Diagnosis of tuberculous pleurisy using the biologicL parameter adenosine deaminase

E A Dosumu, K Osagie, A Shuaib, and J A Momoh

Abstract

Adenosine Deaminase (ADA) isoenzyme levels in the pleural fluid have not been used before in Nigeria to make a diagnosis of tuberculous (TB) pleural effusions. Our objective was to identify the ADA isoenzymes as a diagnostic tool for tuberculosis (TB) in pleural effusions. One hundred and twenty patients (120) with exudative effusions and ADA activities >20 U/l, due to causes that satisfied certain diagnostic criteria, participated in this study. Of the effusions studied, 85 were tuberculous effusions, 20 were infective effusions, 10 were malignant effusions, and 5 were other exudative effusions (pancreatitis and lung embolus). The ADA isoenzymes in the pleural fluid were identified in each case using polyacrylamide gel electrophoresis. Microbiology and cytology (including differential cell counts) were also carried out on each specimen. The study was carried out between 1 January and 31 2005. The differential white cell counts of the effusions were: tuberculous, 90% lymphocytes and 5% neutrophils; empyematous, 15% lymphocytes and 73% neutrophils; and non-empyematous, 22% lymphocytes and 72% neutrophils. ADA_{1c} and ADA_2 were the predominant isoenzymes observed in tuberculous effusions while ADA_{1c} and ADA_{1m} were predominant in infective effusions (non-empyematous). It was concluded that ADA_2 activity is a more efficient diagnostic marker of tuberculosis effusions than total ADA activity.

Introduction

The necessity to improve diagnosis and the need to identify tuberculosis (TB) as a global crisis have been discussed by Iseman.[1] Adenosine deaminase (ADA) activity is greatest in the lymphoid tissues,[2] most especially in T-lymphocytes.[3] ADA can help in the differentiation of lymphoid cells[3,4] and the maturation of macrophages.[5] Cellular immune response, and in particular the activation of T-lymphocytes,[6] is reflected by the presence of ADA in pleural fluid. ADA activity has been implicated in TB, malignancies, collagen vascular disease, e.g. systemic lupus erythematosus (SLE), and empyemas.[6,7]

ADA_1 and ADA_2, having two different molecular forms and with unique properties, have been identified in humans.[8] A different gene locus code for each isoform,[8] ADA_1 may exist in two forms: a monomer, ADA_1, and a dimer, combined by a protein, ADA_1+CP.[8] These ADA subtypes have been referred to as ADA_{1m} and ADA_{1c}, respectively.[9]

ADA_1 has been shown to have the highest activity in lymphocytes and monocytes, whereas ADA_2 originates from monocytes.[10] Serum from both normal subjects and patients with increased ADA activity contains ADA_{1c} and ADA_2 and is the predominant isoenzyme in patients with increased ADA activity, except in acute lymphoblastic leukaemia where ADA_{IC} is found in excess.[10] However, ADA_{1m} has been reported in the serum of one patient with acute lymphoblastic leukaemia.[10]

Ungerer and Grobler,[11] and Kurata and colleagues,[12] have identified ADA_2 and ADA_1 in pleural fluid, with ADA_2 being predominant in TB. These finding were later revalidated by Ugerer and colleagues[9] and showed that para-infective effusions were associated with both ADA_{1m} and ADA_{1c}, while tuberculous effusions were associated with the ADA_2 isoenzyme.

This present work was performed in order to establish whether the different ADA isoenzymes could enhance the diagnosis of tuberculosis pleural effusions.

Patients and methods

Approval was given for the project by the Ethics and Research Committee of the National Hospital, Abuja, Nigeria.

Pleural effusion specimens from patients admitted to medical, surgical, paediatric, and gynaecological wards of the National Hospital, Abuja between 1 January and 31 December 2005 were analysed. Exudates were distinguished from transudates according to Light et al.'s criteria[13] by determining their total protein and lactate dehydrogenase levels. These were determined using a multichannel analyser (Technicon DAX 48).

Pleural fluid samples were sent to the microbiology laboratory for Gram staining, bacteria and tuberculosis culture, and cytology. The differential cell count was also carried out. Other investigations, including histology, were carried out based on the possible diagnosis envisaged in each case by the primary physician. Patients that had an exudate (n=120) had their speciments frozen at -20^0C for determination of ADA and ADA isoenzymes.

Diagnosis was made in all cases by a review of the hospital records according to the following criteria.

