World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Serum immunoglobulin classes, interferon gamma and oxidative stress status of Nigerian multidrug resistant pulmonary tuberculosis patients at Ibadan, Nigeria

Olubayo M AKINOSUN [1, 2, *], EZENKWA Simon Uchenna [1], BOLAJOKO Elizabeth B [1] and ARINOLA Ganiyu O [1]

[1] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.*
[2] *Department of Chemical Pathology, University College Hospital, Ibadan, Ibadan, Nigeria.*

World Journal of Advanced Research and Reviews, 2024, 23(01), 2826–2832

Publication history: Received on 01 June 2024; revised on 25 July 2024; accepted on 27 July 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.1.2246

Abstract

Introduction: Emergence of multidrug resistant strains constitutes a serious threat to global tuberculosis control efforts, with evidence emerging about the possible contribution of humoral immunity and antioxidant status in the control of this disease. This study evaluated correlation of antioxidant status, interferon gamma and immunoglobulin in multidrug-resistant tuberculosis.

Method: Twenty-three multidrug-resistant tuberculosis patients, 28 drug-susceptible patients and 28 healthy participants were enrolled for study. Serum malondialdehyde (MDA), total antioxidant status (TAS), interferon-gamma, and serum immunoglobulins (IgA, IgM, and IgG) were analysed using standard methods. Descriptive statistics, Student t-test, analysis of variance, Pearson's correlation and chi-square tests carried out and significance set at $p < 0.05$.

Results: Tuberculosis occurred more in the young, with male preponderance. Between group correlations was significant for TAS and IgA, as well as IgA and MDA while, IgA was significant in male. Significant difference found in levels of IgA and MDA but not IFN-Ɣ, IgG, IgM and TAS. Between group differences were significant between drug sensitive and control, MDR and control, MDR and drug sensitive.

Conclusion: Multidrug-resistant tuberculosis is associated with increased oxidative stress. A possible synergy also exists between acquired humoral immunity (IgA and IgG) and cellular immunity (IFN-Ɣ) in tuberculosis control.

Keywords: Multidrug Resistant Tuberculosis; Total Antioxidant Status; Oxidative Stress; Immunoglobulins

1. Introduction

Tuberculosis is a major global health problem. The World Health Organisation in 2012 estimated a prevalence of multidrug resistant tuberculosis of 5.7% globally, defined as resistance by the organism, Mycobacterium tuberculosis, to both isoniazid and rifampicin [1]. Of these, 3.5% were new cases while 20.5% represents previously treated TB cases [2]. These patients require prolonged therapy with second line but less effective drugs. Effective immunity against M.TB infection is mediated by T cell activation of macrophages to kill the intracellular bacteria and/or form a granuloma to wall-off the organism preventing its spread [3,4]. The mycobactericidal effects of mononuclear phagocytes are mediated by free radicals generation which concomitantly produce host tissue damage when in excess of antioxidants [5]. Indeed, studies have shown increased oxidative stress in tuberculosis [6,7]. Hitherto, it was believed that B cells have no role in TB immunity. However, data exists suggesting a protective role of immunoglobulins in M.TB infection [8]. Maglione and Chan [9], in their study showed that absence of B cells in acute tuberculosis disease result in granuloma formation dysregulation while active B cell clusters around the granuloma aid in prevention of reactivation disease. Balu et al,

* Corresponding author: Olubayo M AKINOSUN

[10], investigated the effect of preincubation of human monocytes with IgA monoclonal antibody (mAb) and/or recombinant INF-Ɣ prior to infection with mycobacterium bacilli, and found a significant inhibition of infection for 72-108h when IgA and INF-Ɣ were combined than with either alone. Although the evidence is not conclusive, it however suggests a synergy between acquired humoral and cellular immunity in TB immunity, and requires further study as immune therapy with immunoglobulins may help in controlling the human and economic costs of the disease [11]

Studies describing the interplay of these parameters in multidrug resistant tuberculosis are scarce. This informs the need to undertake this study.

