CHAPTER – 2

REVIEW OF LITERATURE

This chapter presents the literature in the area of medicinal chemistry wherein the relation of various bio-chemical parameters with the disease Pulmonary Tuberculosis have been reviewed. Therefore the pulmonary tuberculosis and its parameters have been separately dealt with.

Glycoproteins and Pulmonary Tuberculosis

Morishita\textsuperscript{1} (1965) conducted a prospective study on protein bound polysaccharide in the dermatological field and concluded that variations in the contents of serum polysaccharide which can be regarded characteristic of each disease were hardly recognize, though increase tendencies in the hexose were observed in the exudative stage of acute eczema, urticaria, lupus vulgaries and in certain cancers and decrease tendencies in toxicolclermia, lupus erythematodes discoides, melanosis and in some cases of xanthoma. High levels of hescosamine were observed in acute systemic lupus erythematodes and malignant tumors and decrease were seen in urticaria etc.

Mittae\textsuperscript{2} et al (1966) estimated that the average serum glycol protein levels in 45 untreated new cases and 35 treated chronic cases of proved pulmonary tuberculosis without any complication and of 24 normal healthy adults were estimated rise increases of pulmonary tuberculosis.

Bradley P. William\textsuperscript{3} et al (1977) found out that the correlations among serum protein bound carbohydrates ,serum glycoproteins ,lymphocyte reactivity and tumor burden in cancer patients ,their results showed that serum protein bound carbohydrates or
glycoproteins may be of adjunctive value in assessing tumor burden and immune reactivity in cancer patients.

Leif C. Anderson⁴ et al (1980) observed specific changes in the surface glycoprotein patterns which correlate with the degree of maturation and functional activation of T cells. Surface molecules carrying T cell specific antigens were identified by Immune precipitation from lysates of surface labeled thymocytes and T-Lymphocytes using rabbit anti-human T cell antibodies.

Stefenelli. N⁵ et al (1984-85) determined the levels of sialic acid in patients suffering with various malignant tumors, bacterial infections, rheumatoid diseases and chronic liver diseases. Higher values are found in the groups with neoplasms, inflammatory diseases and active rheumatoid diseases, and chronic liver diseases. Higher values are found in the groups with neoplasms, inflammatory diseases and active rheumatoid diseases.

U Singh⁶ et al (1989) conducted a study on serum glycoprotein in patients of pulmonary tuberculosis and found that the levels of serum glycoprotein were found to increase in all the stages of pulmonary tuberculosis with a marginal difference and the treatment with anti tuberculosis drugs resulted in a decrease in ESR and the various fractions of serum glycoprotein.

Fassbender Klaus⁷ et al (1995) used acute phase reactant x1-acid glycoprotein AGP, orosomucoid as an additional tool in the differential diagnosis of bacterial lung infections and concluded that compared with those in healthy controls the serum concentrations AGP were significantly increased in both tuberculosis and Non tuberculosis bacterial pneumonia.
Shinohara and co-workers (2008) studied the relationship between the sialic acid concentration in serum and whole saliva in rats with naturally occurring gingivitis. They suggested that the amount of sialic acid in saliva can be a useful index of the severity of periodontal disease.

Kalyan Goswami et al (2003) in their research came up with a conclusion that there exists a significant negative correlation between carbonylation and sialic acid content of serum proteins ill hyperthyroidism and enhanced desialylation and carbonylation of serum proteins by vitro H2O2 Treatment suggest that oxidative stress can cause desialylation of serum glycoproteins.

Albillos A. et al (2004) illustrated that increased lipopolysaccharide-binding protein was the only factor independently associated with severe bacterial infection ill a multivariate analysis. Monitoring of serum lipopolysaccharide–binding protein could, therefore help to target cirrhotic patients with ascites for antibiotic prophylaxis.


Song –Ze Ding et al (2007) studied the effect of Helicobacton pylori infection on oxidative stress levels and programmed cell death in human gastric epithelial cells, they could deduce that infection of DCFH2-DA treated gastric epithelial well lines with H. Pylani was associated with a rapid increase in fluorescence compared to levels of
fluorescence measured in uninfected control cells, indicating the increased accumulation of intercellular ROS in infected cell lines.

**H.M. Erdogan** et al (2008) designed a study to disclose some indicators of oxidative stress and inflammation in natural cases of bovine leptospirosis and the results obtained suggest that oxidation damage along with other mechanisms might have taken part in the pathogenesis of bovine leptospirosis should that increased total sialic acid levels lipid bound sialic acid levels, MDA, NO, Uric acid, total protein, glucose and decreased GHS and albumin concentrations were suggestive of inflammation and oxidative stress in diseased bulbs.

**Julien Brillault** et al (2009) Moxifloxacin (MXF) is a fluoroquinolone antibiotic that is effective against respiratory infections. However, the mechanisms of MXF lung diffusion are unknown. Active transport in other tissues has been suggested for several members of the fluoroquinolone family. In this study, transport of MXF was systematically investigated across a Calu-3 lung epithelial cell model. MXF showed polarized transport, with the secretory permeability being twice as high as the absorptive permeability. The secretory permeability was concentration dependent (apparent $P_{max} = 13.6 \times 10^{-6}$ cm.s$^{-1}$; apparent $K_m = 147$ µM), suggesting saturated transport at concentrations higher than 350 µg/ml. The P-glycoprotein inhibitor PSC-833 inhibited MXF transport in both directions, whereas probenecid, a multidrug resistance-related protein inhibitor, appeared to have no effect in the Calu-3 model. Moreover, rifampin, a known inducer of efflux transport proteins, upregulated the expression of P-glycoprotein in Calu-3 cells and enhanced MXF active transport. In conclusion, this study clearly indicates that MXF is subject to P-glycoprotein-mediated active transport in the Calu-3 model. This P-
glycoprotein-dependent secretion may lead to higher MXF epithelial lining fluid concentrations than those in plasma. Furthermore, drug-drug interactions may be expected when MXF is combined with other P-glycoprotein substrates or modulators.

