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RESEARCH ARTICLE

ADENOSINE DEAMINASE LEVEL IN DRUG RESISTANT TUBERCULOSIS

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Abstract

Tuberculosis (TB) remains one of the health problems in Nigeria and worldwide. Adenosine Deaminase acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It also acts in maturation of monocytes transforming them to macrophage. Adenosine Deaminase is a significant indicator of active cellular immunity. Adenosine Deaminase has been proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid. This study aimed to determine the relationship between Adenosine Deaminase and drug Resistant Tuberculosis (DR-TB) among patients attending Tuberculosis Clinic in Government Chest Hospital, Jericho, Oyo State, Nigeria.

Methodology: A prospective case-control study involving thirty (30) Multi-Drug Resistance Tuberculosis patients and thirty (30) apparently healthy participants in Tuberculosis Clinic in Government Chest Clinic Hospital, Jericho, Oyo State, Nigeria. Theparticipant's socio-demographic data was obtained using questionnaire. Sputum samples were collected from each patient from the two groups of participants in leak proof screw capped specimen containers. About 5 mL of venous blood sample was collected from the antecubital fossa of the study participants into vacutainer plain tubes. Sputum samples collected were analysed for Mycobacterium tuberculosis using Gene Xpert. Blood sample collected was analyzed for Adenosine Deaminase using ELISA method. The prevalence of MDR-TB was 0.18%, majority of MDR-TB were within age of 15-30 years with mean age 36.30±13.40 years with female having 63.3% and male 36.7%. Among MDR-TB, the mean±SD Adenosine Deaminase activity was 37.67±15.25 IU/L; and among Healthy controls, the mean±SD of Adenosine Deaminase was 12.26±5.11 IU/L. There was significance increased in ADA activity among MDR-TB participants when compared to Healthy controls at p-value<0.05 (p=0.001). ADA level of 18 IU/L cut off point has a sensitivity of 90.0%, specificity of 87.0% and diagnostic accuracy of 88.0%. In conclusion, there was moderately high prevalence of MDR-TB among the study participants. There was increased in ADA levels among MDR-TB. High sensitivity and specificity of ADA activity was observed among MDR-TB. This might be a useful alternative test to diagnose and rule out drug resistance tuberculosis.

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Introduction:-

Tuberculosis is one of the earliest and fatal ailments also known to be described as a chronic granulomatous inflammation highly prevalent in developing countries resulting in high mortality rate (Mathur *et al*., 2006). About a decade ago, drug resistant tuberculosis (DR-TB) has been a major public health issue, occurring in both young and old age especially immunocompromised patients irrespective of sex (Kaisemann*et al*., 2004; Kumar and Clark, 2005). DR-TB can be defined as the resistance to either rifampicin or isoniazid that is one of the first lines Tuberculosis (TB) drug which serves as a major challenge for TB control that may undermine recent achievements (Alexander and De, 2007).

Timely diagnosis and proper treatment of TB have been identified as essential factors for successful TB control. It is estimated that availability of a widely used rapid diagnostic test for TB could avert 625,000 TB deaths annually (Keeler *et al*., 2006). Also, studies have demonstrated delays in TB diagnosis due to drawbacks of the presently available diagnostic tools (WHO, 2006; Storla*et al*., 2008). Mycobacterium culture that is the gold standard for TB diagnosis takes eight weeks before result is available. Sputum smear microscopy, a quick screening method is not a sensitive method while polymerase chain reaction (PCR) test is expensive, requires sophisticated equipment and cannot be used for monitoring treatment response (Adekambi*et al*., 2015). Hence, there is need for more biomarkers to monitor treatment and diagnosis of TB.

Serum Adenosine deaminase (ADA) is an enzyme of the purine metabolic pathway (Shore, 1981; Swami, 2016). ADA catalyses the irreversible conversion of adenosine and 2' deoxyadenosine to inosine and 2' deoxyinosine respectively (Piras*et al*., 1978; Zavialov*et al*., 2010). ADA acts in proliferation and differentiation of lymphocyte and especially T lymphocyte and is essential in the maturation of monocytes to macrophages. High concentration of adenosine or deoxyadenosine as a result of non-conversion to inosine or deoxyinosine is toxic to lymphocytes and macrophages (Zavialov*et al*., 2010). Also, ADA deficiency has a direct effect on the lungs, as lung damage and inflammation have been associated with elevated adenosine and deoxyadenosine in lungs of ADA-Severe Combined Immuno-Deficient patients (Swami, 2016).

