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INFERENCE OF FREE RADICALS AND ANTIOXIDANT STATUS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Free radicals accept a fundamental part in the pathogenesis of tissue mischief in various clinical issue, including atherosclerosis. Cell reinforcements shield the body from harm brought on by free radicals. In this review we researched oxidative anxiety, cancer prevention agents and incendiary particles in patients with intense myocardial localized necrosis. This review has been done on 106 patients with intense myocardial localized necrosis, (89 men and 17 females). The control gather comprised of 50 sound, age-coordinated subjects (40 men and 10 females). Levels of Glucose, lipid profile, glutathione reduced, glutathione peroxidase, Superoxide dismutase, Glycosylated hemoglobin, fibrinogen, vitamin C, vitamin E, malondialdehyde, ceruloplasmin, adenosine deaminase, lysozyme and sialic destructive were measured.Malondialdehyde and ceruloplasmin levels were fundamentally high and cancer prevention agents, for example, vitamin C, vitamin E, glutathione lessened, glutathione peroxidase and superoxide dismutase were altogether diminished in diabetic and non-diabetic AMI patients as contrasted and control ($p<0.001$). Incendiary markers demonstrated huge ascent in diabetic patients as contrasted and controls. Our outcomes plainly indicate expanded aggravation and oxidative worry in patients with intense myocardial dead tissue. Wretchedness of cell reinforcement framework in these patients affirms this conclusion.

1. INTRODUCTION

Free radicals contain at least one of unpaired electrons. They assume an imperative part in the pathogenesis of tissue harm in numerous clinical issue. Oxygen free radicals are prepared for hurting blends of each and every biochemical class; including nucleic acids, proteins, lipids, lipoproteins, starches and connective tissue macromolecules.Ordinarily, there is a harmony between tissue oxidant and cell reinforcement movement [1]. The last is accomplished by the cell reinforcement forager framework, which incorporates compounds like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and cancer prevention agent vitamins (C, An, E and different carotenoids). Oxidative anxiety is a condition in which oxidant

metabolites apply their dangerous impact in light of an expanded creation or a changed cell instrument of insurance (3).Increased oxidative anxiety and the era of the free oxygen radicals can bring about alteration of LDL to oxidized LDL that could prompt to atherosclerotic sores. Likewise, irritation possesses an essential focal position in all periods of atherosclerosis, which is basic reason for Acute Myocardial Infarction [2].

In our review, numerous territories are secured which may have critical part in the pathophysiology of Acute Myocardial Infarction (AMI, for example, oxidative anxiety atoms (MDA and ceruloplasmin), cancer prevention agent particles (diminished glutathione), cell reinforcement proteins (glutathione peroxidase and superoxide dismutase), cancer prevention agent vitamins (vitamin C and vitamin E),inflammation-related particles (fibrinogen, sialic corrosive, lysozyme and adenosine deaminase), metabolic control (glucose, glycosylated hemoglobin (GHb) and lipid profile),anthropometric parameters (body mass file and midriff to hip proportion). Consequently, we can get an about total biochemical perspective of AMI [3], which is infrequently found in past reviews. In spite of the fact that AMI is broadly considered, part of some conceivable fiery particles like ADA, lysozyme and ceruloplasmin are ineffectively disclosed with reference to AMI. Along these lines, in our review, we researched part of ADA, lysozyme and ceruloplasmin in AMI-related irritation and oxidative anxiety [4].

2. MATERIALS AND METHODS

Study design and patients: The review comprised of 106 patients (89 men and 17 ladies) with a mean age of 43.5 ± 10.1 years and the determination of AMI, admitted to the Intensive Cardiac Care Unit, Appolo Hospital, Delhi Ncr. Patients were further sub gathered as diabetic (58 patients) and nondiabetic (48 patients) as per their history and glucose and GHb levels. The determination of AMI was set up as indicated by clinical criteria: trunk torment, which went on for up to 3 hours, ECG changes (ST height of 2 mm or more in no less than two leads) and CPK rise. The control bunch comprised of 50 solid, age-coordinated subjects, 40 men and 10 ladies, enlisted from a yearly registration program.

Blood gathering and biochemical techniques utilized:-

10 ml of venous blood was gathered after overnight fasting in various holders

moderately steady yellow shading, when 5, 5'-dithio-bis-(2-nitrobenzoic corrosive) or DTNB, is added to sulfhydryl compounds.1.0 ml of blood concentrate was blended with 4.0 ml of 0.3 M Na2HPO4 and 1.0 ml of DTNB reagent [40 mg of 5, 5'- dithiobis (2-nitrobenzoic corrosive) in 100 ml of watery 1% tri-sodium citrate]. Absorbance was perused at 412 nm promptly. Results were communicated as micromoles/liter.

