Role of oxidative stress and antioxidant levels in tubercular, reactive and metastatic lymphadenopathy - A comparative study

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Abstract

Objectives:

1. To evaluate the serum levels of lipid peroxidation product like thiobarbituric acid reacting substance(TBARS) i.e. Malondialdehyde and Reactive oxygen species(ROS) i.e. Nitric oxide in patients of tubercular, reactive and metastatic lymphadenopathy.
2. To evaluate the antioxidants status i.e. level of ascorbic acid, reduce glutathione and superoxide dismutase in patients of tubercular, reactive and metastatic lymphadenopathy.

Materials and Method: Forty cases each of tubercular lymphadenitis, reactive lymphadenitis and metastatic lymphadenopathy was diagnosed on FNAC and evaluated for various parameter i.e. Malondialdehyde, Nitric oxide, ascorbic acid, reduced glutathione and superoxide dismutase by colorimetric method. Thirty healthy individuals were taken as controls.

Results: Levels of malondialdehyde and nitric oxide were significantly raised in patients of tubercular lymphadenitis (6.89 ± 1.48nmol/ml) and metastatic lymphadenopathy (8.03±1.51nmol/ml) as compare to patients with reactive lymphadenopathy (4.62 ± 1.14nmol/ml) and control group (4.62 ± 1.45nmol/ml). Superoxide dismutase, reduced glutathione and Ascorbic acid reduce in tubercular (2.14 ± 0.63units/ml) as well as metastastic lymphadenopathy (1.88 ± 0.52units/ml) than reactive lymphadenopathy (3.19 ± 0.54units/ml) and control group (3.42 ± 0.53units/ml).

Conclusion: ROS are known to play tremendous role in health and disease. A frequent cellular target to ROS is lipid components of the cell membrane resulting in lipid peroxidation. To counter the harmful effects of ROS, antioxidants defense mechanism operates to detoxify or scavenge these ROS. This may be important for better understanding the pathogenesis of the disease and may contribute to its diagnosis and treatment.

Keywords: Oxidative stress, Antioxidants**,** Lymphadenopathy, Tuberculosis, Metastatic.

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Introduction

Oxygen, the very essence of life, can itself become a toxin and endanger cellular components.(1) Salevemini (2002) described oxygen as doubled edged sword. Tetravalent reduction of oxygen produce water but univalent reduction produce a series of reactive radical and non radical, which are collectively known as Reactive Oxygen Species (ROS).The important ROS in biological systems are superoxide anion (o_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical(OH) and singlet oxygen.(2)

ROS are known to play tremendous role in health and disease. A frequent cellular target to ROS is lipid components of the cell membrane resulting in lipid peroxidation.(3)

Lipid peroxidation is one of important mechanism in pathogenesis involved in oxidative stress. Following lipid peroxidation, a great variety of aldehydes are form by degradation of lipid hydroperoxidase in biological system, which react with thiobarbaturic acid and form substances, which are popularly thiobarbituric acid reactive substances (TBARS). Among the many different aldehydes form, the most intensively studied is malondialdehyde (MDA), MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations.(4)

Proteins are also subjected to ROS mediated denaturation which may lead to structural loss or enzymatic inactivation. Nucleic acids are prone to base hydroxylation, cross- linking or strand breakage on exposure to ROS which may result in mutation or even cell death.

To counter the harmful effects of ROS, antioxidants defense mechanism operates to detoxify or scavenge these ROS. Antioxidants act by quenching of superoxide anion, decomposition of H2O2 and sequestration of metal ions.(5) The antioxidants belonging to first line defense includes the enzyme like superoxide dismutase (SOD), catalase(CAT), glutathione peroxidase(GSH-Px), and glutathione reductase (GR). The antioxidants belonging to second line defense includes vitamins like vitamin C, Carotenoides, vitamin E, metabolic antioxidants like glutathione (GSH), Uric acid.

Oxidative stress occurs when the generation of free radical and active intermediates in a system exceeds the system's ability to neutralize and eliminate them.(6) Oxidative stress is implicated in the etiopathogenesis of variety of human disease like atherosclerosis, Diabetes

mellitus, Alzheimer's disease, Parkinson disease, cataract, tuberculosis and cancer.[7]

Tuberculosis like any other chronic inflammatory diseases is characterized by generation of oxygen and nitrogen free radical. Mycobacteria can induce ROS production by activating phagocytes, enhanced ROS generation may promote tissue injury and inflammation. Markers of oxidants mediated tissue damage have shown to be elevated in peripheral circulation in human with active tuberculosis.

