59.88	63.09	65.20
	± SD	7.80	5.86	4.55	6.16	7.46	8.35	8.17	7.99
	P		NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

- **NS: Not significant, P-value: statistical difference before and after irradiation**

Table (4): plasma malondialdehyd (MDA) values relative to doses of radiation (cGy) in control and in diabetic sample and compare between two groups

		Group I (Control)		Group II (Diabetes mellitus)	
		Before Radiation	After Radiation	Before Radiation	After Radiation
0.5 cGy	Mean	5.56	4.75	8.46	7.76
	± SD	1.41	1.11	1.35	1.57
	P1-value		NS		NS
	P2-value			P<0.01	P<0.05
1.5 cGy	Mean	5.56	3.90	8.46	7.42
	± SD	0.14	0.86	1.35	1.33
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
2.5 cGy	Mean	5.56	3.72	8.46	6.97
	± SD	1.41	.90	1.35	1.36
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
3.5 cGy	Mean	5.56	3.52	8.46	6.65
	± SD	1.41	1.16	1.35	1.29
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P>0.01
4.5 cGy	Mean	5.56	3.15	8.46	6.34
	± SD	1.41	1.03	1.35	1.25
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
5.5 cGy	Mean	5.56	2.88	8.46	5.98
	± SD	1.41	1.05	1.35	1.22
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01
6.5 cGy	Mean	5.56	2.50	8.46	5.69
	± SD	1.41	0.88	1.35	1.26
	P1-value		P<0.01		P<0.01
	P2-value			P<0.01	P<0.01

NS: Not significant, P1-value: statistical difference before and after irradiation, P2-value: group II as compared with group I

DISCUSSION

In the present study, the level of erythrocyte glutathione is significantly lower in diabetic subjects than the controls ($P<0.01$) before irradiation, this difference between the two group equal to (39.4%). Our result was in agreement with chough [23] *et al*, (1999); Coleman and Rustioni, (1999).[24]

The reduction in the level of erythrocytes glutathione may be due to the prolonged exposure to hyperglycemia in diabetic subject which will increase the oxidative stress and then lead to increase free radicals generation. This continuous production of free radicals for long period could exhaust the scavenging system.

Our finding and explanation are in harmony with Zhao, (2001) [25] who reached to the same conclusion.

When the blood sample of healthy volunteer was exposed to different doses of radiation, we found that at dose 0.5 cGy there was insignificant increase in the level of glutathione while other doses of radiation cause significant increase ($P<0.01$) in the level of glutathione when compared to un-irradiated sample, this increase was (20.8%, 34.9%, 49.6%, 60.9%, 70.2% and 79.5%) at doses of radiation (1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 cGy) respectively.

The gradual increment in the glutathione level indicates that cellular defense mechanism is well responding to the production of high free radical induced by radiation which stimulate the scavenging system of which GSM is one of the most important antioxidant molecules of the cell. These results were in agreement with Bairtey and Goodwin, 2001[26] (in human), Nakajima and Yukawa, (1996) were reached to the same result when conduction this test on animals. When the blood of the diabetic subjects was exposed to X-ray the level of glutathione was insignificant increased at dose 0.5 cGy. However, the level had also changed with an increment criteria by the following percentage (28.9%, 47.3%, 65.2%, 80.1%, and 96.19%) at doses (1.5…6.5 cGy) respectively.

It was not possible to compare our result with others as there was no previous report to compare with. This was a novel observation to study the effect of radiation on cellular defense mechanism expressed by erythrocytes glutathione in diabetic subjects. This proportional increase in glutathione level with the doses of radiation have multiplied the free radical production and stimulated the glutathione.

So because as glutathione is a broad spectrum free radical scavenger agent and its level was increased as a cellular response to the increased free radical level.

In our study we found that the level of malondialdehyde (MDA); the product of lipid peroxidation was significantly higher ($P<0.01$) in diabetics than the controls (34.3%) before irradiation, this results was in agreement with the previous studies (Chugh *et al*, 1999[23]; Slatter *et al*., 2000) [28] which revealed increased level of oxidative products of lipid in diabetic (Abdella *et al*, 1990[29]; Aremstrong *et al*, 1992).[30]

The elevation in the level of MDA may be due to elevated oxidative stress and this is attributed to the fact that increased production of reactive oxygen species and protein glycation during the course of diabetes and also altered intracellular ratio between free radicals and antioxidant system, all will promote lipid per oxidation in diabetic patients (Oranje et al., 1999).[31]

After irradiation the same scenario of erythrocytes glutathione is repeated but in the reverse direction, the level of MDA at dose 0.5 cGy, showed insignificant decrease in the healthy and diabetic blood but there was significant decrease ($P<0.01$) by (29.9%, 33.1%, 36.7%, 43.3%, 48.2%, and 55%) in healthy blood and by (12.3%, 17.6%, 21.4%, 25.1%, 29.3%, and 32.7%) in diabetic blood at radiation doses (1.5…...6.5 cGy) respectively. These results were in agreement with Nakajim and Yukawn, (1996) [27], who conduct this procedure on rats. No study was performed to link between radiation and diabetes, so we the first team who studied the effects of radiation on the level of malondialdehyde (MDA) in

diabetic subjects. The decrease in the level of MDA could be connected to the observation that increase in the level of glutathione which is a powerful scavenging system have protected the cell form the harmful effect of free radical produced by radiation and its level, by not allowing the attack of phospholipids in the cell membrane and producing MDA.

CONCLUSION

After broad observation to our study we would conclude the following:

1. Diabetes mellitus has a powerful cause for free radicals' production through hyperglycemia. Diabetic subjects have lower scavenging system (glutathione, catalase enzyme and uric acid) with high lipid per oxidation product malondiadehyde as compared with controls.
2. Diagnostic X-Ray was also a second independent cause for enhancement of free radicals production in normal and diabetic blood through all doses of radiation that was used in our study except the first dose which was 0.5 cGy.

It is possible to extend our study to evaluate the effect of diagnostic x-ray on experimental animals in vivo. We could use higher doses of radiation and estimates its effect.

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