Given the roles T cells and macrophages play in protection (cellular immunity) against *Mycobacteriam tuberculosis* (Mtb) infection, ADA levels increases in various body fluids simultaneously with Mtb infection. Data regarding ADA levels in PTB concentrated in pleural, peritoneal and pericardial fluids has been documented (Greco *et al*., 2003; Cimen*et al*., 2008). Moreover, in Nigeria, few reports regarding blood levels of ADA in TB have been recorded. This study determined the role of adenosine deaminase enzyme in drug resistant tuberculosis in Ibadan northwest local government, Nigeria.

Materials and Methods:-

Description of study site

This study was carried out in Government Chest Hospital , Jericho, Ibadan. Ibadan is the capital city of Oyo State which is domiciled in the South-West geopolitical zone of Nigeria. The city of Ibadan has an estimated population of 6,000,000 inhabitants spread over a total area of 3,080 square kilometres. Government Chest Hospital is located at North-West Local Government Area of Oyo State. It headquarters is located in Ibadan City.

Subjects and consent

Ethical approval was obtained from the Ethical Committee of the Oyo State Ministry of Health. At an individual level, inform consent was received from each of participants before sample and Data collection, a copy of the consent form was included in the appendix section. Respondents received a detailed description of the study, confidentiality provisions and the fact that their participation is voluntary and they could withdraw at any point if they wish. The principal privacy and confidentiality were upheld.

This study collects demographic information, data on Tuberculosis disease history as well as Demographic and risk information (age, sex, HIV status, TB History, previous treatment, alcoholism, smoking, history of diabetes, history of asthma) was obtained through questionnaires from each participant. All data was collected anonymously and unlinked to participants' personal, health or treatment records. Sample collected from each participant after laboratory analysis was cross referenced with participant data using codes devised

solely for the purpose of the study. Therefore, participants will not be traceable from collected data or specimensThe study population was divided into 3 Categories:

Group 1 (Healthy): This group comprised healthy individuals who are free of any disease (healthy normal individuals).
Group 2: Drug Sensitive Tuberculosis (DS-TB)
Group 3: Drug Resistant Tuberculosis (DR-TB): This group includes patients who are resistant to either of the Tuberculosis first line drug.

A minimum of 90 samples (30) MDR TB individual, (30) DS TB individual and (30) individual without tuberculosis was collected from DR-TB individuals was collected respectively.

Inclusion Criteria for Case
All Acid Fast Bacilli (AFB) positive sputumfrom consented patients at the hospitals inclusive of at least a drug resistant strain was included in the study.

DR-TB cases, confirmed by gene expert was enrolled into the study**.**

Exclusion Criteriafor Case
AFB negative sputum samples, from those who consented will be excluded.
Individuals belonging to any high-risk groups (e.g., drug users, sex workers, homeless) and persons with co-morbidities that would interfere with Tuberculin Skin Test (TST) results or increased risk for TB, such as those with immunosuppressive disease (e.g., HIV infection, rheumatoid arthritis or cancer) or taking any immunosuppressive drug (e.g. corticosteroid) was excluded from the study.

Inclusion Criteria for Control
Healthy male and female between age 18-60years was included in the study for control only.

Exclusion Criteriafor Control
Healthy male and female outside this age bracket (18-60years)were excluded from the study.

Individuals belonging to any high-risk groups (e.g., drug users, sex workers, homeless) and persons with co-morbidities that would interfere with Tuberculin Skin Test (TST) results or increased risk for TB, such as those with immunosuppressive disease (e.g., HIV infection, rheumatoid arthritis or cancer) or taking any immunosuppressive drug (e.g. corticosteroid) or any form of diseases capable of affecting the study was excluded from the study.

Patients on any drugs which affected peripheral blood, and known at the time of study to have a chronic disease which may adversely affect the body systems including the bone marrow and the peripheral blood.

Specimen Collection
A total of 90 samples was collected from DR-TB individual. Peripheral blood samples (5ml) were collected in plain tube and then separated for serum using centrifuge 3000rpm for 5 minutes for patients found to be in the category of Drug Resistant Tuberculosis (DR-TB) as well as control subject.