2. Materials and methods

2.1. Study population and location

This is a case-control study conducted in Ibadan, Oyo state, South Western Nigeria. The participants were recruited from individuals attending the Oyo State Government Hospital, Tuberculosis Clinic Jericho, Ibadan North West Local Government Area, who had been diagnosed as having drug resistant and drug susceptible tuberculosis, following the WHO recommended protocol and are receiving therapy [1]. The control group was drawn from apparently healthy staff of the same hospital, postgraduate students of the University of Ibadan, and from voluntary blood donors at the University College Hospital (UCH), Ibadan. The control group was matched with the MDR and drug susceptible tuberculosis patients for age (age ≥18 years) and sex. Exclusion criteria include age < 18 years, HIV co-infection and presence of other chronic disease except tuberculosis. Eighty-eight (88) participants volunteered to participate in this study; 30 each for MDR-TB and drug-sensitive groups and 28 for the control group. Seven (7) of the MDR-TB and 2 of the drug-sensitive volunteers were HIV positive and so were excluded from the study. Of the 79 enrolled participants, 52 were males. Ethical clearance for this study was obtained from the university of Ibadan/University college hospital institutional review board

2.2. Data and sample collection

A structured questionnaire was used to collect the participant's demographic data. The questionnaire was self-administered or interviewer-administered in situations where the participants could not read or write. Items on the questionnaire were interpreted to the participants in their language of understanding via an interpreter(s). The data collected included age, gender, and dietary habits.

Ten millilitres of venous blood samples were collected into 5ml each of plain and heparinized bottles from each participant over the antecubital fossa by venepuncture after overnight fast. The samples were centrifuged at 3000rpm for 10min and stored at –20˚C until analysis. The serum samples were used for analysis of the following parameters;

- Serum malondialdehyde (MDA) concentration.
- Serum level of total antioxidant status (TAS).
- Assay for immunoglobulin classes (IgG, IgA, IgM) and INF-Ɣ.

2.3. Analytical methods and procedures

Serum MDA levels were determined spectrophotometrically (Spectrum lab 23_A spectrophotometer Model No: 23A08390, from Gulfex Medical & Scientific, England) using non-chromatographic assay for malondialdehyde-thiobarbituric acid adducts according to the method of Badcock et al [12]. This method is based on reaction of MDA with thiobarbituric acid (TBA) or its diethyl derivative forming a red MDA-TBA adduct of 2 mol TBA to 1 mol MDA measured at wavelength of 532 nm.

To express the result as units per mg protein, total protein assay was determined by the Biuret method [13] using a total protein assay kit manufactured by Randox laboratories (Randox laboratories limited, 55 Diamond Road, Crumlin, county Antrim, BT29 4QY, United Kingdom. lot number 750TP), and following the manufacturer's instructions. Spectrumlab 23_A spectrophotometer (Model No: 23A08390, from Gulfex Medical & Scientific, England) was used to read the absorbance of the solution at a wavelength of 546nm.

Malondialdehyde was then calculated using this equation

$$\text{MDA}\left(\frac{units}{mgprotein}\right)=\frac{A532\ x\ volume\ of\ mixture}{1.56x10^5\ x\ volume\ of\ sample\ x\ mgprotein}$$

Serum TAS was assayed using spectrophotometry method (Spectrumlab 23_A spectrophotometer Model No: 23A08390, from Gulfex Medical & Scientific, England) as described by Koracevic et al [14] based on the principle that a standardised solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the release of hydroxyl radical. These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substance. The rate of inhibition of colour development measured at 532nm is proportional to the concentration of oxidative activity of plasma. The absorbance was measured at 532nm wavelength.

Immunoglobulins and INF-ϒ were determined using ELISA methods and as specified by the manufacturer. The ELISA kits were procured from Assay PRO (Assapro LLC, 30Triad South Drive, St. Charles, MO 3304), with Lot and Catalog Nos. 021001438, El1023-1(IFN-ϒ), 091651320, El700-1(IgA),031791429, El7200-1(IgG) and 031591430, El7301-1(IgM) respectively. The ELISA technique used in this study employs a quantitative sandwich enzyme immunoassay technique that measures the analyte of interest in less than 5 hours. A polyclonal antibody specific for human IFN-gamma, Ig A, IgG and IgM in each case has been pre-coated onto a 96-well micro plate with removable strips. The standards and samples are sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for the analyte, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The colour development is stopped and the intensity of the colour is measured. At this point the absorbance of the wells was read on a microplate reader at a wavelength of 450 nm immediately. The microplate reader used is spectramax plus 384 by Molecular devices. Readings were taken in triplicate. The mean value of the triplicate readings for each standard and sample were taken. A standard curve was generated by plotting the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line was determined by regression analysis using four-parameter logistic curve-fit. The unknown sample concentration was determined from the standard curve and the value multiplied by the dilution factor 1:60,000, 1:80,000, 1:600,000 (IgM, IgA, IgG respectively).