Reactive oxygen species are directly or indirectly involved in multistage process of carcinogenesis. They are mainly involved in DNA damage leading to mutation in tumor suppressor genes. They also act as initiator and/or promoter in carcinogenesis.[8]

Our study therefore seeks to determine whether free radical and antioxidant level in patients of tubercular lymphadenitis are significantly different from those of patients with other kinds of lymphadenopathies like inflammatory and malignant.

Materials and Method

The present study was carried out in Department of Pathology in collaboration with the Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sewagram. The study was designed as a case-control study.

The present study was approved by the Institutional Ethical Committee. The selected subjects were informed about this research project and included in the study with their consent without any economical burden to them.

Forty cases each of tubercular lymphadenitis, nonspecific reactive lymphadenopathy and malignant/metastatic lymphadenopathy diagnosed on Fine needle aspiration cytology were included in the study. Age and sex match healthy individuals without past or present history of tuberculosis or any other aliments were included as control group. Detailed history was taken including personal details, nutritional status and treatment history. Both Papanicolaou and Giemsa stained smears of these cases were reviewed and their cytological features were recorded. Zheil Nelson (ZN) stain was done on smears of patients suspected with tuberculosis.

About 6ml of blood was collected from antecubital vein of each subject after obtaining informed consent. About 3ml of blood was stored in an EDTA (Ethylene diamine tetraacetic acid) vial for plasma and cells and 3 ml of blood was stored in plain bulb for serum. Plasma and sera were separated by centrifuging at 3000rpm for 10 min and stored separately at -20^{o} C after adding 5% sodium azide 20µl/ml. Plasma was used to estimate Vitamin C, Nitric oxide and reduced glutathione and serum was use to estimate malondialdehyde and superoxide dismutase.

Estimations

Estimation of Malondialdehyde: MDA was assessed in serum by TCA-TBA method of stater T. F. et al.[9]

Estimation of Nitric Oxide: NO was assessed in plasma by Griess reagent assay of Lee D. U. et al.[10]

Estimation of Superoxide dismutase: SOD was assessed in serum utilizes the principle of inhibition of auto- oxidation of pyrogallol by SOD enzyme using the method of Marklund S, Marklund G et al.[11]

Estimation of Reduced Glutathione: GSH was assessed in serum by method of Beutler et al.[12]

Estimation of Plasma Ascorbic Acid: Vitamin C was assessed by colorimetric method as describe by Aye Kyaw.[13]

Results

The study was carried out on 120 subjects with lymphadenopathy. On the basis of FNAC diagnosis, 40 cases each of tubercular lymphadenitis; non-specific reactive lymphadenopathy and metastatic lymphadenopathy were included in this study. 30 healthy subjects were also included as control group in this study.

On distribution of cases according to their ages, it was observed that maximum number of cases (24; 60%) of tubercular lymphadenitis belonged to the third and fourth decade. Similarly, in reactive lymphadenopathy, maximum number (23; 52.5%) of cases belonged to the third and fourth decade. Ninety percent patients with metastatic lymphadenopathy were seen after 40 year of age. The mean age was 32.2 ± 12.2 year, 29.07±10.08 year and 52.17±11.64 year in patients of tubercular, reactive and metastatic lymphadenopathy respectively.(Table 1)

Table 1: Age Distribution of Cases

Age Groups(In Year)	Tubercular Lymhadenities (n%)	Reactive Lymphadenopathy (n%)	Metastatic Lymphadenopathy (n%)
0-9	0(0.0)	0(0.0)	0(0.0)
10-19	6(15.0)	10(25.0)	0(0.0)
20-29	12(30.0)	11(27.5)	1(2.5)
30-39	12(30.0)	12(30.0)	3(7.5)
40-49	5(12.5)	5(12.5)	13(32.5)
50-59	4(10.0)	2(5.0)	10(25.0)

60+	1(2.5)	0(0.0)	13(32.5)
Total	40 (100.0)	40 (100.0)	40 (100.0)

[n= Number of subject]

Most of the cases of Tubercular Lymphadenitis and Metastatic Lymphadenopathy were male, while most reactive lymph nodes sampled were from female patients.