Glutathione Peroxidase (GPx) : The glutathione peroxidase action was controlled by the methodology of Paglia et al (6). Quickly, the oxidized glutathione delivered amid GPx chemical response was promptly diminished by NADPH and glutathione reductase. Along these lines, the rate of NADPH utilization was checked as a measure of arrangement of oxidized glutathione. Results were communicated as units of GPx per gram of hemoglobin [5].

Superoxide Dismutase (SOD) : Superoxide dismutase was controlled by technique for Winterbourn et al (7). This strategy depends on the capacity of SOD to restrain the decrease of nitro blue tetrazolium (NBT) by superoxide which is created by the activity of photograph lessened riboflavin and oxygen. The blue shading shaped was perused at 560 nm. One unit of protein action was measured as the measure of SOD, which causes half of the most extreme hindrance of lessening of nitro-blue tetrazolium. Results were communicated as units of SOD per gram of hemoglobin. Hemoglobin was measured by Drabkin's strategy [6].

Sodium fluoride bulb: - 1.0 ml of blood for glucose

Glycosylated hemoglobin (GHb) :was determined byestimation.

- EDTA knob: - 5.0 ml of blood was included. 0.2 ml of blood was utilized for diminished glutathione estimation. Remaining blood was centrifuged. Isolated plasma was utilized for the estimation of fibrinogen and vitamin C. Red platelets were washed 3 times with super cold typical saline and utilized for the estimation of glutathione peroxidase, superoxide dismutase and glycosylated hemoglobin [7].
- Plain globule:- Remaining blood was included and serum was isolated. Serum was utilized for the estimation of lipid profile, malondialdehyde, vitamin E, ceruloplasmin, sialic corrosive, adenosine deaminase and lysozyme [8].
- Glucose and plasma lipids (add up to cholesterol and HDL-cholesterol, triglycerides) were measured utilizing business packs from Accurex, India, on mechanized analyzer. All chemicals of logical review were acquired from Sigma or Merck India [9].

Reduced Glutathione (GSH) : The level of diminished glutathione in erythrocytes was controlled by a strategy for Beutler (5). This technique depends on the advancement of a with 2 [10], 6-dichlorophenol indophenol in corrosive arrangement. On titration with an ascorbic corrosive arrangement, this compound is decreased to the dull leucobase. The ascorbic corrosive is oxidized to dehydroascorbic corrosive. End-indicate was blue red to lackluster. Mg of vitamin C/100 ml of plasma are 1.6/ml of titration.

Vitamin E: Vitamin E was dictated by strategy for Baker and Frank as given in Varley et al [11]. Serum vitamin-E diminish ferric to ferrous particles, which then shape a red shaded complex with α, αl-dipyridyl. Tocopherols and carotenes are initially extricated into Xylene and the absorbance is perused at 460 nm to quantify the carotenes. A revision for the carotenes is made in the wake of including ferric chloride and perusing at 520 nm. Results were communicated as mg/dl of vitamin E.

MDA Method :MDA levels were assessed by thiobarbituric corrosive (TBA) response. Utilizing 40% trichloroacetic corrosive, proteins were accelerated from 0.5 ml serum, and encouraged proteins were hatched with TBA reagent in a bubbling water shower for a hour. The shaded complex that happened was refrigerated to room temperature and measured by utilizing a spectrophotometer at 533 nm. 1,1,3,3-tetraethoxypropane (1 µmol/L) was utilized as a standard for MDA estimation. Convergences of MDA were communicated in µM/l [12].

Ceruloplasmin: Ceruloplasmin was resolved utilizing its copper oxidase action by strategy for Ravin (13). In this technique, activity of ceruloplasmin on p-phenylenediamine is utilized to quantify the measure of ceruloplasmin present in the serum. Dull lavender shading was perused at 530 nm utilizing control tube as clear. Centralization of ceruloplasmin in mg/dl is absorbance X 87.5. [13]

Adenosine deaminase (ADA) :was controlled by technique for Martinek [14] in light of Berthelot response. In a soluble arrangement, and within the sight of hypochlorite, smelling salts will consolidate with phenol, to give an exceptional blue shaded indophenol. The response is typically upheld by the nearness of an impetus like sodium nitroprusside. Shading was perused at 640 nm. 1 unit of adenosine deaminase action is the sum that frees 1 µg of smelling salts nitrogen per ml of serum every hour at 370C.

