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Effect of low level ionizing radiation on endothelial progenitor cells in atherosclerotic patients with lower limb ischemia

Hoda El-sayed Mohammed[1], Mohammed Helmy EL-batanony [2], Soheir Saad Korraa[3], Eman Fayez Said Taha*[3]

[1]Biochemistry department, Faculty of Pharmacy, Zagazig University, Egypt.
[2]General and Vascular Surgery department, Faculty of Medicine, Cairo University, Egypt.
[3]National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt.
Corresponding author e-mail: emanfayez81@yahoo.com

ABSTRACT

Various studies had underlined the important role of bone marrow–derived endothelial progenitor cells (EPCs) in vasculogenesis and angiogenesis of ischemic tissue, but only a few studies had concentrated on the role of low doses of ionizing gamma radiation on EPCs in the prevention and therapy of atherosclerosis. Extended endothelial cell damage by cardiovascular risk factors can result in endothelial cell apoptosis with loss of the integrity of the endothelium. The consequences are an increased vascular permeability of the endothelium followed by facilitated migration of monocytes and vascular smooth muscle cell proliferation, resulting in a premature manifestation of an atherosclerotic lesion. A growing body of evidence suggests that circulating EPCs play an important role in endothelial cell regeneration.

The present study included 30 patients complaining of lower limb ischemia attributed to atherosclerosis and the presented data were statistically evaluated in relation to a control group of 30 normal healthy volunteers, age and socioeconomic matching volunteers.

The current study focuses on the role of low level ionizing radiation on increasing number of EPCs in atherosclerotic patients with lower limb ischemia. Present study demonstrated that low doses of ionizing radiation at 0.25 Gy caused a significant increase in the levels of $CD34^+$, $CD133^+$, KDR^+ and $CD133^+KDR^+$ blood mononuclear endothelial progenitor cells in atherosclerotic patients and decrease apoptosis of these cells. Irradiation of blood of atherosclerotic patients appeared to be effective in minimizing lipid peroxidation as well as increasing the antioxidant activity such as superoxide dismutase and the level of nitric oxide which may be involved in multiple biological processes.

Low dose of ionizing radiation has an ameliorative effect on endothelial progenitor cells.

Key words: Atherosclerosis, Endothelial progenitor cells, CD34+, CD133+, KDR+, Low dose ionizing radiation.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality throughout the developed world (Williamson *et al.*, 2012). Coronary artery disease (CAD) or atherosclerotic heart disease is a chronic life-threatening disease, which is characterized by reduced blood supply to the heart as a result of the accumulation of atheromatous plaques within the walls of the arteries supplying the myocardium.

Progressive atherosclerosis in the coronary arteries may lead to intimal thickening and eventual artery occlusion. Coronary artery occlusion can cause acute myocardial ischemia as a result of reduced oxygen supply or increased oxygen demand (Luthje and Andreas, 2008). Convincing evidence indicates that atherosclerosis is associated with endothelial dysfunction at the early stage of the disease process (Chiang *et al.*, 2012).

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In recent years, endothelial progenitor cells (EPCs) have gained a central role in vascular regeneration and endothelial repair capacity through angiogenesis and restoring endothelial function of injured blood vessels. These bone–marrow-derived cells are capable of promoting neovascularization, improving blood perfusion, and facilitating the recovery of ischemic tissues through differentiation into functional endothelial cells and secretion of angiogenic mediators (Berger and Lavie, 2011).

Ionizing irradiation had been shown to have angiogenic potential in malignant and nonmalignant diseases. It was demonstrated for the first time that ionizing radiation stimulates hypoxia-inducible factor-1 (HIF-1α) up-regulation in endothelial cells (ECs) , a HIF-1α–independent up-regulation of stromal cell–derived factor-1 (SDF-1), as well as endothelial migration, all of as which are essential for angiogenesis. Ionizing radiation activates a novel pathway stimulating ECs migration directly through the expression of SDF-1 independent of HIF-1 α induction. Low doses ionizing gamma radiation result in up-regulation of the vasculogenic chemokine SDF-1 and subsequent improved EPCs chemotaxis (Lerman *et al.*, 2010). The aim of the present study is to identify the relationship of number of $CD34^+$, $CD133^+$ and KDR^+ blood mononuclear endothelial progenitor cells to direct measures of atherosclerosis compared to control and to elucidate the enhancing effect of low dose ionizing radiation on cultured blood mononuclear endothelial progenitor cells number after 24 h with respect to, decreasing apoptosis and oxidative stress and also increasing the level of NO in atherosclerotic patients.

PATIENTS and METHODS

The present study includes 30 patients complaining of lower limb ischemia attributed to atherosclerosis. Participants for this study were recruited from the department of general and vascular surgery unit in El-kasr El-eny hospital, Faculty of Medicine, Cairo University.The presented data were statistically evaluated in relation to a control group of 30 normal healthy volunteers, age and socioeconomic matching volunteers. All the studied patients were conducted to laboratory investigations including serum fasting blood glucose level and complete serum lipid profile. Moreover, all the patients were subjected to Duplex ultrasonographic evaluation of the lower limb, and accordingly selected patients were further investigated by arteriography.