On analyzing the site of lymph node enlargement in these patients, 76(63.34%) of the cases showed cervical lymph node enlargement, while 22(18.34%) of them presented with Axillary lymphadenopathy and 11(9.16%) of the cases presented with supraclavicular lymph node enlargement. as shown in Table 2.

Table 2: Site of Lymph Node Enlargement

Site of Lymph Node Enlargement	Tubercular Lymphadenitis (n%)	Reactive Lymphadenopathy (n%)	Metastatic Lymphadenopathy (n%)	Total (n %)
Cervical	28(70.0)	25(62.5)	23(57.5)	76(63.34)
Supraclavicular	3(7.5)	3(7.5)	5(12.5)	11(9.16)
Axillary	8(20.0)	4(10.0)	10(25.0)	22(18.34)
Inguinal	0(0.0)	4(10.0)	2(5.0)	6(5.0)
Generalized	1(2.5)	4(10.0)	0(0.0)	5(4.16)
Total(n)	40(100)	40(100)	40(100)	120(100)

[n= Number of subject]

All the cases and control were evaluated for marker of oxidative stress and antioxidants levels. Table 3. Shows the level of malondialdehyde(MDA) detected in patients of all three categories and in control group. The mean MDA level of patients with tubercular lymphadenitis was 6.89±1.48nmol/ml, while it was 4.62 ±1.14nmol/ml, 8.03±1.51nmol/ml and 4.62 ±1.45nmol/ml for patients with reactive and metastatic lymphadenopathy and for control group respectively.

Table 3 shows the mean MDA levels of patients tubercular, lymphadenitis and metastatic lymphadenopathy were significantly higher against the control as well as the patients with reactive lymphadenopathy (p value < 0.01).

Table 3: Level of Malondialdehyde in Cases and Controls

	Mean MDA Level ± Std deviation (nmol/ml)	P value(with control)
Tubercular Lymphadenitis	6.89±1.48	< 0.01
Reactive Lymphadenopathy	4.62 ±1.14	>0.05
Metastatic Lymphadenopathy	8.03 ± 1.51	< 0.01
Control	4.62 ± 1.45	

Mean levels of Nitric oxide (NO) detected in patients of all three study groups and in control group were analyzed as shown in Table 4. The mean NO level of patients with tubercular lymphadenitis was 0.47±0.22µM, while it was 0.32±0.11µM, 0.64±0.20µM and 0.28±0.10 for patients with reactive and metastatic lymphadenopathy and for control group respectively.

Table 4: Levels of Nitric Oxide in Cases and Controls

	Mean NO Level ± Std deviation (µM)	P value(with control)
Tubercular Lymphadenitis	0.47 ± 0.22	< 0.01
Reactive Lymphadenopathy	0.32 ±0.11	>0.05
Metastatic Lymphadenopathy	0.64± 0.20	< 0.01
Control	0.28 ± 0.10	

The Mean NO levels of patients with tubercular lymphadenopathy and metastatic lymphadenopathy were significantly higher than control as well as patients with reactive lymphadenopathy (p value < 0.01) as shown in Table 4.

The mean level of antioxidants superoxide dismutase (SOD) detected in patients of study group and control group has shown that the mean SOD level of patients with tubercular lymphadenitis was 2.14±0.63 units/ml, while it was 3.19±0.54units/ml, 1.88±0.52 units/ml and 3.42±0.53 units/ml for patients with reactive and metastatic lymphadenopathy and for control group respectively.

The mean SOD levels of patients with tubercular lymphadenitis and metastatic lymphadenopathy were significantly lower than control as well as than patients with reactive lymphadenopathy (p value < 0.01), as shown in Table 5.

Table 5: Levels of Superoxide Dismutase in Cases and Controls

	Mean SOD Level ± Std deviation (unit/ml)	**P value(with control)**
Tubercular Lymphadenitis	2.14 ± 0.63	< 0.01
Reactive Lymphadenopathy	3.19 ±0.54	>0.05
Metastatic Lymphadenopathy	1.88± 0.52	< 0.01
Control	3.42 ± 0.53	

Table 6 shows, the mean GSH level of patients with tubercular lymphadenopathy was 0.13±0.08 mg/dl, while it was 0.29± 0.08mg/dl, 0.12 ±0.04mg/dl and 0.32±0.08 mg/dl for patients with reactive and metastatic lymphadenopathy and for control group respectively.