Microbiology Analysis
Genexpert system made by Cepheid inc. Sunnyvale, USA was used for detection of MTB/RIF and drug susceptibility cases. Manufacturer's instruction was followed strictly throughout the analysis.

Biochemical Analysis
Enzyme-linked immunosorbent assay (ELISA) was used for the measurement of adenosine deaminase (Human ADA; Lot: AK0016MAR21058), Elabscience, China,) The Assay protocol was followed as specified by the manufacturer and the absorbance was measured at 450nm with an ELISA reader (SpectraMax Plus 384, Molecular Devices LLC, USA).

Data Analysis

Epi Info, version 6.0 software (center for disease control and prevention, Atlanta, GA) was used to analyze the data. The significant difference in adenosine deaminase levels with respect to DR-TB for case and controls was assessed using the Anova for the 3 groups. p value<0.05 was considered statistically significant at 95% confidence level. Mean ±SD was employed to show results in tables, also result was shown in bar charts and histogram for both case and control.

Results:-

A total number of ninety (90) participants were recruited for this study. Thirty (30) Multi-drug Resistance Tuberculosis patients (MDR-TB), Thirty (30) Drug sensitivity Tuberculosis (DS-TB) patients and Thirty (30) Healthy controls.

Distribution O f MDR-T B, DS-TB A nd Healthy Control

As shown on Figure 1, the prevalence of Multi-drug resistance tuberculosis (MDR-TB) was 33.3%.

Figure 1:- Distribution O f MDR-TB, DS-TB And Healthy Control.

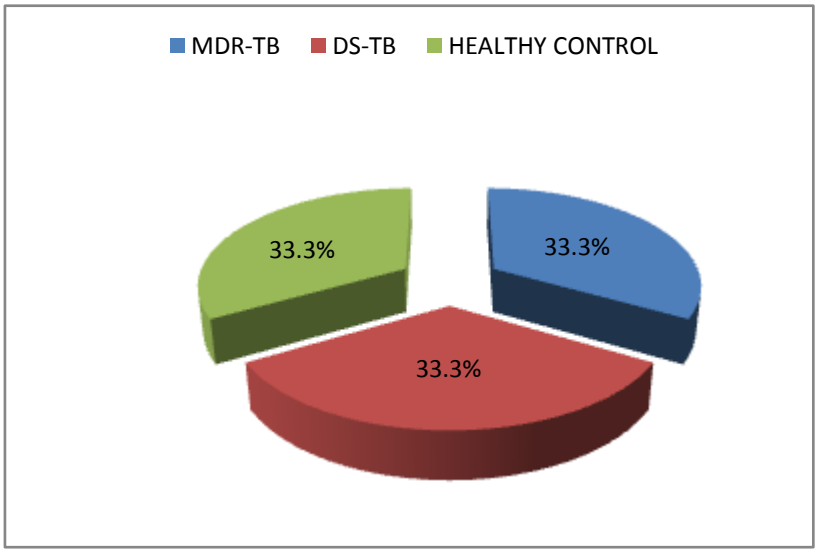


Figure 2:- Sex of Participants.