2.4. Statistical analysis

Statistical evaluation was carried out using the statistical package for social sciences (SPSS) version 16 for the analysis of data. Pearson correlation coefficient was used to test the correlation between analytes of interest and study groups. Independent student t-test was used to compare differences in means between patients responding to anti-TB and the MDR-TB patients and with the control groups. Analysis of variance (ANOVA) was used to test the difference in means among the three groups. Categorical variables were expressed as frequencies; the level of statistical significance was set at 5% (0.05).

3. Results

Of the 79 enrolled participants, 52 were males. The mean and standard deviation of the age of the study population is 34.25±9.25 years. This shows that tuberculosis is more prevalent in the younger age group in this population. Keeping in mind that this is a hospital based study, it may not be a true reflection of the prevalence in the general population.

Table 1 shows the pattern of vegetable intake among the study groups. This table shows that the participants in DSTB group ate fruit and vegetables more frequently, followed by those in Control group and then those in MDR group.

Table 1 Vegetable and Fruit Intake among the Study Populations

Response	MDR (%)	DS (%)	Control (%)
Daily	5	48.4	28
Weekly	10	22.6	48
Occasionally	85	29	24
Total	100	100	100

DS: Drug sensitive; MDR: Multidrug resistant

The descriptive statistics, analysis of variance and student t-test for the serum MDA, IFN-ϒ, and immunoglobulins are shown in Table 2. Serum IgA and MDA levels show significant difference in the mean of their values among the three groups ($p = 0.013$, 0.044 respectively). The pattern of variation in the values of IgA is remarkable in that the value is lower in MDR-TB than in the DSTB group. Malondialdehyde level in the three groups showed consistent variation, being

highest in the MDR-TB group and lowest in the control group suggesting that oxidative damage increases with increased burden of the disease.

Table 2 Descriptive statistics, ANOVA and t-test for MDA, IFN-Ɣ, TAS and immunoglobulin.

Variable	MDR-TB (mean ± SD)	DSTB (mean ± SD)	Control (mean ± SD)	ANOVA	MDR/DSTB (t-test)	MDR/CONTROL (t-test)
IgA(mg/dl)	718±1.02	$2.99x10^6$±$1.58x10^7$	614.65±1.5x10^2	0.013	0.104	0.006
IgM(mg/dl)	99.32±3.01	99.57±32.69	93.10±27.01	0.61	0.211	0.923
IgG(mg/dl)	1211.49±574.42	948.43±343.43	1214.61±589.36	0.077	<0.001	0.65
IFN-Ɣ(ng/ml)	10.33±6.64	10.12±5.58	12.78±5.45	0.058	0.141	0.035
MDA(units/gprotein)	5.76±1.10	4.81±1.15	3.05±2.48	0.044	0.068	0.658
TAS(mmol/l)	19.68±4.99	19.29±9.53	21.93±7.00	0.38	0.094	0.004

The significant difference between the MDR-TB and the drug-sensitive groups (p=0.004) suggests that reduced total antioxidant status is associated with MDR-TB. However, total antioxidant status may not explain the presence of tuberculosis in the DSTB patients and not in the control group because of the non-significant difference between their means. Other factors may account for this.

When taken in pairs, the between group differences in the means of immunoglobulins showed significant difference in IgG between the MDR and DSTB groups (p<0.001). The other immunoglobulins showed no significant difference between the MDR and DSTB groups. MDR group had a higher level of IgG, a finding that suggests that in drug resistant TB, the plasma cells produce more antibodies against the M.TB. Interferon-gamma is produced by activated Th 1 lymphocytes and is an indicator of acquired cellular immunity in tuberculosis infection. In this study, the difference in means of INF-Ɣ between MDR and DSTB was insignificant (p=0.141) indicating that both groups of patients mount same level of T cell response to the disease. However, the difference in means between the MDR and control groups was significant (p=0.035) while that between the DSTB and the control groups was non-significant.

There is no significant correlation between any pair of analytes among the study groups.

Further correlation analysis within individual study groups showed significant associations only within the control group as shown in Table 3. Within this group, significant association was found between TAS and IgA (p=0.042), and between IgA and MDA (p=0.011). The inverse relationship between TAS and IgA suggests that antioxidant levels increase when the IgA levels is deficient thereby ameliorating the oxidant stress from reactive oxygen and reactive nitrogen species generated by phagocytes in response to the infectious agent. Additionally, the positive correlation between IgA and MDA suggests that when infectious agents invade the respiratory mucosal surfaces, ROS produced bring about tissue damage. At the same time in the host capable of mounting antibody response, IgA levels increase also. These observations suggest interplay between antioxidants and immunoglobulin in protecting the mucosal surfaces against infections and the consequent oxidant damage that ensue from the host's response to them.