Table 6: Levels of Reduced Glutathione in Cases and Controls

	Mean GSH Level ± Std deviation (mg/dl)	**P value(with control)**
Tubercular Lymphadenitis	0.13 ± 0.08	< 0.01
Reactive Lymphadenopathy	0.29 ±0.08	>0.05
Metastatic Lymphadenopathy	0.12 ± 0.04	< 0.01
Control	0.32 ± 0.08	

The mean GSH levels of patients with tubercular lymphadenitis and metastatic lymphadenopathy were significantly lower than control as well as than patients with reactive lymphadenopathy (p value < 0.01).

Table 7 shows, the mean vitamin C level of patients with tubercular lymphadenopathy was 0.35± 0.14mg/dl, while it was, 0.55±0.13mg/dl dl, 0.29±0.11 mg/dl and 0.58±0.15mg/dl for patients with reactive and metastatic lymphadenopathy and for control group respectively.

Table 7: Levels of Ascorbic Acid in Cases and Controls

	Mean Vitamin C Level ± Std deviation (mg/dl)	**P value(with control)**
Tubercular Lymphadenitis	0.35 ± 0.14	< 0.01
Reactive Lymphadenopathy	0.55±0.13	>0.05
Metastatic Lymphadenopathy	0.29± 0.11	< 0.01
Control	0.58 ± 0.15	

The mean vitamin C levels of patients with tubercular lymphadenitis and metastatic lymphadenopathy were significantly lower than control as well as than patients with reactive lymphadenopathy (p value < 0.01).

Discussion

The results of our study indicates that the tubercular and reactive lymphadenopathy was commonly seen in the age group of 20-29 years and 30-39 year respectively(Table 1), where as metastatic lymphadenopathy was seen in patients above 40 years of age. This findings were correlates with Shrivastav A. et al.[14]

Tubercular and metastatic lymphadenopathy was more common in males; whereas reactive lymph nodes were commonly seen in female patients (Table 2).

In our study, the most common group of lymph nodes involved was cervical group (63.34%), which was similar to the findings of Khajuria *et al*.[15]

It was observed that the malondialdehyde levels were highest (8.03±1.51nmol/ml) in the patients of metastatic lymphadenopathy. Higher levels were also observed in patients of tubercular lymphadenopathy (6.89±1.48nmol/ml). The difference between mean malondialdehyde levels of patients with tubercular and metastatic lymhadenopathy were statistically significant than control as well as patients with reactive lymphadenopathy (Table 3)

The mean nitric oxide level was again highest in metastatic lymphadenopathy (0.64±0.20µM) and also higher in tubercular lymphadenopathy (0.47±0.22µM) than reactive lymphadenopathy and control group. The difference was found to be statistically significant.

Similarly, Increased level of reactive oxygen species have been reported by Jack et al in patients of tuberculosis. (Table no. 4). Reddy et al evaluated the concentration of circulating antioxidants and marker of oxidative stress in tuberculosis patients.[17] They reported high free radical activity and low antioxidants level patients of tuberculosis.

The findings of presents study are in agreement with other worker that there is rise in reactive oxygen species and lipid peroxidation products in patients of tuberculosis. It is postulated that during tuberculosis infection, macrophages produce higher level of reactive oxygen species to kill the bacteria effectively. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) which can serve as marker of free radical mediated processes also induce lipid peroxidation.

In present study, reactive nitrogen intermediates (RNI) and lipid peroxidation products are present in

higher levels in patients of tuberculosis than reactive lymphadenitis indicating that oxidative stress do play a role in specific pathogenic mechanism that work in tuberculosis in comparison with reactive lymphadenitis.