Collection of blood sample:

Whole blood: 16 ml of peripheral blood was collected into 8 tubes of a 2-ml Vacutainer (Becton Dickinson, Basel, Switzerland) tubes containing liquid tri-potassium ethylene diamine tetra-acetic acid (K3EDTA) as an anticoagulant then mixed well by inversing the tubes up and down several times, shaking was avoided and processed within 2 hrs of collection. After selection, blood samples from investigated patients and from controls were divided into two parts for:

A. Flow Cytometric Investigations: Flow cytometric Investigations included the separation of blood mononuclear cells by density-gradient centrifugation using Ficoll-Paque PLUS (sigma, Saint Louis, MO), which were further cultured for 24 hours with or without exposure to ionizing gamma radiation and then subjected to enumeration by flow cytometry. The flow cytometer used is FACS caliber flow cytometer (Becton Dickinson, Sunnyvale, CA, USA) equipped with a compact air cooked low power 15 mwatt argon iron laser beam (488nm). CD marker and apoptosis histogram derived from flow cytometry was obtained with a computer program for Dean and Jett mathematical analysis (Dean and Jett, 1974). Γ-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using a ^{60}Co Gamma Cell-40 biological irradiator. Cultured MNCs were exposed to 54 unit of γ-radiation that was equivalent to o.25 Gy (25 cGy) of ^{60}Co.

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B. Instrument: ^{60}Co trady-800 from Argentina.

Cultured blood mononuclear cells (BMNCs) from investigated patients were divided into three subgroups besides control group.

Control Group (Normal healthy)

Group I: Non-irradiated BMNCs at zero time in culture media

Atherosclerotic patients:

Group II: Non-irradiated BMNCs at zero time in culture media

Group III: Non-irradiated BMNCs 24 hrs. in culture media.

Group V: Irradiated BMNCs with LDIR (0.25 Gy of 60 Co) 24 hrs. in culture media.

C. Biochemical investigation: Biochemical investigation of plasma and blood for the determination of superoxide dismutase enzyme (SOD) activity according to the method of (Minami and Yoshikawa, 1979), nitric oxide (NO) level according to the method described by (Miranda *et al.*, 2001) and lipid peroxidation measured as malondialdehyde (MDA) according to (Yoshioka *et al.*, 1979). After selection of patients and controls; blood of 30 patients was classified into two subgroups besides control group.

Control (Normal healthy volunteers)

Group I: Non-irradiated blood

Atherosclerotic patients:

Group II: Non-irradiated blood

Group III: Irradiated blood with low dose ionizing gamma radiation (0.25 Gy of ^{60}Co)

Sample preparation

Plasma:

1. Collect blood (2 ml for lipid peroxidation determination and 2 ml for NO) using an anticoagulant (EDTA).

2. Centrifuge at 4,000 for 10 minutes at 4°C.

3. Collect the plasma for assaying and store on ice If not assayed on the same day, freeze at -80°C.

Chemicals

Chemicals used in the present study were of high analytical grade and purchased from: Biosource (Germeny), Spinreact (Germeny), Sclavo (Italy), Biocon (Germany), Merck (Germeny) and Sigma (USA).

Statistical Methods

Continuous data are presented as mean ± standard error. The means of continuous variables were compared using a normalized linear model for data. Categorical data were investigated using Chi-Square test (x^2) of association, where appropriate. Adjustment for parental age was carried out using a generalized linear model for continuous variables and logistic regression for categorical variables. Correlation was measured using Pearson's coefficient. Linear regression analysis was used to investigate the relationship between cultured blood mononuclear EPCs number in atherosclerotic patients with lower limb ischemia and normal healthy, The probability of error (P value) was expressed as follows: P>0.05=non-significant, P value of less than 0.05=significant, and P value of less than 0.01=highly significant. The significance of the results was calculated by the aid of a digital computer, using SPSS version 16.0 program.

RESULTS

The results of the present study are summarized, statistically analyzed and presented in the following tables and figures. The comparison analyses included blood mononuclear cells (BMNCs) of 30 patients with atherosclerosis suffering from lower limb ischemia, compared to BMNCs of control. Table (1) shows that both groups are comparable as regard age, gender and smoking.

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Table (1): Characteristics of atherosclerotic patients suffering from lower limb ischemia and control.

Groups		Control	Atherosclerotic patients	*P*
Age in years (Mean± SD)		50.8± 5.8	58.2± 4.9	NS
Gender (n, %)	Male	16 (53.3 %)	21 (70%)	NS
	Female	14 (46.7 %)	9(30%)	
History of smoking (n, %)	Presence	14 (46.7 %)	18 (60%)	NS
	Absence	16 (53.3 %)	12 (40%)	

NS:non-significant

Effect of ionizing gamma radiations at 0.25, 0.125 and 0.0625 Gy of ^{60}Co on blood mononuclear endothelial progenitor cells in atherosclerotic patients

Results in Figures (1, 2, 3, 4) show that, ionizing radiation at doses of 0.25, 0.125 and 0.0625 Gy of ^{60}Co were found to have significant increase in levels of $CD34^+$, $CD133^+$, KDR^+ and $CD133^+KDR^+$ blood mononuclear endothelial progenitor cells in atherosclerotic patients after 24 hrs. as respect to cells counted without radiation after 24 hrs. Cells exposed to a single dose of 0.25 Gy radiations, showed high significant increase in cells compared to the other doses, also it decreased early apoptosis suggesting an increase in EPCs numbers. Thus, a dose of 0.25 Gy radiations was used for all subsequent experiments.