**Vitamin E and Pulmonary Tuberculosis**

*Fernandez Danares* et al. (1989) studied the vitamin status in patients with inflammatory bowel disease and found depreciated vitamin E levels.

*Kuroki* et al. (1997) studied whether vitamin E depleted in crohns diseases and initial diagnoses and found out that the levels of vitamin E were low in patients of crohns disease.


*Geerling B J* et al. (1999) studied on altered ascorbic acid status in the mucosa from IBD patients and concluded that the antioxidant enzymes and alteration in fatty acid profile in patient with prone disease. There were decreased blood level in vitamin C and vitamin E and decreased intestinal mucosal levels of CUZN super oxide dismutase glutathione vitamin A,C,E and β carotene were reported for crohns disease patients.

*Lettow M*. et al (2003) worked upon micronutrient, malnutrition and wasting in adults with pulmonary tuberculosis in Malami and concluded that lower dietary carotenoids, vitamin E, Vitamin A and selenium are associated with wasting that data support the hypothesis that wasting in tuberculosis is associated with lower plasma micronutrient concentrations.
Reddy, Y.N. et al (2004) studied the Severe oxidative stress has been reported in TB patients because of malnutrition and poor immunity. The purpose of this study was to investigate the serum lipid peroxidation products and important free radical scavenging enzymes i.e. superoxide dismutase (SOD), catalase and antioxidant glutathione levels and total antioxidant status in TB patients. The subjects for this study comprised of normal human volunteers (NHV, n=25), TB patients (n=100) – including, untreated (TB 1, n=55), under treatment (TB2, n=30) and after treatment (TB3, n = 15) with anti-tuberculosis therapy (ATT). The levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in TB 1 & TB2 (P<0.001) and also in TB3 (P<0.01); these levels gradually decreased with clinical improvement with ATT. SOD, catalase, glutathione levels and total antioxidant status were decreased significantly in TB 1 & TB2 (P<0.001), TB3 (P<0.01) patients in comparison with NHV, these levels gradually increased with clinical improvement with ATT. Oxidative stress was observed in all the TB patients (TB 1, TB2, TB3), irrespective of treatment status. The study showed that in TB patients free radical activity is quite high and antioxidant levels are low. A suitable antioxidant therapy may prove beneficial and nutritional antioxidant supplementation may represent a novel approach to fast recovery.

Panjamurthy, K., Manoharan, S. and Ramachandran, C.R. (2005) investigated to assess the degree of oxidative stress in patients with periodontitis by measuring their levels of thiobarbituric acid reactive substances (TBARS), enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GSHPx)), and non-enzymatic antioxidants (vitamins –E and C, reduced glutathione
This study was conducted on 25 adult chronic periodontitis sufferers who were patients in Rajah Muthiah Dental College and Hospital, Annamalai University. The levels of TBARS and non-enzymatic antioxidants, and the activities of enzymatic antioxidants in the patients' plasma, erythrocytes and gingival tissues were assayed using specific colorimetric methods. The periodontitis sufferers had a significantly higher TBARS level than the healthy subjects. In the plasma, erythrocytes, erythrocyte membranes and gingival tissues of the periodontitis sufferers, enzymatic antioxidant activities were found to be significantly higher, whereas the levels of non-enzymatic antioxidants were significantly lower (except for reduced glutathione in the gingival tissues) relative to the parameters found for healthy subjects. The disturbance in the endogenous antioxidant defense system due to over-production of lipid peroxidation products at inflammatory sites can be related to a higher level of oxidative stress in patients with periodontitis.

**Nwanjo, H. U. and Oze, G.O.** (2007), studied the oxidative imbalance and non-enzymic antioxidant status in plasma of pulmonary tuberculosis patients in Nigeria were investigated. Forty HIV /AIDS seronegative pulmonary tuberculosis patients with the active infection (24 males, 16 females) aged 20-60 years diagnosed by Ziehl Neelsonstaining/demonstration of mycobacterium tuberculosis in sputum and sputum culture Lowenstein Jensen medium visiting Federal Medical Centre, Owerri were selected for the study. Sixty normal subjects free from pulmonary tuberculosis and HIV /AIDS (30 males and 30 females) ages 20-60 years were also used as control. Patients with complication such as renal diseases, viral and other bacterial infections, etc. were excluded from the study. In the analysis of the
results using Duncan multiple range test, pulmonary tuberculosis infected subjects presented significantly higher mean values of plasma lipid peroxide (p<0.05) when compared with control. Also the levels of non-enzymic antioxidants such as Vitamin C, vitamin E and reduced glutathione in plasma were significantly depleted in the pulmonary tuberculosis infected subjects (p<0.05) when compared with control. This shows that pulmonary tuberculosis could probably be associated with excess ROS production.