Table 3 Correlations of TAS, MDA, IFN-Ɣ, and IgA in the Control group

Variable	IgA	
	r	t-test
TAS	-0.387	0.042
MDA	0.474	0.011
IFN-Ɣ	-0.045	0.821

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

4. Discussion

The burden of tuberculosis is worsened by the emergence of drug resistant strains. Acquired cellular immunity against the organism has been extensively and conclusively studied. Little is known about the role of humoral immunity in TB. Tuberculosis is associated with oxidative stress with reduced total antioxidant status [15]. It is at the backdrop of the above that this study was conceptualised. We set out to investigate possible roles of immunoglobulin, antioxidant status and interferon gamma in multidrug resistant tuberculosis.

This study shows a prevalence of tuberculosis among the young population. Although not statistically significant, ($p=0.346$), it agrees with research study by Skrahina et al, [16]. These researchers found a positive association between age of <35years and MDR-TB. Explanation for this trend in this present study is not clear. As expected, TB cases' being more prevalent in the young population will lead to loss of man hour at work.

The serum level of lipid peroxidation product, MDA, is a marker for free radical and/or reactive oxygen and nitrogen species damage to host tissues. The result of this study showed a significant difference in mean of serum MDA among the groups. However, between groups, mean difference in MDA serum levels was only significant between the drug sensitive and control groups. The data also found a significant difference in the mean of serum TAS between the MDR-TB and drug sensitive groups, but not between the MDR/Control nor between Drug-sensitive/Control groups.

The data presented here also show lowest level of serum MDA in the control group, followed by DSTB group with the MDR group highest. It could therefore be asserted that oxidative stress is characteristic of MDR-TB states and improves with response to therapy, being lowest in the healthy state. These results agree with the findings of Wiid et al, [15]. These researchers were able to show that as treatment progressed, antioxidant level approached that of healthy control group significantly. They concluded that this might be due to reduction in free radicals as treatment progressed. They however did not measure the levels of free radicals in the study groups. The findings in this study of significant difference in TAS between the MDR and DSTB groups, and the within group significant difference (ANOVA) in MDA supports the assertion that TB and indeed, MDR-TB, is associated with oxidative stress [17]. Increased generation of free radicals and reduced intake of vitamin-rich foods such as vegetables and fruits in the MDR group could explain the increased oxidative stress [17]. From this study, MDR group participants ate vegetables and/or fruits less often compared to the DSTB and the Control groups in that order.

Of the three study groups in our study, MDR group had the least value of IFN-ϒ, followed by drug sensitive group. There was a significant difference in IFN-ϒ level between MDR and control group, but not with MDR/Drug-sensitive and Drug-sensitive/Control groups. This suggests that higher level of IFN-ϒ is associated with TB disease-free state. Whether reduced level of IFN-ϒ is a risk factor for MDR-TB is not certain since there was no significant difference between levels of IFN-ϒ in MDR and drug sensitive groups. Given that the level of IFN-ϒ in drug sensitive group was higher than in MDR group, one may say with some level of confidence that reduced IFN-ϒ is a risk factor for MDR-TB. These findings confirm the earlier observations by [18], who in their research study, found impaired expression of IFN-ϒ in MDR-TB and susceptible TB (S-TB) patients, irrespective of the strain. They believed that this might be due to altered Th1/Th2 profile characteristic of advanced disease. Other researchers have found conflicting outcomes with regard to IFN-ϒ and TB infection [19]. Analysis of variance among the three groups was however non-significant suggesting that T cells may have other roles besides IFN-ϒ secretion in curtailing the infection or disease.

Tuberculosis affects the respiratory system predominantly, hence the significant difference in serum IgA among the groups studied. This result supports the research findings of [20,21,22]. The finding of higher value of IgA in the drug sensitive group compared to MDR group in our study may suggest a reduction in B cell synthesis of IgA in the MDR-TB patients, thus enabling multidrug resistance by the mycobacterium.

We also report here a significant association between male gender and IgA only in the MDR group. Explanation for this is not readily clear. A study among healthy Caucasians showed that serum concentrations of IgA were significantly higher in men than women [23]. There is however paucity of data in this regard among TB patients.