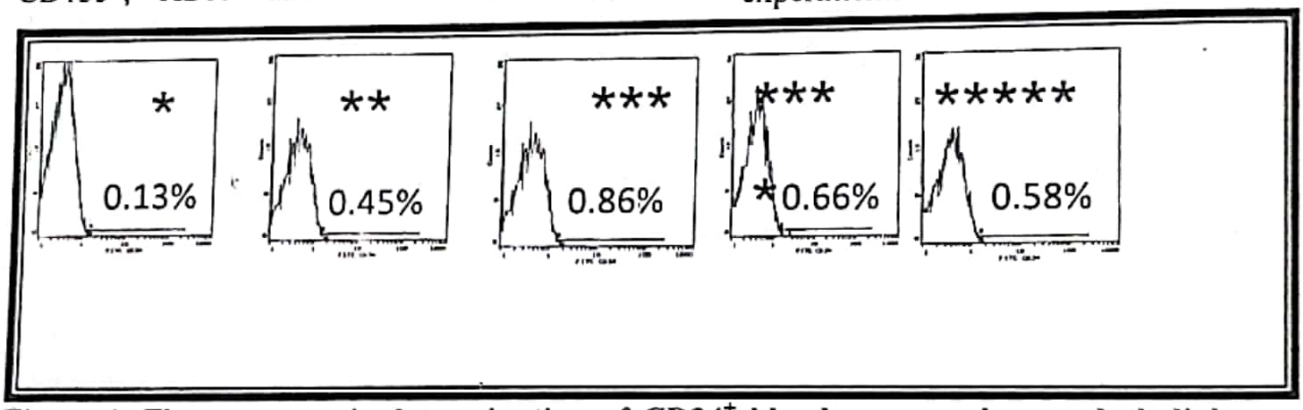

Figure 1. Flow cytometric determination of $CD34^+$ blood mononuclear endothelial progenitor cells in atherosclerotic patients with lower limb ischemia before and after low doses of ionizing radiation.

(*) $CD34^+$ cells (%) at zero time in culture media before ionizing radiation ,(**) $CD34^+$ cells (%) after 24 hrs. in culture media before ionizing radiation, (***) $CD34^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.25 Gy of γ-radiation, (****) $CD34^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.125 Gy of γ-radiation,(*****) $CD34^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.0625 Gy of γ-radiation

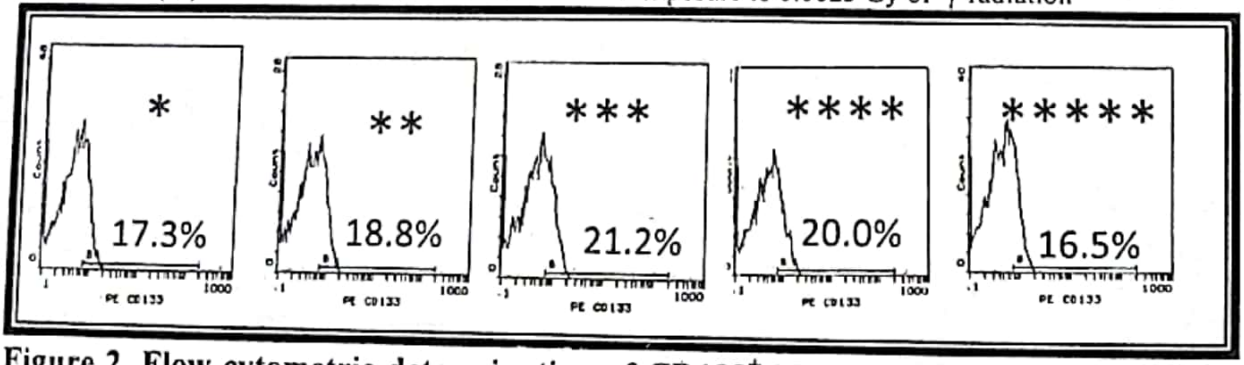

Figure 2. Flow cytometric determination of $CD133^+$ blood mononuclear endothelial progenitor cells in atherosclerotic patients with lower limb ischemia before and after low doses of ionizing γ- radiation.

(*) $CD133^+$ cells (%) at zero time in culture media before ionizing radiation ,(**) $CD133^+$ cells (%) after 24 hrs. in culture media before ionizing radiation, (***) $CD133^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.25 Gy of γ-radiation, (****) $CD133^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.125 Gy of γ-radiation,(*****) $CD133^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.0625 Gy of γ-radiation .

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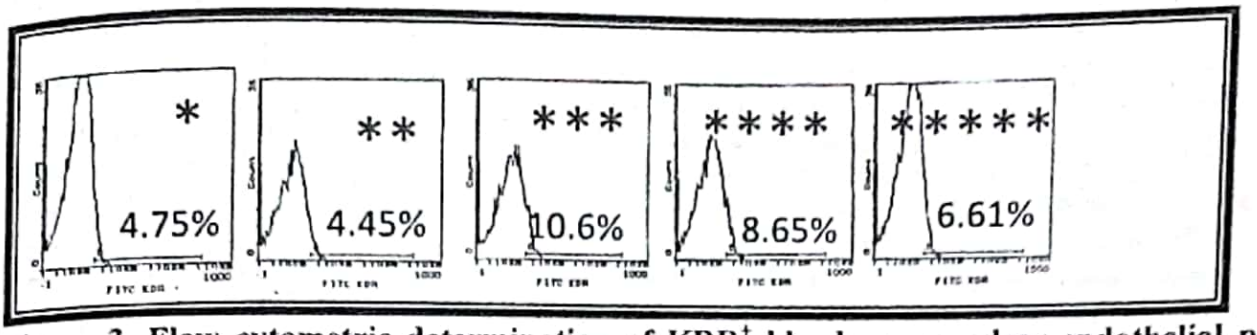
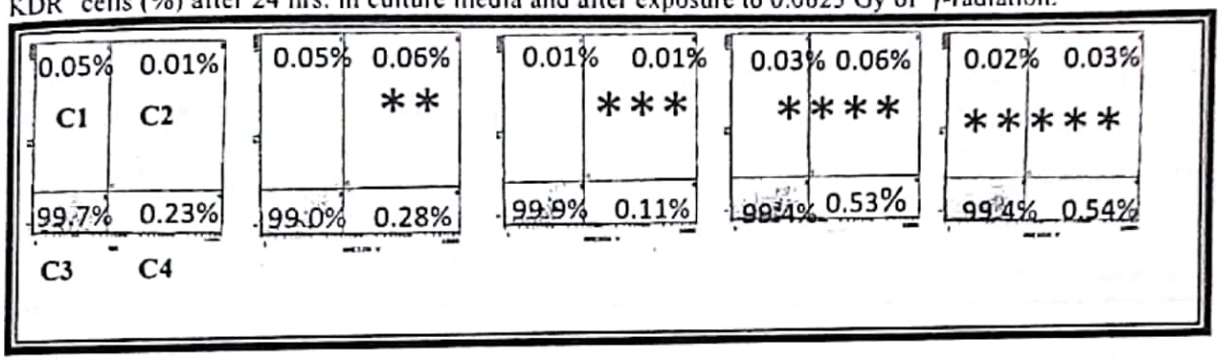


Figure 3. Flow cytometric determination of KDR$^+$ blood mononuclear endothelial progenitor cells in atherosclerotic patients with lower limb ischemia before and after low doses of ionizing γ- radiation.