Seyedrezazadeh, E. et al. (2008) studied the increased production of reactive oxygen species secondary to phagocyte respiratory burst occurs in pulmonary tuberculosis (TB). The present study evaluated the efficacy of vitamin E-selenium supplementation on oxidative stress in newly diagnosed patients treated for pulmonary TB. A double-blind, placebo-controlled trial including patients with newly diagnosed TB was conducted. The intervention group (n = 17) received vitamin E and selenium (vitamin E: 140 mg α-tocopherol and selenium: 200 μg) and the control group (n = 18) received placebo. Both groups received standard anti-TB treatment. Assessment of micronutrient levels, oxidative markers and total antioxidant capacity were carried out at baseline and 2 months after the intervention. Malondialdehyde levels were significantly reduced in the intervention group (P = 0.01), while there was minimal reduction in the control group. The mean plasma level of total antioxidants was increased significantly (P = 0.001) in both the intervention and the control groups. A 2-month intervention with vitamin E and selenium supplementation reduces oxidative stress and enhances total antioxidant status in patients with pulmonary TB treated with standard chemotherapy.
Reddy, Y. Narsimha et al (2009) studied the pleural fluid was aspirated from the tuberculous patients both untreated and under treatment with three months anti-tuberculosis therapy. The amount of nalondialdehyde, lactate dehydrogenase, and total protein content in pleural fluid of untreated tuberculous patients were found to be significantly higher when compared with under treatment group. The pleural fluid total antioxidant levels were significantly lower in untreated cases in comparison to under treatment. Decrease in the total antioxidant status was more pronounced in untreated cases, established that antioxidants were nearly completely utilized to scavenge the free radicals. Our findings further support the importance of antioxidants in the treatment of tuberculous patient. This work is licensed under a Creative Commons Attribution 3.0 License. You are free to copy, distribute and perform the work. You must attribute the work in the manner specified by the author or licensor phase of methanol: water (70: 100) containing 550 mL of H3PO4 with 80 nM of NaOH and 20 mL sample was injected. Catalase measurement was done based on the ability of catalase to oxidize hydrogen peroxide (Beers and Sizer, 1952). The change in absorbance was measured for was measured at 240 nm for 3 min. The results were expressed in terms of IU/mL of pleural fluid. For the estimation of total antioxidant status, we used a stable free radical α,α-diphenyl-β-picryl hydrazyl (DPPH), at the concentration of 0.2 mM in methanol (Blios, 1958; Kalpana et al., 2001), ascorbic acid was used as a reference standard. The standard graph was plotted using different concentrations of ascorbic acid and the antioxidant status values were expressed in terms of nM of ascorbic acid. Lactate dehydrogenase is zinc containing intracellular enzyme concerned with reversible oxidation of pyruvate to lactate, involves in glycolytic cycle. The reaction velocity is determined by a decrease in
absorbance at 340 nm resulting from oxidation of NADH (Varley et al., 1980). Pleural fluid total protein content was estimated by the method of Lowry et al., 1951. Proteins form chromophoric complex with phenol reagent, which was measured at 610 nm using UV-VIS spectrophotometer (Elico, SL-150). The protein content was calculated from standard curve prepared with bovine serum albumin and expressed in terms of g%. Statistical evaluation was done using student t-test. Lipid peroxidation product and malondialdehyde level estimated in the pleural fluid of tuberculous patients (Table 1) was significantly decreased (p<0.001) in under treated cases when compared with untreated cases. The levels were decreased with clinical improvement with anti-tuberculosis therapy. The pleural fluid lipid peroxide levels were found to be significantly high (p<0.001) in untreated cases in comparison with under treated cases of all three categories used different treatment regimens, the lipid peroxidation levels were more in category 2. In the present study catalase level in pleural fluid of the both untreated and under treated cases it was found that the difference was not statistically significant and the catalase level was low even after completion of antituberculosis therapy. The antioxidant levels were increased significantly (p<0.01) in under treated cases in comparison with untreated cases. The pleural fluid antioxidant status in under treatment patients with different treatment regimen, in category 1 and 3 was significantly increased (p<0.01) in comparison with untreated patients, there was no significant variation in category 2 (Table II). Lactate dehydrogenase level was decreased significantly (p<0.01) in under treated cases in comparison with untreated cases. In tuberculous patients based on treatment regimen, the pleural
Fluid lactate dehydrogenase levels were decreased

Samudram, P. et al (2009), investigated the case-control study followed by a longitudinal cohort study was undertaken to evaluate the level of lipid peroxidation product malondialdehyde (MDA) and nitrite as an indirect measurement of nitric oxide vis-à-vis the levels of antioxidants vitamin C and vitamin E in pulmonary tuberculosis. Fifty-six sputum smear-positive cases or pulmonary tuberculosis based on Ziehl-Neelsen (ZN) staining and 50 healthy controls without any systemic disease were included in this study. Thirty-five cases were longitudinally followed up with standard antituberculosis chemotherapy (ATT) for two months. Serum levels of malondiadehyde (MDA), nitrite, and plasma levels of vitamins C and E were measured. The mean serum MDA level was significantly higher (8.1 ± 1.61 nmoles/ml) in PTB patients before commencement of ATT as compared to healthy controls (3.45 ± 1.7 nmoles/ml) (p=0.0001) and decreased significantly after 2 months of ATT (3.84 ± 1.28 nmoles/ml) (p=0.0001). The mean serum nitrite level (47.19 ± 18.44 µmol/l) was significantly elevated before ATT compared to healthy controls (32.89 ±11.94 µmoles/l) and decreased significantly after 2 months of ATT (27.71 ± 11.97 µmoles/l) (p=0.0001). The mean plasma levels of vitamins C (0.88 ± 0.33 mg/dl) and E (0.79 ± 0.24 mg/dl) in PTB patients before commencement of A TT were lower than healthy controls (1.42 ± 0.38 mg/dl) and (1.35 ± 0.35 mg/dl), respectively (p=0.001). There was a significant increase in vitamin C levels after 2 months of ATT (1.19 ± 0.40 mg/dl) compared to before A TT (0.83 ± 0.31 mg/dl) (p=0.0001), but no significant change in mean plasma vitamin E level before and after 2 months on A TT was found. Elevated malondialdehyde and nitrite levels with concomitant depressed vitamin
C and E levels are indicative of lipid peroxidation and oxidative stress. The decrease in levels of malondialdehyde and nitrite with subsequent increase in vitamin C levels after two months of follow-up indicate a good response to treatment with standard ATT. Hence, the extent of oxidative stress in PTB can be evaluated by analyzing lipid peroxidation product, antioxidant and nitric oxide levels.

**Antioxidant enzymes and Pulmonary Tuberculosis**

Safarian and karapetian\(^{26}\) (1990) studied the serum of pul. T.B. patients and observed an increase in SOD as compared to normal controls.

Thomas\(^{27}\) et al (1992) performed a study on oxidative stress and antioxidants in intestinal disease and found that increased oxidative stress levels and decreased antioxidant levels.

Jacson p\(^{28}\) et al (1995) worked on finding the effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic renal failure and found that there levels showed variation.