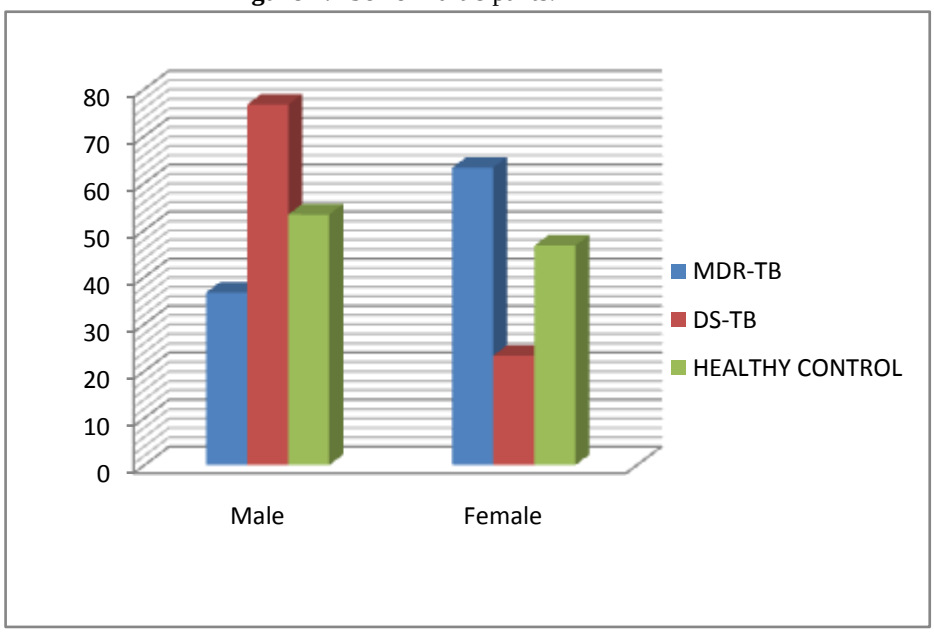
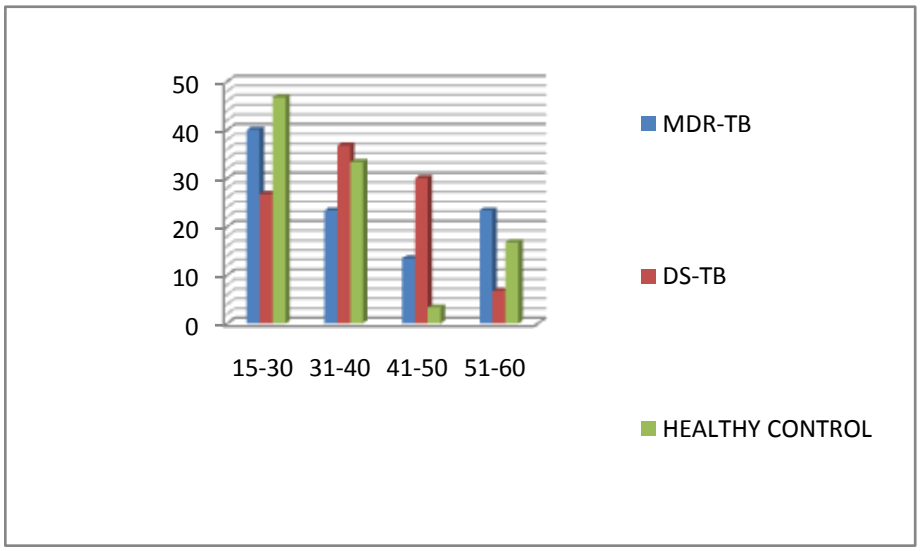


Figure 3:- Age of the Participants.



Socio-Demographic Characteristics

Table 1 showed descriptive analysis of the socio-demographic information of study participants. Majority of the participants among MDR-TB were within 15-30 years (40.0%) with the mean age of 36.30±13.40 years. More than half of the participants were female among MDR-TB (63.3%). Majority of the respondents in MDR-TB were traders (26.7%).

Mean±Sd O f Adenosine Deaminase (Iu/L) Among MDR-TB, DS-TB And Healthy Controls

Table 2 showed the mean±SD of Adenosine Deaminase among MDR-TB (37.67±15.25 IU/L), DS-TB (21.51±10.53 IU/L) and Healthy controls (12.26±5.11 IU/L).

Table 2:- Mean±SD Of Adenosine Deaminase(Iu/L)Among MDR-TB, DS-TB A nd Healthy Controls.

PARAMETERS	ADENOSINE DEAMINASE (IU/L)
MDR-TB	37.67±15.25
DS-TB	21.51±10.53
HEALTHY CONTROL	12.26±5.11

Comparison O f Adenosine Deaminase Between MDR-TB A nd Healthy Control

Table 3 showed the mean±SD of Adenosine Deaminase among MDR-TB (37.67±15.25 IU/L) and Healthy controls

VARIABLES	MDR-TB		DS-TB		HEALTHY CONTROL	
	FREQ.	**PER. (%)**	**FREQ.**	**PER. (%)**	**FREQ.**	**PER. (%)**
AGE (Years)						
15-30	12	40.0	8	26.7	14	46.7
31-40	7	23.3	11	36.7	10	33.3
41-50	4	13.4	9	30.0	1	3.3
51-60	7	23.3	2	6.7	5	16.7
Mean±SD	**36.30±13.40**		**36.37±10.22**		**33.10±12.17**	
	N=30		**N=30**		**N=30**	
SEX						
Male	11	36.7	23	76.7	16	53.3
Female	19	63.3	7	23.3	14	46.7
	N=30		**N=30**		**N=30**	
OCCUPATION						
Trader	8	26.7	9	30.0	3	10.0
Students	7	23.3	5	16.7	8	26.7
Farmer	1	3.3	2	6.7	1	3.3
Driver	3	10.0	4	13.2	2	6.7
Artisan	6	20.0	2	6.7	5	16.7
Civil servants	5	16.7	8	26.7	11	36.7
	N=30		**N=30**		**N=30**	