(*) KDR$^+$ cells (%) at zero time in culture media before ionizing radiation ,(**) KDR$^+$ cells (%) after 24 hrs. in culture media before ionizing radiation, (***) KDR$^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.25Gy of γ-radiation, (****) KDR$^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.125 Gy of γ-radiation,(*****) KDR$^+$ cells (%) after 24 hrs. in culture media and after exposure to 0.0625 Gy of γ-radiation.



C1: necrotic cells (%) C2: Late apoptotic cells (%) C3: Viable cells (%) C4: Early apoptotic cells(%)

Figure 4. Flow cytometric determination of apoptosis, necrosis and viability of blood mononuclear endothelial progenitor cells in atherosclerotic patients with lower limb ischemia before and after low doses of ionizing γ- radiation.

(*) apoptosis at zero time in culture media before ionizing radiation ,(**) apoptosis after 24 hrs. in culture media before ionizing radiation, (***) apoptosis after 24 hrs. in culture media and after exposure to 0.25 Gy of γ-radiation, (****) apoptosis after 24 hrs. in culture media and after exposure to 0.125 Gy of γ-radiation,(*****) apoptosis after 24 hrs. in culture media and after exposure to 0.0625 Gy of γ-radiation.

Concerning the results of Table (2), the levels of CD34$^+$ and CD133$^+$ blood mononuclear endothelial progenitor cells (%) was higher in normal healthy than atherosclerotic patients pre-irradiation at zero time in culture, more over their percentage in atherosclerotic patients post 0.25 Gy of ionizing gamma radiation (^{60}Co) after 24 hrs in culture was also higher as compared to its levels pre-irradiation after 24 hrs in culture.

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Table (2): Statistical analysis for the differences in $CD34^+$ and $CD133^+$ blood mononuclear endothelial progenitor cells percentage in atherosclerotic patients pre and post ionizing gamma radiation as compared to normal healthy

Parameters / Statistics	Normal healthy blood mononuclear cells pre-irradiation at zero time in culture (n=30)	Atherosclerotic blood mononuclear cells pre-irradiation at zero time in culture (n=30)	Atherosclerotic blood mononuclear cells pre-irradiation after 24 hrs in culture (n=30)	Atherosclerotic blood mononuclear cells post-irradiation after 24 hrs in culture (n=30)
CD34				
Mean ± SE	.49 ± .05	.51 ± .05	1.3 ± .35	1.9 ± .37
Minimum-Maximum	.23 - .95	.13 - .96	.18 - 6.9	.24 - 7.1
P^a value <		NS	0.028	.001
P^b value <			.028	.001
P^C value <				.083
CD133				
Mean ±SE	13.4 ± 2.3	4.7 ± 1.2	4.6 ± 1.2	7.4 ± 1.9
Minimum-Maximum	.17 - 42.5	.14 - 20.9	.61-26.3	1.11 - 50.7
P^a value <		.001	.001	.016
P^b value <			NS	.069
P^C value <				.018

Table (3): Statistical analysis for the differences in KDR^+ and $CD133^+$ KDR^+ blood mononuclear endothelial progenitor cells percentage in atherosclerotic patients pre and post ionizing gamma radiation as compared to normal healthy

Parameters / statistics	Normal healthy blood mononuclear cells pre-irradiation at zero time in culture (n=30)	Atherosclerotic blood mononuclear cells pre-irradiation at zero time in culture (n=30)	Atherosclerotic blood mononuclear cells pre-irradiation after 24 hrs in culture (n=30)	Atherosclerotic blood mononuclear cells post-irradiation after 24 hrs in culture (n=30)
KDR^+				
Mean ± SE	3.5 ± 1.04	1.9 ± .37	2.96 ± .33	5.9 ± .82
Minimum-Maximum	.07 - 29.6	.31 - 9.3	.37 - 9.6	.54 -14.9
P^a value <		0.095	NS	.024
P^b value <			.001	.001
P^C value <				.01
$CD133^+$ KDR^+				
Mean ± SE	2.3 ± .44	1.3 ± .32	1.7 ± .28	2.9 ± .36
Minimum-Maximum	.08 - 8.45	.08 - 8.42	.11 - 5.40	.72 - 8.04
P^a value <		0.048	NS	NS
P^b value <			NS	.001
P^C value <				.002

P<0.05: Significant P<0.01: High significant NS: Non- significant
P^a: significant compare to control (Post Hock test)
P^b: significant compare to group (2) according to (Paired test)
P^c: significant compare to group (3) according to (Paired test)

(B) Whole blood for biochemical investigation for determination of SOD activity, NO and MDA level

The concentration of malondialdehyde (MDA nmol/mL) level in plasma, superoxide dismutase activity in blood (SOD U/ml) and NO level (nmol/ml) in plasma were determined in normal healthy (control) and atherosclerotic patients {before and after ionizing radiation}.