Lysozyme : Lysozyme was controlled by strategy for Harrison et al [15].The examine depends on the lysis of Micrococcus luteus cells by lysozyme. To 1.0 ml of substrate containing Micrococcus luteus, 0.1ml of serum/standard was included and kept at 250C.Readings were taken at 30 seconds and 10 minutes. Distinction between two readings was computed. Convergences of lysozyme were communicated in µg/ml.

Antioxidants Status in AMI

Sialicacid : Sialic corrosive was controlled by strategy for Cabezas et al [16]. The rule utilized includes precipitation of serum glycoproteins by 95% ethanol at 0°C: Hydrolysis and the response of N-acetylneuraminic corrosive (NANA) by aHCl-cupric-resorcinol reagent; extraction of the hued part by butyl acetic acid derivation: and the estimation of the shading power at 580mµ.Concentrations of sialic corrosive were communicated in mg/dl.

Statistical analysis: The information from patients and controls were thought about utilizing Student's't'- test. Qualities were communicated as mean ± standard deviation (SD). Sigma detail form 3.0 was utilized for measurable examination. "P" estimation of under 0.05 was considered to demonstrate measurable hugeness.

3. RESULTS

Demographic data of control and AMI group are shown in Table 1.

Age and Sex : Diabetic AMI patients had mean age 44.9 ± 10.3 years, with weight 65.2 ± 10.9 Kg. Non-diabetic AMI patients had mean age 42.1 ± 9.9 years, with weight 64.0 ± 12.6 Kg and Controls had mean age 45.6±9.0 years with weight 56.4± 7.4 Kg.

Body mass index (BMI): It was significantly high in diabeticgroup ($p<0.05$) as compared with control. Compared with controls, there were no significant differences in non diabetic AMI.

Blood Pressure: Systolic circulatory strain was fundamentally high ($p<0.05$) in both the patients bunches (DM and NDM) as contrasted and controls.

The metabolic attributes of the two gatherings (AMI and Control) are delineated in Table 2. Glucose and glycosylated hemoglobin levels were altogether high in diabetic AMI aggregate.

Table 1 : Demographic data in AMI and Control

Variable	Control	AMI	
	n=50	DM (n=58)	NDM (n=48)
Age (Years)	45.6± 9.0	44.9 ± 10.3	42.1 ± 9.9
Sex (Male/Female)	40/10	45/13	43/5
Weight (Kg)	58.4± 7.4	65.2 ± 10.9	64.0 ± 12.6
Body mass index(Kg/m^2)	22.3± 1.9	25.5 ± 4.5	24.7 ± 5.1
Waist/Hip	0.82± 0.04	1.00 ± 0.09	0.99 ± 0.14
Systolic blood pressure (mm of Hg)	111±9	137 ± 18	130 ± 22
Diastolic blood pressure (mm of Hg)	85± 4	95 ± 10	87.0 ± 13

Table 2 : Metabolic markers in AMI and Control

PARAMETER (Normal Range)	Control (n = 50)	AMI	
		DM(n = 58)	NDM(n = 48)
Glucose (mg/dl) (70-110 mg/dl)	90.1± 4.1	161.9 ± 44.1*	102.7 ± 17.1NS
GHb (of total Hb) (3-8% of total Hb)	6.91 ± 0.63	10.25 ± 1.39*	7.89 ± 0.88NS
Total Cholesterol (mg/dl) (150-250 mg/dl)	170.2± 10.2	220.7 ±26.9*	195.8 ± 34.4NS
Triglycerides (mg/dl) (50-150 mg/dl)	91.8 ± 15.3	178.8 ±39.3*	158.1 ±26.4**
LDL-Cholesterol (mg/dl) (100-160 mg/dl)	105.4± 12.7	149.8 ±15.3*	134.2 ± 21.0NS
HDL-Cholesterol (mg/dl) (40-60 mg/dl)	49.6± 6.1	37.1 ± 6.3NS	36.8 ± 9.4NS
VLDL-Cholesterol (mg/dl) (10-30 mg/dl)	23.5± 3.2	39.7 ± 6.7 *	37.4 ± 7.2 **

Values Expressed as Mean ± SD

***P < 0.001, ** P < 0.01, *** P < 0.05, NS-Not noteworthy as Compared to Control (p<0.001) as contrasted and control. Contrasted and controls, there were no noteworthy contrasts in non diabetic AMI.**

Blood lipids demonstrated an altogether higher focus (p<0.001) of aggregate

cholesterol, triglyceride, low thickness lipoprotein and low thickness lipoprotein in diabetic AMI bunch. Malondialdehyde and ceruloplasmin levels in control and AMI gathering are appeared in Table 3. MDA and CER levels were altogether high in diabetic and non diabetic AMI patients as contrasted and control ($p<0.001$). A negative relationship was seen between ascend in MDA and fall in GSH levels in diabetic AMI ($r=-0.62$) and non diabetic AMI ($r=-0.60$). Cell reinforcement status in control and AMI gathering is displayed in table 3.

