

Assessment of Oxidative Stress & Antioxidant Status in Acute Pancreatitis by Measurement of Ischaemia Modified Albumin, Super Oxide Dismutase & Tocopherol

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ABSTRACT

Acute pancreatitis (AP) can present either as a mild, self-limiting localized disease to fatal widespread multi-organ failure with high mortality rates. There is increased oxygen free radical production & it causes oxidation of lipids in the pancreatic cell membrane and oxidatively modify proteins, depolarize mitochondrial membrane, and induces DNA fragmentation. In this study, 50 diagnosed acute pancreatitis patients (32 gallstone, 15 alcoholic and 3 idiopathic) and age and sex matched healthy controls coming to Dept. of Biochemistry, Medical College and Hospital Kolkata as patient parties between January 2012 to August 2013 were tested to assess the oxidative stress & antioxidant status in acute pancreatitis patients by measurement of ischaemia modified albumin (Albumin Cobalt binding assay), super oxide dismutase (Kakkar's method) & tocopherol (Baker & Frank method). The result of this study suggests that there is increased antioxidant status (super oxide dismutase and tocopherol) in healthy controls than acute pancreatitis patients and increased oxidative stress (which is indicated by raised ischemia modified albumin) is present in acute pancreatitis patients. There is scope to elaborate this field with larger sample size, inclusion of different race, and with other oxidative stress parameters.

Keywords: Acute pancreatitis, oxidative stress, super oxide dismutase, ischaemia modified albumin, tocopherol.

INTRODUCTION

Oxidative stress results from an imbalance between reducing agents and enzymes involved in the removal of free radicals (FR) and/or reactive oxygen species (ROS).^[1]

Acute pancreatitis (AP) can present either as a mild, self-limiting localized disease to fatal widespread multi-organ failure with high mortality rates. Mild pancreatitis is characterized by

inflammation and edema of the pancreas, with additional features of necrosis and secondary injury to extrapancreatic organs in severe pancreatitis.^[2-4] Feature of AP is acute inflammation and necrosis of pancreatic parenchyma, necrosis of pancreatic fat, hemorrhage, and inflammatory infiltration.^[5] Pancreatic cell death occurs due to either necrosis or apoptosis mechanisms. The study of Booth et al^[6] found that oxygen free radical (OFR)

induction in acinar cells promoted apoptosis as well as inhibition of OFR generation led to an increase in necrosis accompanied by reduced ATP. These researches suggest that OFR production within acinar cells may be a protective response during pancreatitis. The main reason behind necrotic or apoptotic cell death is the early activation of pancreas zymogens, especially cathepsin and trypsinogen inside the pancreas. Study of Scott et al [7] showed that excessive OFR in a pathologic state can cause tissue and cell damage. The OFR is one of the main reasons behind pancreatic edema process in AP, and may participate in the pancreas necrosis process. The OFR, directly attacks lipids and proteins in the biological membranes and thus disrupts their functions. The action of OFR includes oxidation of lipids in the pancreatic cell membrane and oxidatively modified proteins, depolarization of the mitochondrial membrane, and induction of DNA fragmentation. [8]

In this study, 50 diagnosed acute pancreatitis patients (32 gallstone, 15 alcoholic and 3 idiopathic) and age and sex matched healthy controls coming to Dept. of Biochemistry, Medical College and Hospital Kolkata as patient parties between January 2012 to August 2013 were tested to assess the oxidative stress & antioxidant status in acute pancreatitis patients by measurement of ischaemia modified albumin, super oxide dismutase & tocopherol.

REVIEW OF LITERATURE

Antioxidant is any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The main antioxidant defence comprises of Superoxide dismutase, Catalase, Glutathione Peroxidase (they catalytically remove free radicals and other reactive species). Super oxide dismutase converts superoxide to hydrogen peroxide and oxygen. Alpha tocopherol, retinol, Ascorbic acid, Bilirubin, Uric acid, these agents scavenge the reactive oxygen species with

help of their large molecular size and presence of double bonds. [9] Ischemia/reperfusion induced oxidative stress changes the structure of the amino terminus of albumin in such a way that causes the loss of its Co^{2+} binding capacity leading to the formation of an 'ischemia-modified albumin'. HPLC, LC-MS and NMR analysis have shown that the N terminal region of human serum albumin Asp-Ala-His-Lys binds the transition metals cobalt and nickel, modification of this region by way of N acetylation or the deletion of one or more amino acid resulted in no binding of cobalt, an assay that detects this reduced binding could be useful in the diagnosis of ischaemia. [10]