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It was found that high significant increase in MDA level (nmol/mL) in plasma of atherosclerotic patients pre and post 0.25 Gy of ionizing gamma radiation (^{60}Co) by 107.5% and 79.8% than in normal healthy plasma. Significant decrease in MDA level (nmol/mL) in plasma of atherosclerotic patients post 0.25 Gy of ionizing gamma radiation (From 151.7 nmol/mL to 131.5 nmol/mL; P<0.073) compared to its group pre irradiation. A high significant decrease in SOD activity (U/mL) and NO level (nmol/mL) in blood of atherosclerotic patients pre-irradiation by 32.2% and 24.21% respectively compared to normal healthy. Also significant increase in SOD activity (U/mL) and non-significant increase in NO level (nmol/mL) in blood of atherosclerotic patients after exposure of blood to 0.25 Gy of ^{60}Co compared to blood pre-irradaition (From 8.2 U/mL to 9.7 U/mL; P<0.03 and from 21.6 nmol/mL to 26.4 nmol/mL; P>0.05 respectively). The main results are given in Table (4) which showed the correlation between estimated parameters and other variables.

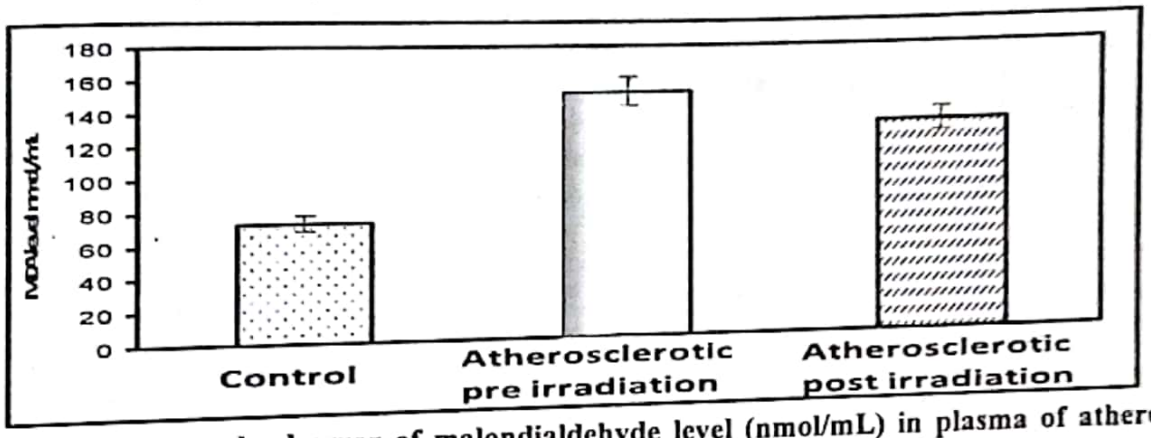

Figure 5. Mean and standard error of malondialdehyde level (nmol/mL) in plasma of atherosclerotic Patients pre and post irradiation compared to control.

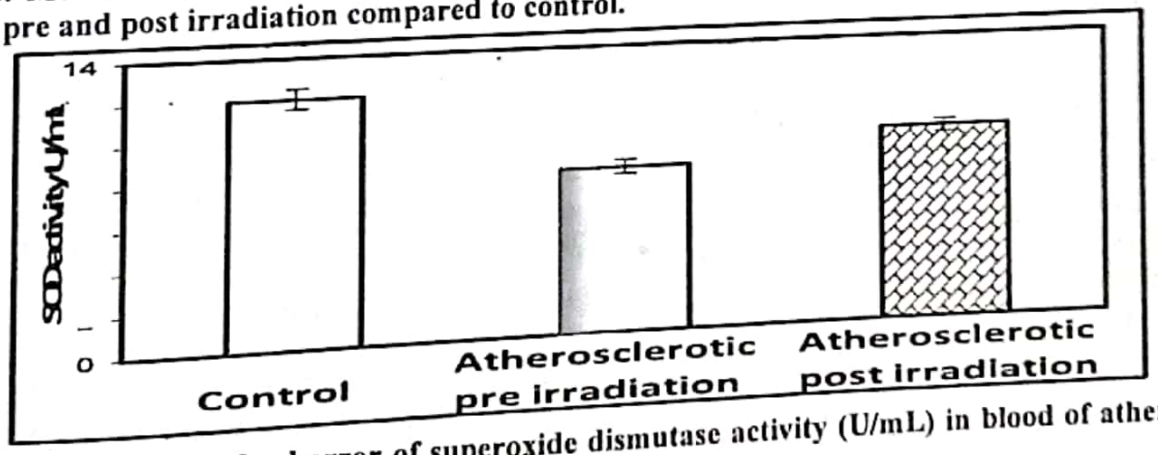

Figure 6. Mean and standard error of superoxide dismutase activity (U/mL) in blood of atherosclerotic Patients pre and post irradiation compared to control