(12.26±5.11 IU/L). As shown on the table, the p-value = 0.001. This show there was a statistically significant difference in Adenosine Deaminase activitybetween MDR-TB and Healthy controls at p-value < 0.05.

Parameters	Mdr-tb Mean±SD	Healthy control Mean± SD	P-value	
Adenosine deaminase (iu/l)	37.67±15.25	12.26±5.11	0.001	S

Table 3:- Comparison Of Adenosine Deaminase B etween Mdr-Tb A nd Healthy Control (T- Test).

Comparison Of Adenosine Deaminase Between MDR-TB And DS-TB

Table 4 showed the mean±SD of Adenosine Deaminase among MDR-TB (37.67±15.25 IU/L) and DS-TB (21.51±10.53 IU/L). As shown on the table, the p-value = 0.001. This show there was a statistically significant difference in Adenosine Deaminase activitybetween MDR-TB and DS-TB at p-value < 0.05.

PARAMETERS	MDR-TB Mean±SD	DS-TB Mean±SD	P-VALUE	
ADENOSINE DEAMINASE (IU/L)	37.67±15.25	21.51±10.53	0.001	S

Table 4:- Comparison Of Adenosine Deaminase B etween MDR-TB And DS-TB (T-Test).

Comparison O f Adenosine Deaminase B etween Ds-Tb A nd Healthy Control

Table 5 showed the mean±SD of Adenosine Deaminase among DS-TB (21.51±10.53 IU/L) and Healthy controls (12.26±5.11 IU/L). As shown on the table, the p-value = 0.001. This show there was a statistically significant difference in Adenosine Deaminase activitybetween DS-TB and Healthy controls at p-value < 0.05.

PARAMETERS	DS-TB Mean±SD	HEALTHY CONTROL Mean±SD	P-VALUE	
ADENOSINE DEAMINASE (IU/L)	21.51±10.53	12.26±5.11	0.001	S

Table 5:- Comparison O f Adenosine Deaminase Between Ds-Tb And Healthy Control (T-Test).

Comparison Of Adenosine Deaminase Between MDR-TB, DS-TB And Healthy Control Using One –Way Anova

Table 6: showed the association of Adenosine Deaminase among MDR-TB (37.67±15.25 IU/L), DS-TB (21.51±10.53 IU/L) and Healthy controls (12.26±5.11 IU/L) using One-way ANOVA. As shown on the table, the F-value = 40.28 and the P-value = 0.001. This show there was a statistically significant difference in Adenosine Deaminase activitybetween the three groups at p-value < 0.05.

PARAMETERS	MDR-TB Mean±SD	DS-TB Mean±SD	HEALTHY CONTROL Mean±SD	F	P-VALUE	
ADENOSINE DEAMINASE (IU/L)	37.67±15.25	21.51±10.53	12.26±5.11	40.28	0.001	S

Table 6:- Comparison Of Adenosine Deaminase Between Mdr-Tb, Ds-Tb And Healthy Control Using One –Way Anova

Sensitivity, Specificity, Accuracy, Positive Predictive Value, Negative Predictive Value Of Adenosine Deaminase (ADA) Among MDR-TB

As showed on **Table 7**; among 30 of MDR-TB patients, ADA activity ≥ 18 for diagnosis of multi-drug resistance tuberculosis had Sensitivity = 90.0%, Specificity = 87.0%, diagnostic accuracy = 88.0 %, Positive Predictive Value = 88.0%, Negative Predictive Value= 87.0%.