Lih-brody L\(^{29}\) . et al (1996) performed a study on increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease and found that and imbalance between the increased ROS and the decreased antioxidant defenses occurs in IBD patients.

Durak\(^{30}\) et al (1996) studied total cytoplasmic Cu, Zn-SoD and mitochondrial MnSoD activities in serum and pleural fluids from patients with lung tuberculosis, SoD activities were found to be higher in all patients compared to control. They concluded that enzymatic
activity might be used as a non specific prognostic marker is assessing cellular and mitochondrial tissue destruction.

**Rock** et al (1996) investigated that low food intake, nutrient malabsorption, insufficient nutrient release from the liver, acute phase response and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations.

**Geerling BJ** et al. (1999) studied the relation between antioxidant status and alteration in fatty acid profile in patient with Crohn's disease and control.

**Chan and goldkorn** (2000) undertook a study to investigate the role of SOD as one of the ROS that mediate lung injury.

**Valerie Gouaze** et al (2001) selectively investigated the interaction of seleno-glutathione peroxidase 1 (GPx no. 1) with the cytotoxic response of T470 human breast cancer cells to doxorubicin, an anticancer drug known to promote production of ROS and apoptotic mediator ceramide. Reduced glutathione and N-acetylpaetaine can inhibit both apoptosis and necrosis of several cell types, suggesting a critical role for reactive oxygen species (ROS) in cell death and results indicated that GPx 1 can regulate doxorubicin induced cell death signaling at least in part by interfering with the activation of the sphingomyelin ceramide pathway.

**Jingxiang Bai** et al (2001) conducted a study on mitochondria dysfunction induced by ROS and found that mitochondrial catalase protects cell from oxidative injury induced by hydrogen peroxide and antimycin A. However, it increased the sensitivity of cells tumor necrosis factor-induced apoptosis by changing the redox-oxidative status in the mitochondria. They could conclude that the antioxidative
effectiveness of catalase when expressed in the mitochondrial compartment is dependent upon the oxidant and the locus of ROS production.

**Jose M. Mates, Cristina Perez-Comez** (2002) studied the importance of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and catalase working together in human cells against toxic reactive oxygen species and their relationship with several pathophysiologic process and their therapeutic implications and found that low concentrations of ROS may be beneficial or even dispensable in process such as intracellular signaling and defence against microorganisms. Nevertheless, higher amounts of ROS play a role in the ageing process as well in a number of human disease states, including cancer, ischemia and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, several non enzymatic and enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as consequence of a pathologic event, a defense system promotes the regulation and expression of the antioxidant enzymes.

*Nobuya Ishibashi* (2002) studied on inflammatory spouse and glutathiones peroxidare in a model of stroke and found out that glutathione peroxidase sensitive oxygen species play an important role in regulation of cell death during cerebral IIR by modeling intrinsic neuronal sensitivity as well as brain inflammatory reactions.

*Kolanjiappan, K., Manoharan,S. and Kayalvizhi, M* (2002), examined the structural integrity of red blood cells in cervical cancer patients by measuring the concentrations of thiobarbituric acid reactive substances (TBARS), antioxidant status, cholesterol/phospholipid (C/P) molar ratio, enzyme activity and osmotic fragility of
erythrocytes. Methods: This study has been conducted on 32 adult female cervical cancer patients and an equal number of age- and sex-matched normal subjects. Erythrocyte concentrations of lipids, TBARS, vitamin E, reduced glutathione and enzymic activities of catalase and Na+K+-ATPase were measured as well as plasma concentrations of sodium and potassium. The present study also examined the changes in erythrocyte osmotic fragility in cervical cancer patients and normal subjects. The red cell fluidity and permeability were determined by estimating the C/P ratio and Na+K+-ATPase activity, respectively. Results: The release of thiobarbituric acid reactive substances was significantly higher in cervical cancer patients as compared to normal subjects. The increased lipid peroxidation with concomitant decrease in antioxidants was notable in cervical cancer patients. Red blood cells of cervical cancer patients were more fragile than those from normal subjects. Increase in red cell membrane C/P ratio and Na +K+-ATPase activity was noticed in cervical cancer patients as compared to normal subjects. Conclusions: Increased lipid peroxidation, insufficient antioxidant potential and changes in C/P molar ratio as well as activity of Na +K+-ATPase cause structural and functional abnormalities in the erythrocytes of cervical cancer patients.

Reddy Y, N. (2003) conducted a study on the effect of antioxidant on patients suffering from leprosy and reported that antioxidant therapy is suitable to reduce the oxidative stress.

Blankenberg (2003) worked on patients with suspected coronary artery disease to assess the effect of cationascular events associated with red cell erythrocyte glutathione peroxidase 1 ad superoxide dismutancare activity and found that was among thetosteg
univariate predictors of cardiac events whereas SOD has no association with risk based on the background that cellular antioxidants enzymes such as GPx 1 and superoxide dismutase have a central role in the control of reactive oxygen species and data and studies in animals models suggest that these enzymes may protect against atherosclerosis but little is known about their relevance to diseases.

James D. Crapo et al (2003) investigated that neutrophils are second line of defence when alveolar macrophages fail and the accumulation of eosinophils in the lung is mediated by chemokines, he performed a study in which he deduced that presence of inflammatory cells such as eosinophils or neutrophils in the lungs is associated with increased oxidant injury. Lung is uniquely designed to control oxidative stress and lung lining fluids are enriched in the antioxidant glutathione which reaches a higher level.