Table 3 :Malondialdehyde and ceruloplasmin levels in AMI and Control

Variable	CONTROL	AMI	
	(n = 45)	DM(n = 58)	NDM(n = 48)
Malondialdehyde (µM/l)	2.35 ± 0.11	4.57 ± 0.88*	4.36 ±0.85*
Ceruloplasmin (mg/dl)	19.1 ± 3.1	35.2 ± 3.4*	28.7 ± 4.9*

Values Expressed as Mean ± SD

Table 4. All antioxidants, superoxide dismutase, reduced glutathione, glutathione peroxidase, vitamin C, and vitamin E were significantly decreased in DM and NDM groups of AMI ($p < 0.001$) as compared with controls.

Table 4: Antioxidant Status in AMI and Control

Variable	CONTROL	AMI	
	(n = 45)	DM(n = 58)	NDM(n = 48)
Superoxide dismutase (U/gmHb)	4461 ± 481	2300±276*	2626 ±451*
Glutathione reduced (µM /l)	76.9 ± 4.7	48.2 ± 4.9*	53.5 ± 7.9*
Glutathione peroxidase (U/gmHb)	1.51 ± 0.17	0.75 ±0.13*	0.95 ± 0.11*

Vitamin-C mg/dl	1.28 ± 0.13	0.64 ± 0.10*	0.68 ± 0.13*
Vitamin -E mg/dl	1.39 ± 0.17	0.80 ± 0.09*	0.83 ± 0.14*

Values Expressed as Mean ± SD *P < 0.001 as Compared

Provocative parameters in AMI patients are demonstrated Table 5. Every provocative parameter were fundamentally brought up in diabetic AMI patients when contrasted with controls (p < 0.001) In non-diabetic patients, fibrinogen and adenosine deaminase levels were altogether high when contrasted with controls (p< 0.001 and P<0.01).

Table 5: Inflammatory parameters in AMI and Control

Variable	CONTROL	AMI	
	(n = 45)	DM(n = 58)	NDM(n = 48)
Fibrinogen (mg/dl)	327.3± 32.0	590.8 ±56.7*	514.3 ±77.7*
Adenosine deaminase (U/L)	2.85 ± 0.33	4.79 ± 0.52*	3.98 ± 0.71*
Lysozyme (µg/ml)	12.3± 1.4	16.0 ± 1.4*	13.3 ± 2.0NS
Sialic Acid (mg/dl)	51.5± 3.8	62.2 ± 8.4*	54.5 ± 12.1 NS

Values Expressed as Mean ± SD

*P < 0.001, NS-Non-Significant as Compared to Control

4. DISCUSSION

The underlying driver of AMI is fundamentally atherosclerosis. In spite of prior conviction, examine in the most recent two decades has demonstrated that atherosclerosis is neither a degenerative ailment nor unavoidable because of maturing. In actuality, atherosclerosis is by all accounts an endless incendiary condition that is changed over to an intense clinical occasion by the enlistment of plaque crack, which thusly prompts to thrombosis. Subsequently Inflammation possesses a critical focal position in all periods of atherosclerosis, in spite of the fact that aggravation must seethe for a considerable length of time before bringing about a clinical occasion, as AMI.

Noteworthy ascent in MDA levels ($p<0.001$), a lipid peroxidation item, in our patients is characteristic of lifted oxidative worry in AMI patients. This is like work of Dubois Rande et al [17] demonstrated a decline in cancer prevention agent compound exercises and increment in lipid peroxidation items (MDA, TBARS) in patients with insecure angina and unending heart failure.

An expanded level of serum ceruloplasmin in AMI patients ($p<0.001$) proposes that this atom may go about as an oxidative anxiety pointer, however system stays vague. It is an aggravation delicate protein and an intense stage reactant. It was demonstrated that ceruloplasmin displays master oxidant action and causes oxidative adjustment of LDL. This shows ceruloplasmin is an autonomous hazard figure for cardiovascular ailments. A positive relationship was seen amongst ceruloplasmin and sialic corrosive ($r = +0.64$) in diabetic AMI gather. As sialic corrosive is a notable incendiary marker, ceruloplasmin may have conceivable part in irritation. In diabetic AMI gather, we additionally discovered positive connection amongst'sceruloplasmin and fibrinogen ($r = +0.62$), add up to cholesterol ($r = +0.70$), triglycerides ($r= +0.62$). Every one of these outcomes demonstrate that ceruloplasmin might be considered as a provocative atom.