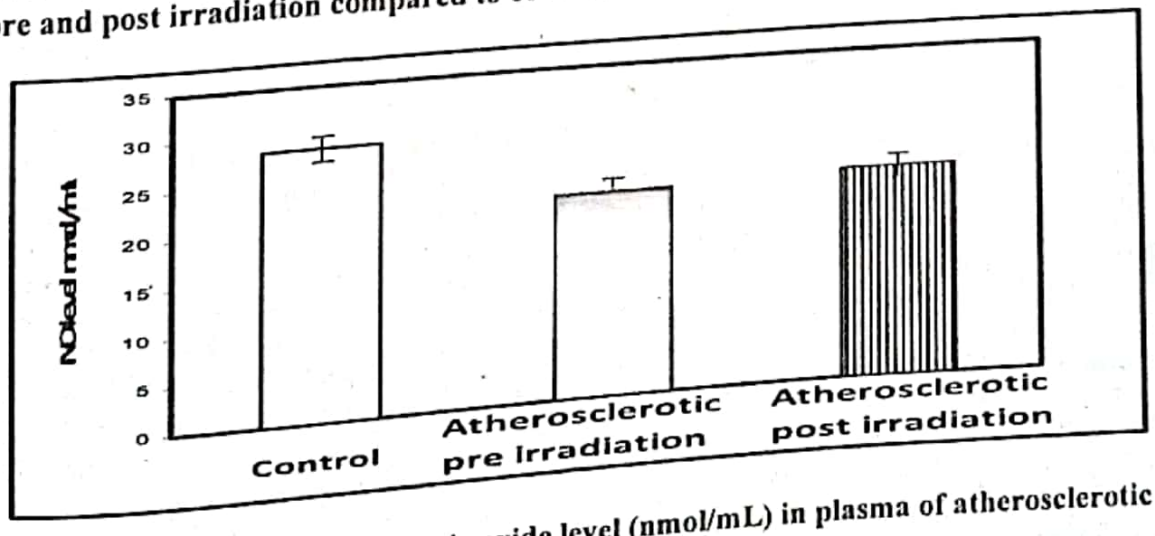

Figure 7. Mean and standard error of nitric oxide level (nmol/mL) in plasma of atherosclerotic Patients pre and post irradiation compared to control.

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Table (4): Significant correlations between some studied parameters (Pearson correlation)

Estimated parameters	Correlated Parameters	r- value	P value<
$CD34^+$ blood mononuclear endothelial progenitor cells	SOD	-.229*	.032
	NO	-.093	NS
	MDA	-.004	NS
$CD133^+$ blood mononuclear endothelial progenitor cells	SOD	-.174	NS
	NO	-.063	NS
	MDA	-.275**	.009
KDR^+ blood mononuclear endothelial progenitor cells	SOD	.048	NS
	NO	.128	NS
	MDA	.003	NS
Early apoptotic blood mononuclear endothelial progenitor cells	SOD	-.241*	0.024
	NO	-.067	NS
	MDA	-.260*	0.013
Late apoptotic blood mononuclear endothelial progenitor cells	SOD	-.115	NS
	NO	-.090	NS
	MDA	-.050	NS
Necrotic blood mononuclear endothelial progenitor cells	SOD	.025	NS
	NO	.066	NS
	MDA	.16	NS
Viable blood mononuclear endothelial progenitor cells	SOD	.129	NS
	NO	-.006	NS
	MDA	0.155	NS

r:Pearson correlation P: Significance

**: Correlation is significant at the 0.01 level (2-tailed).

*: Correlation is significant at the 0.05 level (2-tailed).

-: Negative correlation

DISCUSSION

Atherosclerosis is a progressive disease characterized by endothelial injury and lipid aggregation in the arterial walls (Ma *et al.*, 2006), gradual arterial wall thickening and formation of an atherosclerotic plaque. The integrity of the functional endothelial monolayer, which lines the lumen of all blood vessels, plays a critical role in the development of this process. Damage can result in apoptosis, or inflammatory conditions can stimulate EC dysfunction, consequently resulting in monocyte infiltration, formation of foam cells and the initial stages of a developing atherosclerotic plaque. It was suggested that endothelial injury in the absence of sufficient circulating EPCs may affect the progression of cardiovascular disease (Hill *et al.*, 2003; Vasa *et al.*, 2001), and that the amount of circulating EPCs, measured in terms of circulating blood mononuclear cells (BMNCs) that express $CD34^+$, $CD133^+$ and KDR^+ surface markers offer ideal markers for assessing environmental effects (Kondo *et al.*, 2004). Little is known about the effects of ionizing radiation on the levels of circulating EPCs. Accordingly, the aim of the present study is to identify effect of ionizing radiation on circulating EPCs in vitro. Apoptosis within such cells and changes in lipid peroxidation and nitric oxide were evaluated in order to estimate the effect of ionizing radiation on biochemical environment surrounding EPCs. In the present study, a non-significant increase in $CD34^+$ blood mononuclear cells surface marker among atherosclerotic patients compared to controls (0.51 ± 0.05 vs. 0.49 ± 0.05). A decreased number of circulating EPCs has been shown to be significantly associated with cardiovascular disease (CVD) (Schmidt-Lucke *et al.*, 2005; Werner *et al.*, 2005). Decreased EPCs numbers also have been associated with aging, increased number and level of coronary artery disease risk factors, and increased 10-year risk of coronary artery disease in clinical patients. Incidence of death from cardiovascular causes was observed in patients with low baseline levels of EPCs (Werner *et al.*, 2005); however, this finding has not been consistently confirmed in healthy individuals (Chen *et al.*, 2006). Peter *et al.*, (2009) reported that, increasing presence of traditional cardiovascular risk factors, such as hypertension, diabetes, hyperlipidimia and smoking through