Joppa, P. Petrasova, D. Stancak, B et al (2006-07) investigated that oxidative stress plays an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Oxidant/antioxidant imbalance has also been reported in various forms of pulmonary hypertension. The present study aimed to assess systemic oxidative stress, as reflected by serum malondialdehyde (MDA) concentrations and activities of antioxidant enzymes in erythrocytes [glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT)] in patients with and without pulmonary hypertension secondary to COPD. Seventy-five patients (58 male) with COPD (mean age 65.1 ± 1.2 years; mean smoking history 35.6 ± 3.8 pack-years) were studied. Twenty-one healthy non-smokers served as a control group. Pulmonary function was evaluated with body plethysmography; mean and systolic
pulmonary artery pressures (Ppa) were assessed with Doppler echocardiography. Serum concentrations of MDA and activities of GPX, SOD and CAT in washed red blood cells were measured using spectrophotometry. Pulmonary hypertension was present in 28 patients with COPD (systolic Ppa: 46.4 ± 2.3 mmHg; mean Ppa: 26.0 ± 1.9 mmHg) and absent in 47 (systolic Ppa: 22.9 ± 0.8 mmHg; mean Ppa: 13.4 ± 0.6 mmHg). Compared with the healthy control group, all the patients (with or without pulmonary hypertension) had higher serum MDA concentrations (1.5 ± 0.1 versus 2.3 ± 0.1 versus 2.3 ± 0.1 nmol/mL, ANOVA, P < 0.001) and lower erythrocyte GPX activity (51.3 ± 3.2 versus 42.2 ± 2.0 versus 41.3 ± 2.5 U/g Hb, P = 0.029), whereas SOD (1121.1 ± 29.0 versus 1032.6 ± 21.8 versus 1032.7 ± 36.2 U/g Hb, P = 0.063) and CAT activities (4.9 ± 0.2 versus 4.6 ± 0.1 versus 4.7 ± 0.2 U/g Hb; p = 0.454) were similar. No differences were observed in serum MDA concentrations or activities of GPX, SOD and CAT in erythrocytes between COPD patients with and without pulmonary hypertension.

CV Balasubrahmanya prasad et al (2007) conducted a study on erythrocytic superoxide dismutase (AD) and (catalase) (CAT) activities and hydrogen peroxide peroxide induced lipid peroxidation in leprosy and found the induced peroxidation was significantly high and the enzyme activities were significantly in leprosy total patients) as compared to controls. A progressive increase in peroxidation was detected along the leprosy spectrum from tuberculid leprosy to lepromatous leprosy and the increase was significant in Bordieline leprosy, Borduline lepromotous, leprosy and lepromatous leprosy as compared to controls.

GA Jacobson et al (2007) investigated the plasma glutathione peroxidase concentration GPX activity plasma selenium and oxidative stress in acute severe asthma and that the levels of MDA increased but
no differences in plasma selenium levels or GPx activity. GPx levels measured by Elisa were elevated in severe asthma. These results are consistent with an adaptive up regulation of GPx to protect against oxidative stress.

Halil Ciftci, Ayhan verit, Ercan yeni, Murat Saves\textsuperscript{46} (2008) found that in the urine of patients with UTI have higher total antioxidant spacity (TAC) and lower total peroxide and oxidative stress, index levels, However TAC and plasma Vitamin C concentration and higher total peroxide and as 1 levels were observed in UTI. This condition may be a factor which facilitates the development of the infection on is secondary to UTI.

Naguva Abdallah ismail\textsuperscript{47} et al (2009) studied the oxidative stress status in children with glycogen storage disease by determining activities of GPx SOD and CAT in liver tissue. They concluded that oxidative stress could play an important role in the pathogenesis of glycogen storage disease and increased levels of SOD and GPx were observed in the study.

**ADA and Pulmonary Tuberculosis**

Giblet\textsuperscript{48} et al (1972) observed the relationship between immunological dysfunctions and ADA deficiency by pointing out the ADA deficiency concepts for the first time.

Stranfcinga\textsuperscript{49} et al (1987) found that serum ADA level in pleural fluid were significantly higher than serum ADA levels in both tuberculosis and non tuberculosis pleural effusion. Adenosine deaminase in the diagnosis of pulmonary tuberculosis and monitoring the efficiency of therapy. The ADA activity was (mean ± SD) 21.77 ± 8.51 u/h in pulmonary tuberculosis, 6.24 ±3.25 u/L in old tuberculosis patients, 8.58 ±
4.38 u/L in healthy control subjects, whereas the mean for the patients with bronchial cancer was 18.51 ± 7.85. In pulmonary tuberculosis patients ADA activities were determined both before and after treatment and significant decrease was observed in ADA activation after treatment and serum ADA activity is increased in pulmonary tuberculosis patients, it may be helpful parameter for monitoring therapy.

Segura^50 et al (1989) investigated ADA activity in body fluid and found out that it is a useful diagnostic tool in tuberculosis.

Gupta D.K^51. (1990) revealed that 53 cases of pleural effusion out of which 36 were of tuberculosis etiology. The mean ADA level in tuberculous was 50.57 U/L while in malignant and parapneumonic effusion it was 14.47 U/L and 28.65 U/L respectively. The sensitivity and specificity for diagnosing tuberculosis were 100% and 94.1% respectively.

Jeronimo Jaqueti^52 et al (1990) reported that ADA enzyme, is essential for the differentiation of lymphoid cells and has been used for monitoring several diseases in which immunity is altered. Excess of intracellular reactive oxygen species result in an environment that may modulate gene expression or damage cellular molecules.

Ida^53 et al (1990) observed that abnormally high levels of serum ADA activity were seen in patients with pulmonary tuberculosis, indicating that serum ADA is a good diagnostic tool for tuberculosis.

Bhargava et al And Al-shamrnary^54 et al (1990) concluded from their study that the ADA level in the serum of children with T.B was significantly higher than that of healthy children and in the TB diagnosis the cut off value of serum ADA LEVEL WAS DECLARED
AS 78, 12, 32, 8 respectively in comparison to the cut off value of 53, 76 U/L as found by other studies.

Mukesh Kumar Agarwal\textsuperscript{55} et al (1991) found that the mean serum ADA levels in patients of pulmonary tuberculosis was 38.48 +/- 1.5634 U/Lt. The value was significantly higher than the mean value for healthy controls.