E A Dosumu, K Osagie, A Shuaib, Department of Medicine, National Hospital, Abuja, Nigeria; and J A Momoh, Department of Chemical Pathology, National Hospital, Abuja. Corresponding author: Professor E A Dosumu, P O Box 8683, Wuse Post Office, Abuja, Nigeria. Email: adedos2002@yahoo.com

1. Tuberculous pleuritis – three categories:
   a) finding of the bacillus in pleural fluid or biopsy specimen by stain or by culture or by the presence of granulomas in pleural biopsy tissue;
   b) positive sputum culture in the presence of clinical and radiological evidence of TB and in the absence of any other obvious cause associated with pleural effusions;
   c) clinical and radiological evidence for TB in the absence of any other obvious cause associated with pleural effusions and associated with a positive response to anti-TB therapy.
2. Infective effusions – two categories:
   a) empyematous effusions, characterised by the find ing of frank pus in the pleural cavity;
   b) non-empyematous effusion, including: pneumonic effusions associated with acute febrile illness, purulent sputum, cough, pulmonary pneumonic infiltrates, and definite response to antibiotic treatment; or identification of the causative organism in the pleural fluid, septicaemia, radiological evidence of pulmonary infiltrates and positive blood cultures in the absence of any other cause known to be associated with pleural effusions.
3. Malignant effusions:
   a) presence of histological or cytological evidence of malignant pleural effusion;
   b) histological evidence of a malignant tumour with exclusion of any other cause known to be associated with pleural effusions;
4. Other exudates: effusions caused by pancreatitis, and lung infection in sickle cell disease patients. In all these patients there was an absence of malignancy and disease-causing transudates.

Methods

All reagents and chemicals used were of the purest grade commercially available.

ADA activity determination

ADA activity (U/l) was determined on all samples of pleural fluid according to the method described by Giusti.[14] Adenosine is deaminated by ADA and the free ammonia estimated by Berthelot's reaction. One unit (IU) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine under standard assay conditions. The enzyme is stable for at least 24 hours at 25^0C, 7 days at 4^0C, and 3 months at -20^0C.[15,16.]

Electrophoresis

ADA isoenzymes were determined in all exudates with an ADA activity >20 U/l. The methods of Buel and MacQuarrie[17] and Ungerer et al.[10] employing polyacrylamide gel electrophoresis (PAGE; LKB Instruments Inc,Rockville, MD, USA) with slight modification were used to identify the different isoenzymes. For a 5% gel, a phosphate buffer system consisting of a 0.1M bridge buffer and a 0.05 M gel buffer at pH 6.7 was used. Pleural fluids with an ADA activity >40 U/l were diluted to yield values between 30 and 40 U/l, and 10/μl of the samples applied to the gel. Electrophoresis was carried out horizontally at 200 mA for 2.5 hours at 4^0C.

To make the isoenzymes visible[18] an ADA staining reaction was used. The staining reaction mixture consisted of the following: 24 mg adenosine (Sigma, St Louis, MO, USA), 1 U of nucleoside phosphorylase, and 0.5 U of xanthine oxidase (Boehringer Mannheim, Mannheim, Germany), 12 mg 3-(4, 5–dimethylthiazol -2- yl)-2, 5-diphenyl 2H-tetrazolium salt (Sigma), 1 mg phenazine methosulphate (Sigma) and 16 mg sodium phosphate in 8 ml staining buffer (0.3 M Tris, 0.2 M histidine HCl, pH 7.8). A cellulose acetate sheet soaked with this mixture was applied to the gel and incubated at 37^0C for 60 min.

Statistical analysis

For Mann-Whitney U tests and percentile analyses, the Statgraphics Version 5 programme was used.

Results

ADA isoenzyme patterns were obtained for the following effusions: tuberculous, 85 (15–60 years, female: male 1:1.2, infective), 20 (15–60 years, female: male1:1.2); malignant, 10 (32–60 years, female: male 1:2.2); others 5 (pancreatitis and pulmonary embolus, 30–60 years, female: male 1: 1.5) (see Table 1).

The median (25th, 75th percentile) effusion ADA values were as follows: tuberculous 102 (74, 143) μ/l; infective, 51 (35, 90) μ/l; malignant, 31 (25, 42) U/l; others, 35 (24, 45) U/l. ADA activity was significantly higher for tuberculous effusion compared to the other diagnostic groups ($p<0.005$ for each group).

The white cell counts (differential) of the effusions were as follows: tuberculous, 90% lymphocytes, 5% neutrophils; empyematous, 15% lymphocytes, 73% neutrophils; non-empyematous, 22% lymphocytes, 72% neutrophils.

Three patterns of ADA isoenzyme were identified: ADA_{1C} + ADA_2; ADA_{1C}; and ADA_{1m} in combination with ADA_{1c} and/or ADA_2. Tables 2 to 4 summarise the isoenzyme patterns.

Table 1 Patients demographic and clinical data

Type of effusion	No. of patients	Sex ratio (male:female)	Age range (years)
Tuberculous	85	37:48	15–60
Infective	20	9:11	15–60
Malignant	10	3:7	32–60
Others	5	2:3	30–60

Discussion

In this study, ADA activity was found to be highest in pleural effusions from tuberculosis and infective causes. This finding confirms previous work on this subject. [19,20]. Electrophoretic analysis of the pleural effusions showed differences only between the tuberculous and infective non-empyematous groups. In the former group, ADA_{1c} and ADA_2 predominated, while the latter group was characterised by ADA_{1c} and ADA_{1m}.