ADA TEST RESULTS	MDR-TB	HEALTHY CONTROL	
>18 IU/L	TP=27	FP=4	31
<18 IU/L	FN=3	TN=26	29

	30	30	**60**

Table 6:- Comparison Of Adenosine Deaminase Between MDR-TB, DS-TB And Healthy Control Using One –Way Anova

VARIABLES	MDR-TB	HEALTHY	P-value	
	FREQUENCY (%)	**FREQUENCY (%)**		
AGE (Years)				
15-30	12(40.0)	14(46.7)	0.695	NS
31-40	7(23.3)	10(33.3)	0.467	NS
41-50	4(13.4)	1(30.0)	0.180	NS
51-60	7(23.3)	5(16.7)	0.563	NS
SEX				
Male	11(36.7)	16(53.3)	0.336	NS
Female	19(63.3)	14(46.7)	0.384	NS
OCCUPATION				
Trader	8(26.7)	3(10.0)	0.132	NS
Students	7(23.3)	8(26.7)	0.796	NS
Farmer	1(3.3)	1(3.3)	1.000	NS
Driver	3(10.0)	2(6.7)	0.655	NS
Artisan	6(20.0)	5(16.7)	0.763	NS
Civil servants	5(16.7)	11(36.7)	0.134	NS

1. FN = FALSE NEGATIVE
2. Sensitivity = tp/(tp + fn)
   = 27/(27 + 3) = 0.90**sensitivity (%)** = 90.0%
3. Specificity = tn/(tn + fp)
   = 26/(26 + 4) = 0.87**specificity (%)** = 87.0%
4. Accuracy = (tp + tn)/(tp+tn+fp+fn)
   = (27 + 26)/(27 + 26 + 3 + 4) = 0.88**accuracy** = 88.0%
5. Positive predictive value = tp/(tp + fp)
   = 27/(27 + 4) = 0.87

positive predictive value (%) = 87.0%

6. Negative predictive value = tn/(tn + fn)

= 26/(26 + 3) = 0.90

negative predictive value (%) = 90.0%

Table 8:-Association Of Socio-Demographics Characteristics Between Mdr-Tb And Healthy (Chi-Square)

Association Of Socio-Demographics Characteristics Between Mdr-Tb And Healthy Controls

Table 8 showed the p-value for **age** [15-30 years (0.695), 31-40 years (0.467), 41-50 years (0.180), 51-60 years (0.563), **sex** [Male (0.336), Female (0.384)], **Occupation** [traders (0.132), students (0.796), farmers (1.000), driver (0.655), artisans (0.763), civil servant (0.134)]. This show there was no statistically significant association of **s**ocio-demographic characteristicsbetween MDR-TB and Healthy controls at p-values < 0.05.

Discussion:-

The prevalence of MDR-TB in this present study was 0.18%. This is low comparable to the study done in Kano with prevalence of 53.6% (Adamu and Hafiz, 2015).Varying rates of MDR tuberculosis have also been reported worldwide. These have ranged from very low rates of 0.2% reported from Japan (Hattori et al., 2016),to MDR tuberculosis rates as high as 69% reported from Pakistan (Akhtar et al., 2016). Differences in prevalence rates could be due to different sample size, difference locations and different test methods employed for the different studies.

The mean age of the participants was 36.30±13.40 years. Majority of the participants in MDR-TB were within 15-30 years. Based on assumption, young adults are likely going to be non-compliance in taken their drugs regularly and stop follow-up visits especially when they feel better. Self medication is common among Young adult, which could have led to high resistance to drug in this age group. This is consistent with the study carried by Ifebunandu and Ukwaja, 2012. The data in this study revealed that 63.3% of MDR-TB participants were female. We could assume it

was due to the fact that female MDR-TB participants in this study spent most of their life time in home, where there is no proper ventilation and this unhygienic and limited environment leads to such infection. Findings of this study corroborate with a study conducted by Ayaz *et al*., (2012); Baloch *et al*., (2013) and Ullah *et al*., (2008), who also reported that in female population prevalence of tuberculosis is more. Majority of the MDR-TB patients in this study were traders. Traders used to be busy with their daily business thus making compliance with anti-Tb drugs difficult for them.