Meftun Unsal\textsuperscript{56} et al (1992) undertook a study to assess the levels of ADA in patients suffering with active and inactive pulmonary tuberculosis and derived the result that average serum ADA levels were found to be higher in active disease patient group than in the inactive patients group there was no statistical difference between serum ADA levels of two groups.

Lakshmi\textsuperscript{57} et al (1992) found that the average serum ADA values in 61 patients with active pulmonary tuberculosis patients in their study as follows: sputum –ve PPD patient group 13.13+/-5.97µl. Sputum +ve (PPD)+/- patient group 33.52+/-15.22 and they stated that serum ADA values in patients that may have active pulmonary tuberculosis can be helpful for diagnosis.

Burgess L. J\textsuperscript{58}. (1995) showed ADA activity in tuberculous effusion was higher than in any other diagnostic group. At a level of 50 U/L the sensitivity and specificity for the identification of tuberculosis was 90\% and 89\% respectively. Voight studied 41 cases which were bacteriologically confirmed tuberculosis cases with other causes the mean ADA levels according to tubercular etiology was 99.8 U/L with sensitivity and specificity for diagnosis tubercular ascities was 95 \% and 98\% respectively. DwivediM studied on 49 patients with ascities of which 19 were of tubercular etiology with mean ADA level of 98.8
At an ADA level >33 U/L. The sensitivity, specificity, positive negative predictive values were 100%, 96, 95% and 100 % respectively.

**Kelbel** et al (1995) deduced from their study that serum ADA was a selective marker of immune stimulation in tuberculosis but not in cancer when compared the serum ADA activity of pulmonary tuberculosis patients with the patients of lung cancer pre-treatment and healthy individuals.

**Rohani M. Yu** et al (1995) performed a study on the CSF from patients with meningitis and other conditions with CN system symptoms and ADA activity was determined. T.B meningitis patients had ADA activity greater than the cut off value of 9.0I/uL. ADA is an adjunctive rapid marker for tuberculosis.

**Ferrer** et al (1997) found that ADA measurement is commonly used in European and asian countries where there is a high incidence of tuberculosis.

**Palival R. Shahikr** (1998) conducted a study to evaluate the efficiency and usefulness of serum ADA activity for diagnosis of pulmonary tuberculosis and other common non tuberculosis respectively conditions. Serum ADA levels were determined in 10 healthy individuals and 90 patients which included 65 patients with pulmonary tuberculosis, 15 patients with supportive lung disease and 10 patients with lung carcinoma. It was fond that serum ADA levels were significantly higher in patients with pulmonary tuberculosis.

**Collazos** et al (1998) found in his study that the serum ADA level of 52% of active pulmonary tuberculosis patients was high.
Kuyucu\textsuperscript{64} et al (1999) conducted a study on serum ADA activity in childhood pulmonary tuberculosis. Serum ADA levels were measured in 20 children diagnosed with pulmonary tuberculosis (group 1) and 150 children (group 2) including 128 with tuberculosis infection and 22 healthy children. In group 1 the mean serum ADA activity was 74.06±18.5 which was significantly higher than that of group 2 (40.36±12.00). So it was found serum ADA activity was a useful diagnostic tool in childhood pulmonary tuberculosis.

Raintawan\textsuperscript{65} et al, 1999, Valdes\textsuperscript{66} et al 1996, Burgess\textsuperscript{67} et al 1996, Aggarwal\textsuperscript{68} et al 1999 & Roth\textsuperscript{69} suggested that ADA diagnostic test has 90 to 100% specificity and is inexpensive. Collazazos\textsuperscript{70} et al, 1998; Bansal\textsuperscript{71} et al, 1991; Segura\textsuperscript{72} et al, 1989; Conde\textsuperscript{73} et al, 2002 in their studies have suggested the use of serum adenosine deaminase levels for the diagnosis of pulmonary tuberculosis.

Mishra\textsuperscript{74} et al (2000) noticed raised serum adenosine deaminase activity in a group of tuberculosis cases compared with healthy individuals.

According to Yash P Kataria and Imtiyaz Khurshid\textsuperscript{75} (2001) the reliability of early diagnosis of plural TB has been greatly improved by the use of biochemical markers such as ADA interferon $\gamma$ and lysozyme. The determination of ADA level in the suspected pleural fluid appears to the most promising marker because of care, rapidly and cost-effectiveness of the ADA assay. The determination of ADA activity was first proposed as a serologic diagnostic matter for lung cancer in 1970.

Dilmac A 2002, leolu K\textsuperscript{76} et al (2002) found the diagnostic value of ADA activity in sputum in pulmonary tuberculosis.
Conde M.B. et al. (2002) studied ADA levels in serum for the diagnosis of tuberculosis and ADA levels have been followed during the tuberculosis treatment. 14 is accepted as cut off value for ADA. Bhargave et al and al shamary et al accepted the cut off value of serum as a level as 78, 12 and 32, 8.

Verma et al (2004) studied the serum ADA activity in serum and pleural fluid in patients affected with pulmonary tuberculosis and other common non tubercular chronic respiratory diseases. The study was carried out on patients suffering from various pulmonary diseases, the study revealed that the serum ADA activity was higher in patients of pulmonary tuberculosis and pleural diseases and non tuberculosis pulmonary diseases than in control subjects. The mean serum ADA activity in the patients group was 35.5±6.93 μl as compared to 16.60±2.85.

K. Pratibha et al (2004) reported that patients with liver disease showed significantly higher levels of serum bilirubin and enzymes as compared with control group, a comparison between patients and controls for ADA, 5'NT and MDA, all three parameters were found to be significantly higher in patient group.