In AMI patients, we discovered essentially bring down levels of vitamins E and C, ($p<0.001$) contrasted and controls. This is as per investigations of Singh et al (21), who exhibited that there was a huge drop in vitamins C, E, An and ß-carotene, though lipid peroxides were altogether higher in AMI patients, contrasted and controls. This demonstrates serious harm to cell reinforcement framework, which can't battle oxidative anxiety and aggravation.

Our information demonstrated that diminished glutathione levels and glutathione peroxidase movement are essentially brought down in AMI patients ($p<0.001$), which is like

Kharb (22). Negative connection was seen between decreased glutathione and malondialdehyde levels in diabetic AMI bunches (r = - 0.62). This infers glutathione framework, which is the imperative defensive framework against oxidative harm, is seriously hindered in AMI patients. Our discoveries show the presence of an unusual harmony between the oxidative and defensive systems in AMI patients, especially in diabetic AMI Antioxidants Status in AMI patients.

We additionally found that the action of SOD was essentially lower in patients aggregate ($p<0.001$). Like our perception, Kumar and Das (23) have recommended that an expansion in free radical era and a synchronous reduction in the generation of NO and cancer prevention agent protein, for example, superoxide dismutase and vitamin E happens in fundamental hypertension which is an outstanding danger consider for AMI.

5. CONCLUSION

In this manner, our review shows an irregularity amongst oxidant and cell reinforcement particles in AMI patients, and extent of awkwardness is more prominent in diabetic AMI patients, potentially due to more prominent aggravation in diabetic patients. Additionally, it is proposed that adenosine deaminase, lysozyme and ceruloplasmin can fill in as markers of irritation, which are inadequately examined concerning AMI.

REFERENCES

1. Carrol CE. Oxygen free radicals and human disease. Ann Int Med 1987; 107: 526–45.
2. Shrinivas K, VijayaBhaskar M , ArunaKumari R , Nagaraj K, Reddy KK. Antioxidants, lipid peroxidation and lipoproteins in primary hypertension. Indian Heart J 2000; 52: 285-8.
3. Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Cann B, et al. Factors associated with oxidative stress in human populations. Am J Epidemiol2002 ; 156: 274-85.
4. Libby P. Vascular biology of atherosclerosis: Overview and state of art. Am J Cardiol 2003; 91(suppl): 3A-6A.
5. Beutler E, Duran O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61(5): 882-8.
6. Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70:158-69.
7. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975; 85 (2): 337-41.
8. Fluckiger R, Winterhalter KH. In vitro synthesis of hemoglobin A_{1C}. FEBS Lett 1976; 71: 356-60.
9. Varley H, Gowenlock AH, Bell M. Determination of plasma fibrinogen by tyrosine method (Lampert). In “Practical Clinical Biochemistry” 1986, Heinemann Medical Books, London, Vol. I; 5TH edition, Chapter 19, 557-8.

10. Varley H. Determination of plasma ascorbate by 2,6-Dichlorophenolindophenol titration. In "Varley's Practical Clinical Biochemistry" Ed: Gowenlock AH, McMurray JR and McLauchlan DM, Heinemann Medical Books, London 1988; Vol. I; 6 TH edition; Chapter 35: 927.

11. Baker H, Frank O. Determination of serum tocopherol, In " Varley's Practical Clinical Biochemistry" Ed: Gowenlock AH, McMurray JR and McLauchlan DM, Heinemann Medical Books, London 1988; Vol. I; 6TH edition, Chapter 35: 902.

12. Sasikala M, Subramanyam C, Sadasivudu B. Early oxidative change in low density lipoproteins during progressive chronic renal failure. Ind J ClinBiochem 1999; 14(2); 176-83.

13. Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med 1961; 58(1): 161-8.

14. Martinek RG. Micromethod for estimation of serum adenosine deaminase. ClinChem 1963; 9(5): 620-25.

15. Harrison JF, Lunt GS, Scott P, Blainey JD. Urinary lysozyme, ribonuclease and low molecular weight protein in renal disease. Lancet 1968; 1: 371-5.

16. Cabezas JA, Vazquez Porto J. Sialic acid in human serum. ClinChem 1964; 10(11): 986-90.

17. Dubois-Rande JL, Artigou JY, Darmon JY, Habbal R, Manuel C, Tayarani I, et al. Oxidative stress in patients with unstable angina. Eur Heart J 1994; 15(2): 179-83.