The role of oxidative stress in cancer remains complex and controversial. Some of the studies have observed that the apparent increase of free radical in tumours was actually due to change in shape of electron spin resonance (e.s.r.) signal and not due to true increase in free radical content.[18] However, subsequently there are substantial experimental evidence that provides implication that reactive oxygen species are involved in both initiation and promotion of carcinogesis.[19]

The cumulative production of reactive oxygen and nitrogen species through either endogenous or exogenous insults is common for many types of cancers. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cell compared with normal cells. This redox imbalance may be related to oncogenic stimulation.[20]

MDA, a byproduct of lipid peroxidation, is said to be involved in DNA adduct formation, which is believed to be responsible for carcinogenesis. Nitric oxide, on other hand plays a dual role in cancer. At high concentration it kills tumor cells but at low concentration it promotes tumor growth and metastasis. It cause breaks of single and double stranded DNA.[8]

Large body of evidences has suggested that MDA is mutagen and potential carcinogen.[21] Several reports have also suggested increase MDA level in breast cancer,[22] in solid tumors and human tumors cell lines and in gastric cancer patients.[23] Higher plasma malondialdehyde level in breast cancer patients was also found by Ray et al[24] which is in agreement with the result of present study.

The higher levels of nitric oxide in patients of metastatic lymphadenopathy in our study are also in agreement with Ray G et al.[25]

All the cases and control were also evaluated for antioxidants level by measuring the level of superoxide dismutase (SOD), reduced glutathione (GSH) and vitamin C in serum. It was observed that the mean level of superoxide dismutase was lowest (1.88±0.52 units /ml) in patients of tubercular lymphadenitis. The mean SOD levels of patients with tubercular lymphadenitis and metastatic lymphadenopathy were significantly lower than control as well as patients with reactive lymphadenopathy. (Table 5).

The mean reduced glutathione (GSH) levels of patients with tubercular lymphadenitis (0.13± 0.08 mg/dl) and metastatic lymphadenopathy (0.12 ± 0.04 mg/dl) were also significantly lower than patients with reactive lymphadenopathy and the control group in the present study. (Table 6) Similarly, the mean vitamin C levels were again lower in metastatic lymphadenopathy (0.29 ±0.11mg/dl) and in tubercular lymphadenitis (0.35 ± 0.14 mg/dl) and in tubercular lymphadenitis (0.35 ± 0.14 mg/dl) than in patients with reactive lymphadenopathy and the control group. The difference was found to be statistically significant (< 0.01) (Table 6)

Our findings were similar to Reddy et al who suggest lower antioxidant capacity and higher oxidative stress in tuberculosis patients than in healthy volunteers. They showed a significant reduction in enzymatic antioxidants (superoxide dismutase, catalase) and non-enzymatic antioxidants (glutathione).

Reddy et al also suggested that increased utilization by ROS is an important contributing factor in lowering the concentration of antioxidants in tuberculosis patients. They felt that the combination of malnutrition leading to decreased supplementation of antioxidants and enhanced ROS generation leading to increased utilization of these compounds may result in a pathologic loop that results in markedly enhanced oxidative stress during tuberculosis infection.[17]

Wild et al also suggested that total antioxidants status of TB patients should be considered more effective for disease control and that diets low in antioxidants may render individuals susceptible to tuberculosis.[26]

The findings of decreased level of antioxidants, SOD, reduced glutathione and vitamin C in patient of metastatic lymhadenopathy in our study were in agreement with study by Beevi et al.[27]

In contrast to present study, the increase level of various antioxidants enzyme in cancer patients have been suggested by several workers.[24] To counter the deleterious action of reactive oxygen species, antioxidants enzyme may also be synthesized in higher amount in response the higher production of ROS.[28]

We also found that mean vitamin C level of patients with metastatic lymphadenopathy were significantly lower than patients with reactive lymphadenopathy and control group (Table 7). Mahadavi R et al showed significantly lower plasma vitamin C levels in cancer patients.[29] Low levels of vitamin C in different type of cancer patients may be due increase utilization to scavenge lipid peroxides as well as their sequestration by tumor cells.

Conclusion

Overall findings of our study suggest there was significant rise in reactive oxygen species and lipid peroxidation products in patients with tuberculosis and malignancy. Though the role of oxidative stress in cancer remains complex and controversial, the present study has observed the evidences of oxidative stress in the form of increased malondialdehyde and nitric oxide levels which were found to be raised statistically significant than the control group. Malondialdehyde (MDA), a byproduct of lipid peroxidation is said to be involved in DNA adduct formations which is believed to be responsible for carcinogenesis by mutations.

There was significant decrease in the values of antioxidant in both tuberculosis and malignancy patients.

More studies on oxidative stress and antioxidants will give new insights on their role in pathogenesis of various diseases. This is an exciting possibility because antioxidants are drugs of low toxicity and hence can be used quite harmlessly in patients.

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