The study shows a significant difference in the mean of IgG between the MDR and drug sensitive groups. Titres were higher in MDR compared to the drug sensitive groups. This result agrees with earlier research findings by Demkow and colleagues [20], and Mizusawa et al [24]. Both groups of researchers showed that IgG antibody titres increased significantly with severity of pulmonary TB. Possible explanation for our results includes the prolonged duration of the disease in MDR patients, and the person-to-person variation in antigenic determinants recognition by antibodies [25]. Poor antigenic recognition may therefore be associated with MDR-TB. Plausible basis for the difference in IgG levels between MDR and DSTB groups needs further studies.

The difference in the means of IgM among the groups (ANOVA) was not significant. Likewise, the differences in means of IgM between groups were not significant. It is important to note that the level of IgM across groups is nearly the same. Other studies have also not found association between IgM and clinical or radiological outcomes of Tuberculosis except for gender [20]. This is not unexpected, given that, IgM plays a role in primary immune response, tuberculosis being a reactivation or a secondary response. We also did not find association between IgM and gender or age. Effect of sample size may not be ruled out.

Where correlation exists between analytes of interest among the study groups, it was mostly weak. At the group levels, there was no significant correlation between the analytes in the MDR and drug sensitive groups. This suggests that outcomes observed in these groups are independent of one another. The situation was, however, different in the control group. The negative correlation between TAS and IgA in this group suggests that when the body's antioxidant system is reduced, more IgA is produced to protect the mucous membranes. In other words, when the body's IgA is reduced, the mucous membranes are colonized more readily more readily by the mycobacterium bacilli. The hosts macrophages responds by increasing free radicals generation that necessitates increased production of antioxidants by the host to limit the damage caused by these free radicals. This explanation finds credence in the finding of a positive correlation between IgA and MDA in this same group.

Also, the release of intracellular mycobacterial antigens is accompanied with concomitant release of ROS and RNS produced within the phagolysosome. This will account for the increase in MDA while IgA will be secreted more in response to the released antigens. Further studies will be required to test these hypotheses.

Limitations of this study

Of note is the size of the studied population, 79 participants valid in all. This might have affected the outcomes of interest. Even where significant findings were seen, a larger population size is needed to validate the results obtained.

Another area of concern in this study is the process of participant selection. Hospital based studies are often not representative of the entire population. In addition to that, participants volunteered to participate. However, no one was excluded from the study except on the basis of the exclusion criteria outlined for this study. This most probably limited the possibility of bias.

5. Conclusion

Multidrug resistant tuberculosis is associated with a higher oxidative stress than in drug sensitive tuberculosis.

A possible role for humoral immunity in tuberculosis immunity has been highlighted by this study. A further study on a larger population is required to evaluate further this possible synergy with T cell mediated immunity in tuberculosis.

Compliance with ethical standards

Acknowledgments

We wish to acknowledge the support of the University of Ibadan Senate Research grant 2010 for this work.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] The WHO world Health Organisation. Global tuberculosis report 2012. Geneva

[2] World health Organisation. (2014). Multidrug-resistant tuberculosis (MDR) 2014 update

[3] Saunders BM and Britton WJ (2007). Life and death in the granuloma: immunopathology of tuberculosis. Immunology and Cell Biology; 85:103-111

[4] Maglione PJ, Xu J, Casadeval, A and Chan J. (2008). FCϒ Receptors regulate Immuneactivation and susceptibility to M. tuberculosis Infection. *J. of Immunology*; 180:3329-3338.

[5] Dalvi SM, Palti VW and Ramiraje NN. (2012). Carbonyl protein and antioxidantvitamins in pulmonary and extrapulmonary tuberculosis. *J Phys Pharm Adv.*; 2(5):210-215A

[6] Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. (2004).Role of free radicals and antioxidants in tuberculosis patients. Indian J Tuberc;51:213-218

[7] Palanisamy, G.S., Kirk-NMAckert, D.F., Shanley, C.A., Ome, I.M. and Basaraba, R.J.(2011). Evidence for oxidative stress and defective antioxidant response in guinea pigs with tuberculosis. *PlosONE.*; 6(10):1-13.

[8] Abebe F and Bjune G. (2009).The protective role of antibody responses during Mycobacterium tuberculosis infection. *Clinical and experimental immunology;* 157:235-243.