K Tsai et al (1998) [11] found that In addition to being a possible initiator of acute pancreatitis, Reactive Oxygen Species can also be generated and contribute to the progression of acute pancreatitis. In acute inflammatory disorders like acute pancreatitis, various pathogenetic mechanisms induce accelerated production of ROS (damaged mitochondria, tissue or splanchnic ischaemia-reperfusion with activation of xanthine oxidase, and metabolic activated polymorphonuclear leucocytes). Once produced, oxygen radicals act as a molecular trigger of various inflammatory processes. They can directly attack biological membranes, stimulate arachidonic acid metabolism with increased production of prostaglandins, thromboxane, and leukotrienes, and trigger the accumulation of neutrophils [12] and their adherence to the capillary wall. [13] According to their study it is very likely ROS play a central role in the perpetuation of pancreatic inflammation and development of extrapancreatic complications. [14,15]

According to them, in the initiation stage of acute pancreatitis, oxidative stress is postulated to play an important role in the exocytotic derangement of the acinar cell. The evolution of pancreatic inflammation is presumed to be a result of imbalances between intrapancreatic

oxidative stress and natural antioxidation defences. Activated xanthine oxidase, recruited PMN leucocytes, and microsomal cytochrome P450 have all been incriminated as a source of this “pathogenetic” oxidative stress, but the fundamental pathogenetic mechanisms leading to enhanced production of ROS have yet to be explored. [11]

M Esrefoglu et al (2012) [16] showed that Oxygen Free Radical indirectly act on the arachidonic acid cascade by increasing the production of thromboxane, which lowers tissue circulation by its potent platelet-aggregating and vasoconstricting effects. Additionally, OFR enhance the production of leukotriene B4 which promotes activation of leukocytes and discharge of lysosomal enzymes. As a secondary effect, polymorphonuclear leukocytes are responsible for the respiratory burst that leads to an enhanced production of radical species and activated enzymes and further cell damage.

The results of Rau et al [17] indicate that OFR play an important mediator function in early and later courses of AP (acute pancreatitis). Their findings suggest that OFR species are important mediators but not necessarily triggers of tissue damage in AP. The degree of oxidant-antioxidant balance changes in the early phase of human AP, correlating with the clinical severity of pancreatitis.

Park et al [18] reported higher plasma levels of lipid peroxides and myeloperoxidase and lower superoxide dismutase (SOD) activity in patients with severe AP than in those with mild AP. OFR may thus be closely associated with the inflammatory process and the severity of AP.

Thareja et al [19] reported that high oxidative stress was observed during the early phase of AP and that gradually improving antioxidant status was associated with a better clinical outcome in patients with AP. The concentration of plasma lipid peroxides is a particularly meaningful index

for determining the severity of the disease in humans.

L Robies et al (2013) [20] found that AP results in an oxidative stress which amplifies the inflammatory process through the recruitment and release of pro inflammatory mediators, leading to a systemic inflammatory response. Two striking features determine the clinical course and the severity of AP: (1) Development of systemic inflammatory response syndrome in the early phase of the disease, and (2) Development of pancreatic necrosis leading to infection, sepsis, multi-organ system failure and death.

AP can be caused by various etiological factors however about 80% of all cases are related to either bile stones or excessive alcohol consumption. Alcohol induced damage is, therefore, a relevant model to study the mechanism and pathophysiology of pancreatitis. Alcohol's toxicity is mediated through the action of alcohol itself or through its oxidative and non-oxidative metabolism. In oxidative metabolism; alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde followed by chemical transformation of acetaldehyde to acetate by isoform 2 of aldehyde dehydrogenase (ALDH2), which is specifically localized in the mitochondria. These reactions are coupled with depletion of oxidized nicotinamide adenine dinucleotide (NAD⁺) and accumulation of reduced nicotinamide adenine dinucleotide (NADH) since both ADH and ALDH consume NAD⁺ and produce NADH. In non-oxidative metabolism, fatty acid ethyl ester (FAEE) synthase converts alcohol to FAEE. Thus, alcohol metabolism results in accumulation of acetaldehyde, acetate, NADH, and FAEE, as well as changes in cellular and mitochondrial NAD⁺ and NADH levels. Excessive alcohol consumption results in pancreatic damage through a number of potential mechanisms. Oxidative metabolism of alcohol has been shown to produce ROS and inhibit secretion from pancreatic acinar cells, events that trigger oxidative stress and inflammation

through activation of NF- κ B, and formation of TNF- α , IL-6, and other inflammatory mediators. It also alters cell permeability, increases cell fragility and promotes necrosis by inhibiting apoptosis. [20]

In this study, acute pancreatitis patients were tested to assess the oxidative stress & antioxidant status in them by measurement of ischaemia modified albumin, super oxide dismutase & tocopherol.