ADA is a significant indicator of active cellular immunity. ADA has been proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid (Dinnes*et al.,* 2007). This present study found significance increased in ADA levels in both MDR-TB (37.67±15.25 IU/L) and DS-TB (21.51±10.53 IU/L) patients when compared with healthy controls (12.26±5.11 IU/L). This shows that ADA level is useful in differentiating mycobacterium tuberculosis infected patients from uninfected controls. Our finding is supported by previous studies that reported increased ADA in serum of pulmonary TB patients (Afrasiabian*et al.,* 2013; Srinivasa Rao *et al.,* 2010; Cimen*et al.,* 2008). The increased in this present study might be due to activation, proliferation and differentiation of monocytes to macrophages which presents Mtb antigen to CD4+ T cells. Full functionality of Cell Mediated Immunity has been associated with normal lymphocyte metabolism regulated partially by the purine salvage enzyme such as ADA (Giblette*et al.,* 1972). Studies have also shown that monocytes undergoing differentiation and macrophages continuously secrete ADA which induces proliferation of CD 4+ T cells (Zavialov*et al.,* 2010). This is therefore indicative of continuous activation of Cell Mediated Immunity in TB patients, thus supporting clinical usefulness of plasma ADA as a biomarker for PTB diagnosis.

ADA activity decreased significantly in DS-TB (21.51±10.53 IU/L) patients when compared with ADA activity in MDR-TB (37.67±15.25 IU/L) at $p<0.05$. This shows response to anti-tuberculosis drugs reduces the ADA levels in TB patients. Thus, plasma ADA level may be a useful biomarker to monitor treatment in DS-TB and MDR-TB patients. Reduced ADA level among DS-TB might be explained by reduced MTB specific monocyte and T cell activation as a result of reduced Mtb antigen or other regulatory immune factors. A previous study reported a shift in Cell Mediated Immune response during anti-TB treatment (Cardoso et al., 2002) which was attributed to sequestration of MTB-specific T cells at the site of disease leading to reduced frequency in peripheral blood, the release of anti-inflammatory cytokines by PBMCs and depression of T-cell responsiveness (Wilkinson et al., 1988).

The current study showed that ADA level of 18 IU/L cut off point has a Sensitivity of 90.0%, Specificity of 87.0% and diagnostic accuracy of 88.0% in patients with MDR-TB. From the previous studies, the cut off value range from 15 to 53.5 IU/L for ADA, the sensitivity and specificity ranged from 12% to 100%, and 86% to 100%, respectively. Our finding agrees with study carried out by Shokrollah*et al.,* (2015) with 100% sensitivity of Adenosine Deaminase in diagnosis of MDR-TB.

Conclusion:-

This study showed moderately high prevalence of MDR-TB among the study participants. There was significant increase in ADA activity among MDR-TB compared to DS-TB and healthy controls. High sensitivity and specificity of ADA activity was observed among MDR-TB. This might be a useful alternative test to diagnose and rule out drug resistance TB.

References:-

1. Adamu AU, Hafiz TR. Multi-drug resistant tuberculosis pattern in Kano metropolis, Nigeria. J Am Sci. 2015;11:293–296.
2. Adekambi, T., Ibegbu, C.C., Cagle, S., Kalokhe, A.S., Wang, Y.F., Hu, Y., Day, C.L., Ray, S.M. and Rengarajan, J. (2015). Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response. *Journal of Clinical Investment*. 125(**5**): 1827-1838.
3. Afrasiabian S, Mohsenpour B, Bagheri KH, Sigari N, Aftabi K (2013). Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis. J Res Med Sci 18(3):252-254.
4. Akhtar AM, Arif MA, Kanwal S, Majeed S. Prevalence and drug resistance pattern of MDR TB in retreatment cases of Punjab, Pakistan. J Pak Med Assoc. 2016;66:989–983.
5. Alexander, P.E. and De, P. (2007). The emergence of extensively drug-resistant tuberculosis (TB): TB/HIV coinfection, multidrug-resistant TB and the resulting public health threat from extensively drug-resistant TB, Globally and in Canada. *Canadian Journal of Infectious Diseases Medical Microbiology*. 18(**5**): 289-291.