[9] Maglione PJ and Chan J. (2009). How B cells shape the immune response againstmycobacterium tuberculosis. *Eur J Immunol*.; 39(3):676-686

[10] Balu S, Reljic R, Lewis MJ, Pleass RJ, McIntoshs R, van Kooten C, van Egmond M,Challacombe S, Woof JM, IvanyiJu. (2011). A novel Human IgA monoclonal antibody protects against tuberculosis. *J Immunol,*; 186(5): 3113-3119

[11] Roy E, Stavropoulos E, Brennan J, Coade S, Grigorieva E, Walker B, Dagg B, Tascon,Lowrie DB, Colston MJ, Jolles S. (2005). Therapeutic efficacy of high-doseintravenous immunoglobulin in Mycobacterium tuberculosis infection in mice.*Infection and immunity;* 73(9): 6101-6109

[12] N. R. Badcock, G. D. Zoanetti, and E. S. Martin. Nonchromatographic assay for malondialdehyde-thiobarbituric acid adduct with HPLC equivalence.." The Free Library. 1997 American Association for Clinical Chemistry

[13] Gornall AG, Bardawill CJ, David MM. (1949), Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949 Feb; 177(2):751-66.

[14] Koracevic D, Koracrvic G, Koracevic V, Andrejevic S, Cosic V. (2001). Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54:356-361

[15] Wiid I, Seaman T, Hoal EG, Benade AJS, Van Helden PD. (2004). Total antioxidantlevels are low during active TB and rise with Anti-tuberculosis therapy. *IUBMB Life;* 56 (2):101-106

[16] SkrahinaA,Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, Hoffner S, Rusovich V, Dadu A, Pierpaolo de Colombani, Dara M, Wayne van Gemert,Zigno M. (2013). Multidrug-resistant tuberculosis in Belarus: the size of the problem and associated risk factors. *Bulletin of the World Health Organization*; 91:36-45

[17] Alli J.A, Kehinde A. O, Kosoko A. M., Ademowo O. G. (2014). Oxidative stress and reduced vitamins C and E levels are associated with multi-drug resisitant tuberculosis. Journal of Tuberculosis Research; 2:52-58

[18] Geffner L, Yokobori N, Basile J, Schierloh P, Balboa L, Romero MM, Ritacco V,Vescovo M, Montaner PG, Lopez B, Barrera L, Aleman M, Abatte E, SasiainMC, de la Barrera S. (2009). Patients with Multidrug-Resistant tuberculosisdisplay impaired Th responses and enhanced regulatory T-cell levels in response to an outbreak of multidrug-resistant Mycobacterium tuberculosis M and Ra Strains.*Infection and immunity;* 77(11): 5025-5034

[19] Raja A. (2004). Immunology of tuberculosis. Review article. *Indian J Med Res.;* 213-232

[20] Demkow U, Filewska M, Michalowska-mitczuk D, kus J, Jagodzinski, Zielonka T,Zeolska Z, Wasik M, Rowinska-Zakrzewska E. (2007). Heterogeneity of antibody response to Mycobacterial antigens in different clinical manifestation of pulmonary tuberculosis. *Journal of physiology and pharmacology* 58(5):117-127

[21] Rohini k, Srikumar PS, Mahesh KA. (2012). A study on the serum immunoglobulinlevels in pulmonary tuberculosis patients. *International Journal of Bioscience,biochemistry and bioinformatics*; 2(4): 280-28

[22] Alvarez et al, (2013) Alvarez N, Otero O, Camacho F, Borrero R, Tirado Y, Puig A, Aguilar A, Rivas C, Cervantes A, Falero-Diaz G, Cardiz A, Sarmiento ME, Norazmi MN, HermandezPando R, Acosta A. (2013). Passive administration of purified secretory IgA from human colostrum induces protection against Mycobacterium tuberculosis in a murine model of progressive pulmonary infection. *BMC Immunology;* 14(1):S3

[23] Berth M, Delanghe J, Langlois M, De buyzere M. (1999). Letters, *Clinical Chemistry;* 45(2): 309-310

[24] Mizusawa M, Kawamura M, Takamori M, Kaahiyama T, Fujita A, Usuzawa M, Saitoh H, Ashino Y, Yano I, Hattori T. (2008). Increased synthesis of Antituberculous glycolipid immunoglobulin G (IgG) and IgA with cavity formation in patients with pulmonary tuberculosis. *Clinical and vaccine immunology;* 15(3):544-548

[25] Lyashchenko K, Colangeli R, Houde M, Jahdali HA, Menzies D, Gennaro ML. (1998).Heterogeneous antibody in tuberculosis.*Infection and immunity;* 66(8): 3936 - 3940