In recent years whether to treat the acute pancreatitis patients with antioxidants or not has been the big question.

Sweiry JH et al [21] showed that treatment with antioxidants has been shown to reduce acinar cell injury and oedema in various animal models of pancreatitis, suggesting that the sustained generation of reactive oxygen species depletes cellular antioxidant defences. Evidence for a role for bradykinin and nitric oxide in pancreatitis has been conflicting with some studies suggesting these agents might ameliorate pancreatic dysfunction by enhancing pancreatic blood flow and secretion in response to bradykinin-stimulated generation of nitric oxide from endothelium, while other studies suggest that nitric oxide potentiates pancreatic oxidative stress.

Thus, there is clearly a need for well-designed clinical trials to evaluate the oxidative stress & protective role of antioxidant therapy in acute pancreatitis.

MATERIALS AND METHODS

Subject selection and sample collection: 50 patients of Indian origin coming to the dept. Of Biochemistry of Medical College and Hospital with confirmed diagnosis of acute pancreatitis (by Ranson's & APACHE II score) were used as subjects. 50 sex and age matched healthy patient parties were treated as controls. They were confirmed to be free of major cardiopulmonary, gastrointestinal, and hepatobiliary-pancreatic diseases after a series of screening tests.

Patients with history of malignancy, renal disease, cardiac disease, hypo or

hyperthyroidism, any other major health problems, history of addiction, patients taking any kind of antioxidants were excluded.

The diagnosis of acute pancreatitis in patients presenting with acute abdominal pain rested on the clinical history, physical examination and laboratory aids. The clinical history comprised of pain abdomen, character and severity of pain, radiation of pain, history of nausea, vomiting, fever, abdominal distension, amount and frequency of alcohol intake, drug intake and previous history suggestive of gall stone disease. Standard imaging technique of ultrasonography was used to label a case as that of gall stone origin. If the above histories were negative and there was no history of operative or diagnostic interventional trauma, the case was labelled as idiopathic.

Blood tests including complete blood picture, electrolytes, urea, creatinine, glucose, lactate dehydrogenase, albumin, calcium, liver function tests, arterial blood gases, and prothrombin time were performed on admission, then daily or at more frequent intervals if deemed necessary. Plasma amylase, lipase, and C reactive protein (CRP) were measured on the first day and then every three days. The total amount of blood taken for testing was less than 20 ml each time.

Fasting venous blood samples were obtained from all patients and controls after overnight fasting. 2 ml blood is collected in fluoride vials (to obtain plasma) & 2 ml into EDTA vials and 3 ml into clot vials to obtain serum.

The samples were taken to the laboratory for prompt analysis. Plasma level of super oxide dismutase was estimated by Kakkar's method, [22] serum levels of tocopherol by Baker & Frank method [23] & serum levels of ischemia modified albumin by Albumin Cobalt binding assay. [24]

RESULTS AND ANALYSIS

Statistical analyses were done by Mann-Whitney U test, as the values were

not normally distributed (by Kolmogorov - Smirnov goodness of fit test).

After statistical analysis, it was found that ischemia modified albumin among acute pancreatitis patients increased significantly, (p Value<0.00001). For acute pancreatitis patients mean +/-2SD was 45.33 +/- 2.7 U/ml. For healthy controls mean+-2SD was 24.12+-1.46 U/ml. (vide Tables 1, 2).

Super oxide dismutase in acute pancreatitis patients (Mean+/-2 SD =3.99+/-

0.81 U/ml) were significantly lower (p Value < 0.001), when compared to healthy controls (Mean +/- 2 SD =9.64+/-0.65 U/ml) (vide Tables 1, 2).

Serum Tocopherol levels in acute pancreatitis patients (Mean+/-2 SD =7.89+/-0.42 mg/lit) were Also significantly lower (p Value <0.001) when compared to healthy controls (Mean +/- 2 SD =13.51+/- 0.52 mg/lit). (Vide Tables 1, 2).