Sharma, S.K. et al (2006) evaluated that diagnostic accuracy and cost-effectiveness of ascitic fluid interferon-γ (IFN-γ) and adenosine deaminase (ADA) assays in the diagnosis of tuberculous ascites. Ascitic fluid from patients with proven tuberculosis (TB) (n = 31) and non-TB ascites (n = 88) was analyzed for IFN-γ and ADA levels. Areas under the receiver operative characteristic (ROC) curves (AUCs) for the two biologic markers were compared. Levels of ascitic fluid IFN-γ, median (range): 560 (104-1600) pg/mL vs. 4.85 (0–320) pg/mL (p < 0.001), and ADA, median (range): 58 (16-331) IU/L vs. 10

98
(0-59) IU/L (p = 0.001), were significantly different between TB and non-TB groups. IFN-\(\gamma\) and ADA assays showed equal sensitivity (0.97) and differed marginally in specificity (0.97 vs. 0.94). Difference in AUCs was not significant (0.99 vs. 0.98, p < 0.62). For differentiating TB from non-TB ascites, optimal cutoff points were 112 pg/mL for IFN-\(\gamma\) and 37 IU/L for ADA. The accuracy of the ADA assay was similar to that of the IFN-\(\gamma\) assay in differentiating of TB from non-TB ascites. Because both material and human costs of the ADA assay are far less than those of the IFN-\(\gamma\) assay, the former is probably the most appropriate diagnostic test for analysis of peritoneal fluid in resource limited settings.

Mohammed A Aezoghaibi\textsuperscript{81} et al (2007) performed the study on lipid peroxide in patients with inflammatory bowel diseases increases level of plasma MDA which were an important indication of oxidative stress. Patients with crohns diseases are more susceptible to oxidative stress than the patient with ulserative colitis.

Cimen, Filiz. and Ulukavak Ciftci, Tansu\textsuperscript{82} . et al (2008) aimed to investigate serum ADA levels in patients with tuberculosis and its relation with drug resistance and tuberculosis categories. The study involved 51 pulmonary tuberculosis patients and eleven healthy controls. All patients classified according to the World Health Organization (WHO) and 60.8% of the patients were category I, 21.6% were category II and 17.6% were category IV pulmonary tuberculosis. Serum ADA levels of the sensitive cases to isoniazid (H), rifampicin (R), ethambutol (E) and streptomycin (S) were compared with those of the resistant cases. Serum ADA levels were higher in Rand E sensitive group then in the resistant group (p=0.046, p=0.045). Serum ADA
levels were similar in Hand S sensitive group and resistant group (P>0.05). Serum ADA levels were higher in category I group when compared with the levels of the healthy group (p: 0.03). Comparison between the serum ADA levels of the groups of category I, II and IV with each other showed that the values of category I were significantly higher than values of category II (p=0.038). Although there were no statistically significant differences, it was shown that when the number of resistant drugs increased, the mean serum ADA level tend to decrease. In conclusion, serum ADA levels of category I patients were higher than healthy group and while the number of resistant drugs increasing, ADA levels were decreasing.

Bandyopadhyay, D. and Gupta, S (2008) studied 179 cases of EPIB were analysed for acid-fast bacilli (AFB) smear, adenosine deaminase activity (ADA) and multiplex polymerase chain reaction (PCR). Although estimation of ADA is helpful, its sensitivity and specificity varies widely. On the other hand, a multiplex PCR using amplicons such as IS6110, dnaJ gene and hsp65 genes has high sensitivity (60-88%) and specificity (81-100%). On comparing AFB and ADA results with PCR, the PCR is clearly more effective than AFB (P < 0.001) and ADA estimation (P < 0.02) in CSF. The same result was observed with peritoneal fluid (P < 0.001 vs. P < 0.05) and pleural fluid (P < 0.001 and P < 0.05). The study shows that multiplex PCR remains the best tool and is a much better marker for diagnosing EPTB.

Kamaldeen Baba et al (2008) found in their study that ADA is sensitive marker of tunerculous pleuritis even in HIV patients with very low CD4 counts in a high TB endemic region. ADA assay is inexpensive, rapid and simple to perform and is of great value for the
immediate diagnosis of TB pleuritis while waiting for culture result and this has a positive impact on patients routine.

**Lipid Peroxidation and Pulmonary Tuberculosis**

Letbowitz\(^5\) and al (1973) found that tuberculosis pleural effusion is thought to result from delayed sensitivity reaction that occurs in response to the presence mycobacterial antigens in pleural space.

Mead\(^6\) (1976) one of the accepted hypothesis to explain the molecular mechanism of cell death is lipid peroxidation. In addition to oxidative alteration of complex lipids free radical mediated events may also affect other cellular components such as protein carbohydrate compound and nucleic acid.

May and Spagnuolo\(^7\) (1987) found in their study that mycobacterium can induce reactive oxygen species production by activating phagocytes.

Halliwell B.\(^8\) (1991) deduced from his study that thiobarbituric acid reactive substances primarily reflect malondialdehyde, a breakdown product of lipid peroxides and are commonly used as the measure of oxidative stress.

DJ Silver Man\(^9\) and al (1992) in their study cells infective by ricketzia rickettsii, the causative agent of rocky mountain spotted fever, display unusual intracellular morphological changes characterized by dilation of the membranes of the endoplasmic reticulum and outer nuclear envelope. This observation suggest that the increased peroxide in infected cell may be lipid peroxide degradation products of free radical attack on poly enoic fatty acid.

J A North\(^10\) and al (1994) found that the extent of lipid radical
formation in response to oxidative stress can be influenced by changes in the polyunsaturated fatty acid composition of cell lipid and suggest the possibility that carbon-centered lipid radicals may interact with extracellular structure.

Jack\textsuperscript{91} et al (1994) and Grimble\textsuperscript{92} (1994) investigated that although an important part of host defense against mycobacteria and enhanced ROS phagocytes generation may promote tissue injury and inflammation that also leads to immunosuppression.

Raviglione\textsuperscript{93} et al (1995) in his study describe T.B as a chronic granulomatous disease caused by mycobacterium tuberculosis with various manifestation, involving most commonly the lung but other system as well.

Sen and Packer\textsuperscript{94} (1996) found that reactive oxygen species generated as a result of ischemic-reperfusion associated with haemorrhage has been proposed to contribute to the progress of lung Injury.