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multiple mechanisms, leads to reduction in levels of EPCs in the circulation, homing of EPCs to sites of endothelial damage occurs. Over time, pools of bone marrow EPCs might become depleted. EPCs mobilization from the bone marrow is also impaired; decreased levels of NO are thought to be responsible for decreased mobilization. Aspects of EPCs proliferation, differentiation and apoptosis are affected by inflammatory mediators. In the present study, markers of oxidative stress were measured in terms of lipid peroxidation measured as malondialdehyde, plasma nitric oxide (NO) and superoxide dismutase (SOD) activity in blood of atherosclerotic patients compared to controls. Results showed that the level of malondialdehyde (MDA) (nmol/mL); an indicator of lipid peroxidation, was significantly higher(107.69%) among atherosclerotic patients with lower limb ischemia compared to normal healthy subjects. The present data show a significant increase in percentage of $CD34^+$ cells in atherosclerotic patients 24 hrs in culture without radiation compared to their level before irradiation (from .51 % to 1.3 %; P<.028). Also, a significant decrease in $CD133^+$ cells percentage {from 4.7 % to 4.6 % P<0.028} and a high significant increase in KDR^+ blood mononuclear endothelial progenitor cells (%) (From 1.9 % to 3.8 %; P<0.001) 24 hrs in culture without radiation compared to cells at zero time. Moreover, a significant increase in $CD133^+$ KDR^+ BMNCs {from 1.3 % to 1.7 %; P< 0.047} in atherosclerotic patients 24 hrs after culture without radiation compared to cells at zero time this may attributed to the effect of culture media cells which have to simulate the individual living conditions of the cells in vitro. Hristov *et al.*, (2004) indicated that isolated adult human EPCs react to apoptotic bodies from mature ECs by increasing their number and differentiation state. The idea of apoptotic bodies as transporters of cell-derived compounds (e.g., DNA, peptides, or oxidized phospholipids) contained in these membrane vesicles to induce the maturation of progenitor cells. It was suggested that apoptotic bodies from ECs are phagocytosed by EPCs, increasing their number and differentiation state. Such a mechanism may facilitate the repair of injured endothelium and may represent a new signaling pathway between progenitor and damaged somatic cells.The effects of LDIR exposure on endothelial regeneration and vascular repair are unclear. Bone marrow-derived EPCs are involved in repair of the endothelium and growth of new vessels. Studies had shown increased mobilization of EPCs, enhanced homing capacity, and reperfusion of ischemic regions upon irradiation, suggesting that LDIR is angiogenic. These benefits were seen in both local and total-body irradiation. NO and endothelial nitric oxide synthase (eNOS) appear to have a central role in EPCs mobilization and function. VEGF and statins have been demonstrated to increase circulating EPCs levels through increasing eNOS activity and therefore NO concentration. However, it is well established that IR increases levels of ROS and that ROS scavenges NO. Excess ROS results in oxidative stress, which is widely accepted as an underlying cause of vascular disease. The balance between ROS and NO may explain the contradictory findings of the effects of IR exposure on vascular repair (Kuo *et al.*, 2011). Present data indicated that there was a negative correlation between $CD34^+$ and $CD133^+$ cells in therosclerotic patients and normal healthy. This inconsistency with previous study may be attributed to the fact that, expression of the stem cell marker $CD34^+$ is also found on a lower level on mature ECs, and the search for more specific stem cell markers led to the discovery of $CD133^+$, which is expressed on immature stem cells but whose expression is lost during the differentiation to mature ECs (Peichev *et al.*, 2000; Yin *et al.*, 1997). These mature ECs increase in the circulation of atherosclerotic patients than in normal healthy as a result of ECs damage occurred. Consistent with this hypothesis, it was reported that mature ECs were increased in the circulation in patients with acute coronary syndromes (Lee *et al.*, 2005).

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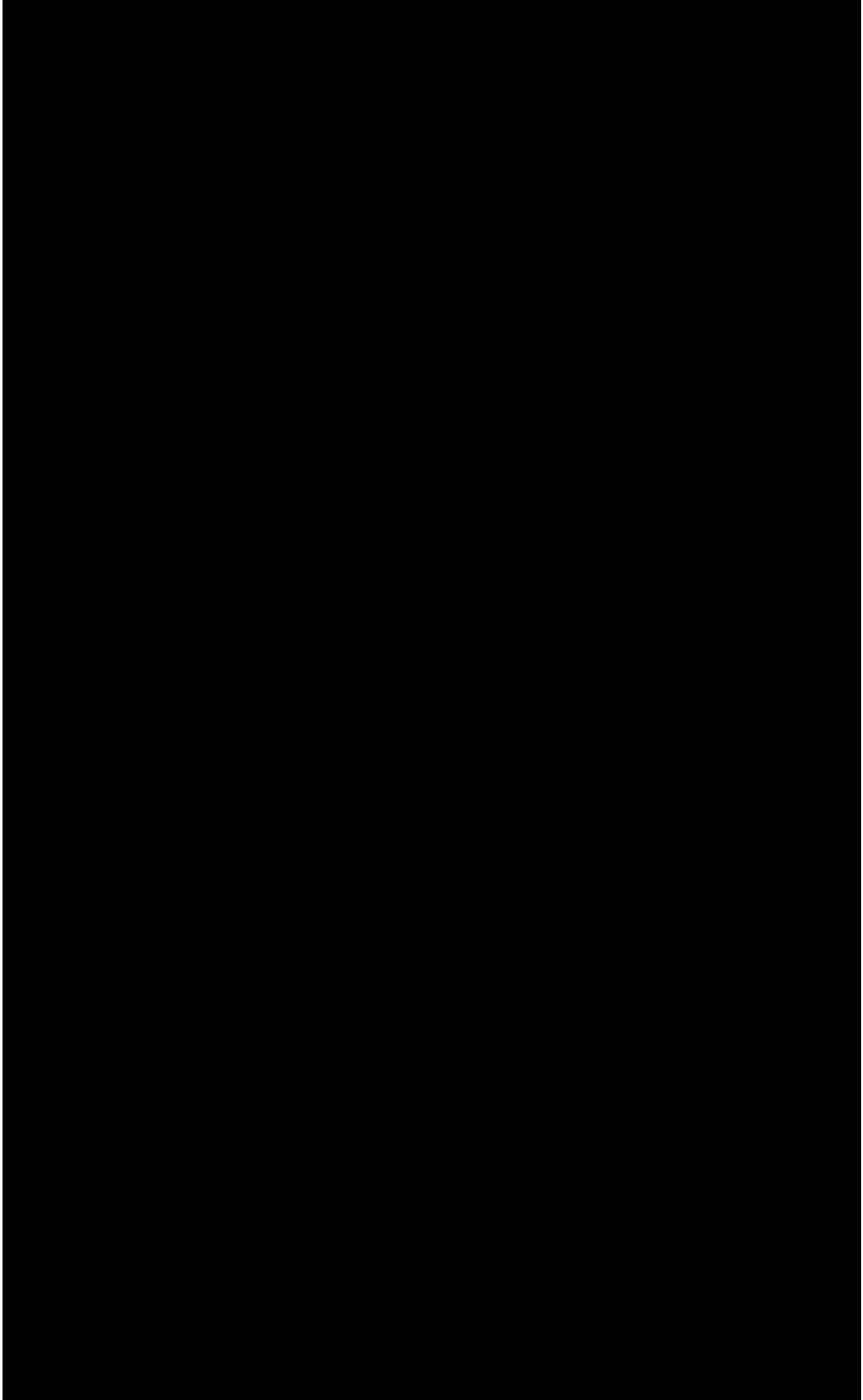