6. Ayaz, S., Nosheen, T., Khan, S., Khan, S. N., Rubab, L., & Akhtar, M. (2012). Pulmonary Tuberculosis: still prevalent in human in Peshawar, Khyber Pakhtunkhwa, Pakistan. *Pak J Life Soc Sci*, 10(1), 39-41.
7. Baloch, S., Devrajani, B. R., & Rahman, A. A. (2013). The prevalence of smear positive pulmonary tuberculosis in Hyderabad, Sindh, Pakistan. *Elixir Human Physio*, *60*, 16447-16450.
8. Cardoso FLL, Antas PRZ, Milagres AS, Geluk A, Franken KLMC, Oliveira EB, Teixeira HC, Nogueira SA et al (2002). T-cell responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 in Brazilian tuberculosis patients. Infect Immun 70(12):6707-6714.
9. Çimen, F., Çiftçi, T.U., Berktafl, B.M., Sipit, T., Hoca, N.T. and Dulkar, G. (2008). The relationship between serum adenosine deaminase level in lung tuberculosis along with drug resistance and the category of tuberculosis. *Turkey Respiratory Journal*. **9**:20-23.
10. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007;11:1-196.
11. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ (1972). Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. The Lancet. 300(7786):1067-1069.
12. Greco, S., Girardi, E., Masciangelo, R., Capoccetta, G.B. and Saltini, C. (2003). Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. *International Journal Tubercle Lung Disease*. **7**: 777–786.
13. Hattori T, Kobayashi N, Nagai H, Chagan-Yasutan H, Telan E, Solante MB. Nationwide HIV-, MDR-TB survey in Japan and collaborative study in the Philippines. Int J Mycobacteriol. 2016;5(5):18. https://doi.org/10.1016/j. ijmyco.2016.09.009
14. Kaisemann, M.C., Kritski, A.L., Pereira, M.F. and Trajman, A. (2004). Pleural fluid adenosine deaminase detection for the diagnosis of pleural tuberculosis. *Journal of Bras Pneumology*. 30(**6**): 1-10.
15. Keeler, E., Perkins, M.D., Small, P., Hanson, C., Reed, S. and Cunningham, J. (2006). Reducing the global burden of tuberculosis: the contribution of improved diagnostics. *Nature*. 444(**1**): 49-57.
16. Kumar, P. and Clark, M. (2005). Infectious diseases. In: *Kumar* and *Clark Clinical Medicine*. (6th Ed). Elsevier, Saunders. 86-91.
17. Mathur, P.C., Tiwari, K.K., Trikha, S. and Tiwari, D. (2006). Diagnostic utility of Adenosine deaminase (ADA) activity in Tubercular serositis. *Indian journal of Tuberculosis*.**53**:92-95.
18. Piras, M.A., Gakis, C., Budroni, M. and Andreoni, G. (1978). Adenosine deaminase activity in pleural effusions: An aid to differential diagnosis. *Britain Medical Journal*. **2**:1751–1752.
19. ShokrollahSalmanzadeh; HeshmatollahTavakkol; Khalid Bavieh; Seyed Mohammad Alavi. Diagnostic Value of Serum Adenosine Deaminase (ADA) Level for Pulmonary Tuberculosis**.**Jundishapur J Microbiol. 2015 March; 8(3): e21760.
20. Shore, A., Dosch, H.M. and Gelfand, E.W. (1981). Role of adenosine deaminase in the early stages of precursor T cell maturation. *Clinical Experimental Immunology***44**: 152-155.
21. Storla, D.G., Yimer, S. and Bjune, G.A. (2008). A systematic review of delay in the diagnosis and treatment of tuberculosis. *Bio Medical Centre Public Health Journal*. **8**:15.
22. Swami, K.K. (2016). Diagnostic and prognostic significance of pleural fluid adenosine deaminase estimation in relation to tuberculosis. Indian Journal of Applied Research. 6(**1**): 232-233.
23. Ullah, S., Shah, S. H., Aziz-ur-Rehman, K. A., Begum, N., & Khan, G. (2008). Extrapulmonary tuberculosis in Lady Reading Hospital Peshawar, NWFP, Pakistan: survey of biopsy results. *J Ayub Med Coll Abbottabad*, *20*(2), 43-46.
24. Wilkinson RJ, Vordermeier HM, Wilkinson KA, Sjölund A, Moreno C, Pasvol G, Ivanyi J. (1988). Peptide-specific T cell response to *Mycobacterium tuberculosis*: clinical spectrum, compartmentalization, and effect of chemotherapy. J Infect Dis 178:760-768.
25. World Health Organization (2016). Guidelines for the programmatic management of drug-resistant tuberculosis, 2016 Update, WHO, Geneva, Switzerland.
26. Zavialov, A.V., Gracia, E., Glaichenhaus, N., Franco, R., Zavialov, A.V. and Lauvau, G. (2010). Human adenosine deaminase 2 induces differentiation of monocyte and stimulates proliferation of T helper cells and macrophages. *Journal of Leukocyte Biology*. **88**:279-290.