Table 1: Descriptive Statistics of Numerical Variables - Group Healthy Control- (Sample Number = 50)
95% CONFIDENCE INTERVAL

	Valid No.	Mean	Std. Dev.	Minimum	Maximum	Lower Bound	Upper Bound
IMA	50	24.12	1.46	22.5	26.82	21.2	27.04
TOCOPHEROL	50	13.51	0.52	12.86	14.12	12.47	14.55
SOD	50	9.64	0.65	8.96	10.14	8.34	10.94

Table 2: Descriptive Statistics of Numerical Variables - Group Acute Pancreatitis- (Sample Number = 50)
95% CONFIDENCE INTERVAL

	Valid No.	Mean	Std. Dev.	Minimum	Maximum	Lower Bound	Upper Bound
IMA	50	45.33	2.7	41.86	49.82	39.93	50.73
TOCOPHEROL	50	7.89	0.42	7.36	8.41	7.05	8.73
SOD	50	3.99	0.81	2.58	5.34	2.37	5.61

*Comparison of group mean of the two Groups by Mann-Whitney U test :- (as the values are not normally distributed)

Parameters	p Value
IMA	<0.00001
TOCOPHEROL	<0.001
SOD	<0.001

Inference:-

- Mean serum ischemia modified albumin levels were higher in acute pancreatitis patients than in healthy controls.
- Mean plasma super oxide dismutase values were more in healthy controls than in acute pancreatitis patients.
- Mean serum tocopherol levels were more in healthy controls than in acute pancreatitis patients.

DISCUSSION & CONCLUSION

After completion of the present study, following salient points that merit further discussion have come to the forefront.

Serum ischemia modified albumin level in acute pancreatitis patients was higher than serum IMA values in healthy controls. Plasma super oxide dismutase values were higher in healthy controls than acute pancreatitis patients & serum tocopherol levels were more in healthy controls than acute pancreatitis patients.

This suggests that there is increased antioxidant status (super oxide dismutase and tocopherol) in healthy controls than acute pancreatitis patients and increased oxidative stress (which is indicated by raised ischemia modified albumin) is present in acute pancreatitis patients.

K Tsai et al (1998) [11] suggested similar explanations. They found Heightened oxidative stress appears early in the course of acute pancreatitis and lasts longer than the clinical manifestations. The dependence of disease severity on the imbalance between oxidants and natural defences suggests that oxidative stress may have a pivotal role in the progression of pancreatitis and may provide a target for treatment. In the blood from patients with acute pancreatitis, they found increased levels of the superoxide radical as well as lipid peroxides.

C D Johnson et al (2007) [25] also suggested that blood antioxidant levels diminish during severe acute pancreatitis

and that supplements of antioxidants can prevent these falls in experimental and clinical pancreatitis.

Lourdes Robles et al (2013) showed that markers for oxidative stress were lower in the treated acute pancreatitis patients than in untreated group. [20]

S Kaur et al (2011) [26] showed that oxidative stress (Total antioxidant status) can not cause pancreatitis but can contribute to worsening of local inflammatory changes after onset of acute pancreatitis. Antioxidant therapy along with other drugs used for acute pancreatitis can decrease the oxidative stress and might help in early recovery by preventing further tissue injury.

Gomez Cambroner LG et al (2002) [27] have shown by experimental and clinical evidences that pro-inflammatory cytokines and oxidative stress are critically involved in the development of local and systemic complications associated with severe acute pancreatitis. Serum levels of pro-inflammatory cytokines, such as TNF-alpha and IL-1beta, increase during the course of acute pancreatitis and they appear to be the driving force for the initiation and propagation of the systemic response. Accordingly, pretreatment with an antibody against TNF-alpha or blockade of TNF-alpha production with pentoxifylline ameliorates experimental acute pancreatitis. In addition, serum IL-6 and IL-8 levels appear to be correlated with severity of pancreatic inflammation. The role of oxidative stress in acute pancreatitis has been evidenced indirectly by beneficial effects of antioxidants as well as directly by pancreatic glutathione depletion and increased lipid peroxidation. Furthermore, circulating xanthine oxidase released by the damaged pancreas acts as a source of systemic oxidative stress.

Sanchez BC et al (2004) [28] showed that in pancreatitic rats, oxidative stress markers like malondialdehyde and protein carbonyl group concentrations were significantly increased in serum, whereas alpha tocopherol concentration diminished.

This study, which was done in a tertiary care hospital in urban eastern India showed that there is decreased antioxidant status & increased oxidative stress in acute pancreatitis patients. But it may not be representative of the rest of the population. There is scope to elaborate this field with larger sample size, inclusion of different race, and with other oxidative stress parameters.

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