Christopher K. W. Lai\textsuperscript{95} et al (1997) found that the clinical manifestations of IB depend on cellular immune responses to the tubercle bacilli characterized by the accumulation of monocytes, macrophages, lymphocytes and polymorphonuclear leukocytes in tuberculous lesions. These responses are initiated on sensitization of T lymphocytes by the bacterial antigen with the release of cytokines that regulate macrophage function. Activation of T lymphocytes in TB is supported by the previous findings that the serum concentration of soluble interleukin (II -2) receptor in patients with active disease was markedly raised. CD4+ Tcells are likely to be the pivot cells in orchestrating the immunologic defence against the mycobacteria as
depletion of these cells is associated with increased susceptibility to TB in both animals and humans.

Subrahmanyam M\textsuperscript{96} et al (1998) in their study B on D effects of topical application on honey on burn wound healing they took 100 burn patients and were divided into two special to be treated with honey dressing or with silver sulphadiazine gauze dressing in honey treated group wounds healed earlier seem lipid peroxide levels were revised in the immediate post burn period in both group. Dacleval cultured revealed that 90\% were rendered sterile in the honey treated group, where as in the silver-sulphadiazine treated group there was persistent infection.

Kwiatkowska\textsuperscript{97} et al (1999) showed that malondialdehyde and lipid peroxidation product levels in sputum smear positive patients with advanced pul tuberculosis and sputum smear negative patients with small radiographic changes were significantly higher than those of healthy control group.

J Bhattacharya and AG Datta\textsuperscript{98} (2001) conducted a study on effects of lipopoly saccharide on lipid peroxidation of erthyrocyte and its reversal by mannital and glyceraol.

Makinskii\textsuperscript{99} et al (2002) showed that high pretreatment levels of free radicals had decreased to normal at the end of the treatment in pul. T.B. cases.

S Kastenbauer, U Koedal\textsuperscript{100} et al (2002) conducted study on oxidative stress in bacterial meningitis in humans and found that tyrosine nitration was strongly increased during meningitis. It was most evident in inflammatory cells and blood vessels in the sub
arachnoid space. High CSF nitrotyrosine concentration were associated with an unfavorable outcome according to the Glasgow outcome score in CSF the increase in nitrotyrosine was accompanied by a depletion of the antioxidant ascorbic acid and an increased oxidation of natural peroxinitrite scavenger uric acid to allantoin.

Peter Barany\textsuperscript{101} et al (2003) worked on inflammation and resistance to erythropoises. Stimulating agents - links to oxidative stress and cardiovascular mortality and found that patients with chronic kidney diseases are thought to have reduced capacity to one thing is there.

Figen Deveei and Nevin Ilhan\textsuperscript{102} (2003) studied on plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis and found that increased amount reactive oxygen species that a produce as a consequence of a phagocytic respiratory burst during pulmonary inflammation are responsible for the increase circulating levels MDA.

Yildiz Guney\textsuperscript{103} et al (2004) investigated the serum malondialdehyde levels and superoxide dismutase activities in pulmonary tuberculosis and lung cancers and came up with the conclusion that serum MDA level in the whole group of patient (lung cancer and TV were much higher than in healthy control) as well as from species of exogenous ori...... such as cigarette.

Kiranjeet Kaur\textsuperscript{104} (2005) reported that there IS an oxidative stress and decreased antioxidant activity in patient of pulmonary tuberculosis which correlate with radiological extent sputum grading and cavity status.
Ceylan\textsuperscript{105} et al (2005) found that in TB patients the levels of serum reactive oxygen metabolites were increased as compared to normal controls, therefore building up oxidative stress in the patients.

Walsh\textsuperscript{106} et al (2006) have primarily shown that TBARS highly correlate with 8-isoprostane and thus it accurately reflects lipid peroxides and that MDA can affect the membrane proteins by cross linkage, rendering them useless as receptors or enzymes.

Silvia Carraro\textsuperscript{107} et al (2008) conducted a study of exhaled breath condensate that is a safe and easy technique that enables several biomarkers of lung disease. EBC has been applied in the study of various sepraty diseases in children (mostly asthma and cystic fibrosis, but also other diseases such as primary ciliary dyskinesia). Several biomarkers of airway inflammation and oxidative stress have been detected in the EBC of these patients, demostaling the role of different inflammatory pathways in the pathophysiology of respiratory diseases.

Critical Appraisal of Studies Reviewed

On analyzing the review of studies in the area of medicinal chemistry, the following observations may be made parameter vise that are considered in the study.

- Most of the researches on ADA are concentrated on drug resistance, nutritional management, anti-oxidation, infection disease, immune stimulation, and gene expression etc. very few researches were found in pulmonary tuberculosis and variation of biochemical parameters in as sputum +ve and sputum –ve patients.
- Studies conducted on lipid preoxidation in the context of
meningitis, honey, cardovas cular disease, poli unsaturated fat, defence mechanism, mico bacterium, lung injury, but hardly any study is traced as is under consideration.

- Researches in the area of antioxidants have been conducted on leprosy, mental disorders, several falinic, hepatic apurecrosis, immunology, asthma, but not even few researches are found related to pulmonary tuberculosis with the combination of parameters as considered in this study.

- Studies on glycoprotein have been reported on levels of glycoprotein, glycoprotein pattern, immuno compromised. Very few studies have been observed that too with the variation from this study.

- Vitamin E has been studied in case of inflammatory disease IDB patients micronutrients, T cells, reactive oxygen species and vitamin E selenium etc. Hence the studies on vitamin E and sputum +ve and sputum -ve pulmonary tuberculosis are very few to report.

Consequently as may be derived from the review of researches in the area of medicinal chemistry, no research with the parameters that are taken in this study, has been reported. Therefore this research study will make a significant contribution in the area of early diagnosis of pulmonary tuberculosis wherein is so frame in its' consequences.
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