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تأثيرالأشعة المؤينة منخفضة المستوى على الخلايا المنتجة لخلايا جدار الأوعية الدموية فى مرضى قصور الدورة الشريانية للأطراف السفلية الناتج من تصلب الشرايين

هدى السيد محمد[1]، محمد حامي البتانوني[2]، سهير سعد قرة[3]، ايمان فايز سعيد طه[3]
[1]قسم الكيمياء الحيوية-كلية الصيدلة-جامعة الزقازيق-مصر، [2]قسم الجراحة العامة وجراحة الأوعية الدموية- كلية الطب- جامعة القاهرة- مصر، [3] المركز القومي لأبحاث الاشعاع والتقنية- هيئة الطاقة النووية-مصر

تصلب الشرايين (التصلب العصيدى) هو حاله تراكم المواد الدهنيه على طول جدار شرايين الجسم تلك المواد التى تصبح مع مرور الزمن كثيفه و قويه مسببه ضيق الشرايين و ربما انسدادها الأمر الذى يؤدى إلى ضعف تدفق الدم عبر هذه الشرايين للأعضاء التى تغذيها مما يعمل على ضعف حيوية و وظيفة هذا العضو و قد تؤدى هذه المواد إلى انسداد كامل لهذه الشرايين مما يؤدى إلى موت هذه الأعضاء و فى حالات أخرى يتم انفصال أجزاء صغيره من هذه التراكمات و انتقالها عبر الدم محدثه جلطات كما وجد أن زياده الضغط التأكسدى الناتج من زيادة العوامل المؤكسده أحد العوامل المؤديه إلى تصلب الشرايين و ذلك لأنها تعمل على نقص أكسيد النيتريك و الذى يعمل بدوره على توسيع الأوعيه الدمويه و انبساط العضلات الملساء و قد أكدت الدراسات على أن الخلايا السلفيه المنتجه لخلايا جدار الأوعية الدموية تقوم بدور هام فى إعاده تكوين هذه الأجزاء التالفه من الغشاء المبطن لجدار الشرايين و ذلك باستخدام الأشعة المؤينة منخفضة المستوى.

استهدفت الدراسة الحالية بشكل أساسي النقاط التالية:
معرفة عدد الخلايا المنتجه لخلايا جدار الأوعية الدموية CD34,CD133,KDR فى الأشخاص المصابين بمرض تصلب الشرايين و مقارنتها بمجموعات التحكم ومعرفه تأثير الأشعة المؤينة على عدد هذه الخلايا.و قد اشتملت الدراسه الحاليه على أخذ عينات دم من مرضى لديهم قصور فى الدوره الشريانيه الناتج من تصلب الشرايين و أشخاص أصحاء كمجموعات تحكم و و قد تم عمل الأتى:
فصل الخلايا أحاديه النواه و زراعتها و تعيين عدد خلاياCD34,CD133,KDR قبل و بعد تعريضها إلى الأشعه المؤينه منخفضه المستوى بإستخدام جهاز التدفق الخلوى وتعيين نسبه SOD,NO,MDA كدليل خاص بالضغط التأكسدى قبل و بعد إستخدام الأشعه المؤينه منخفضه المستوى.
المرضى و الطرق
وقد تضمنت هذه الدراسة استخدام عدد (٣٠) مريض لديهم جلطة فى الطرف السفلى الناتج من تصلب الشرايين و ٣٠ كمجموعات تحكم فى ظروف سنية و إجتماعية و اقتصادية متشابهة وتم فصل الخلايا أحاديه النواه و زراعتها و قد قسمت العينات الى ثلاثة مجموعات بالإضافه الى مجموعة التحكم.
المجموعه الأولى: خلايا أحاديه النواه من مجموعات التحكم
المجموعة الثانية : خلايا احاديه النواه من مجموعه المرضى وتعين عددها بعد زراعتها مباشرة دون التعرض للاشعاع
المجموعه الثالثه : خلايا احاديه النواه من مجموعه المرضى و زراعتها لمده ٢٤ ساعه دون التعرض للاشعاع
المجموعه الرابعه : خلايا أحاديه النواه من مجموعه المرضى و زراعتها لمدة ٢٤ ساعه بعد تعريضها لجرعه منخفضه من الأشعه المؤينه (٠.٢٥ جراى من أشعه الكوبلت ٦٠)
ويمكن تلخيص نتائج البحث كالتالى:
- حدوث نقص فى عدد خلايا CD34 , CCD133, KDR فى مرضى تصلب الشرايين عن مجموعات التحكم و زياده عدد هذه الخلايا فى مرضى تصلب الشرايين بعد استخدام الأشعة المؤينة منخفضه المستوى (٠.٢٥ جراى من أشعه الكوبلت ٦٠)
- حدوث زيادة فى نسبه NO , SOD و نقص فى نسبه MDA فى مرضى تصلب الشرايين بعد أستخدام الأشعة المؤينة