Valdes et al.[21] and Ungerer et al.[10] found that the greatest ADA activity was found in lymphocytes and monocytes/macrophages and that ADA_2 originated from the latter population. Later, Ungerer et al.[19] found that in tuberculous effusions the ADA_2 isoenzyme was primarily responsible for total ADA activity while ADA_{1m} and ADA_{1c} isoenzymes contributed mostly to ADA activity in parainfective effusions. The results of this study essentially agree with these observations. This study further confirmed as shown previously[22,23] that tuberculous effusions were characterised by predominant lymphocyte presence.

It is to be noted that the TB patients that participated in this study were very poor and suffered from malnutrition. They also tended to have super-imposed secondary infections. Another explanation may be the fact that tuberculous pleurisy appears to be due to delayed hypersensitivity caused by *Mycobacterium tuberculosis*.[23] [3]Neutrophils play an important role in the development of tuberculous pleurisy, and following the tuberculous response, macrophages appear to predominate in the pleural fluid for up to 5 days, after which the lymphocytes become the predominant cell in the pleural fluid.

The finding in this study showing ADA_2 isoenzyme to be very high in TB pleural effusion is particularly useful. This is because often in Nigeria, we see a patient with massive pleural effusion and with no history of cough or sputum production. The chest x-ray might not show much because of the pleural effusion. There may be no sputum for Ziehl–Neelson stain. The pleural fluid might show no malignant cells, thus making a diagnosis of the pleural effusion becomes difficult. α1 Interferon level and polymerase chain reaction are usually not readily available in most developing countries. Thus ADA enzyme becomes invaluable in such instances to make the diagnosis of the TB pleural effusion.

In conclusion, ADA_2 activity is a more efficient diagnostic marker of tuberculous effusions than total ADA activity.

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Table 2 ADA activity

Type of effusion	No. of cases	Median ADA activity (U/l (%)
Tuberculous	85	102 (47)
Infective	20	51 (23)
Malignant	10	31 (14)
Others	5	35 (16)
Total	120	219 (100)

Table 4 ADA isoenzyme composition of tuberculous and infective effusions

		Isoenzyme pattern (%)		
Type of effusion	No.	ADA_2	ADA_{1m}	ADA_{1c}
Tuberculous	85	81	3	100
Infective empyematous	5	38	33	100
Non-infective empyematous	15	7	67	100

Table 3 Summary of ADA results in different effusions

		Isoenzyme pattern (%)				Cytology	
Effusion	No.	ADA (median U/l)	ADA_{1c} +ADA_2	ADA_{1c}	ADA_{1m}+ ADA_{1c}/ADA_2	Lymphocytes (%)	Neutrophilis (%)
Tuberculous	85	102	59	38	3	90	5
Infective empyematous	5	51	58	8	33	15	73
Non-infected empyematous	15		7	27	67	22	72
Malignant	10	31	35	32	32	++	
Others	5	35	33	33	33	++	

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Disease burden among inmates in Tarkwa prison, Ghana

J Galley

Abstract

A retrospective cross-sectional study was carried out at Tarkwa prison in Ghana. The objective was to study the disease burden among the inmates. Medical records for 2007 and 2008 were reviewed for all inmates. Upper respiratory tract infections and malaria were the most common causes of morbidity among the inmates. It was concluded that overcrowding in the prison should be addressed and that further studies should be undertaken both in Tarkwa prison and in other prisons for comparison.

Introduction

Prisons are special communities that receive little attention from health authorities. The health sector does not analyse or use the health data generated in prisons. Because the living environments are substandard and overcrowded, it is expected that the inmates will be exposed to many disease conditions, particularly airborne diseases. Knowing that diseases have no boundaries it is prudent to pay serious attention to diseases that are suffered by prison inmates. This study set out to achieve the following specific objectives:

- to explore and describe the disease burden of Tarkwa prison;
- to inform the Municipal Health Directorate in order that these diseases are incorporated into their action plans.

This study is relevant because scientific attention is not given to the data generated in the prisons and these data will contribute to the understanding of the health burden of the inmates of Tarkwa prison. It will also enable the health authorities to take appropriate public health action and will act as a reference document for the district.

Dr Jack Galley, Tarkwa Municipal Medical Officer, Tarkwa, Ghana. Email: jack_galley@yahoo.com

Methods

Tarkwa is the administrative capital of Tarkwa Nsuaem Municipal Assembly in the western region of Ghana. It is a mining community with a population of about 5000, and is the second largest urban city after Sekondi, where another prison is located. Tarkwa prison receives inmates from all over the region.

The prison was chosen because this community has not been studied and is not included as a special population in the municipal health plans. Being a retrospective study no ethical clearance was required.

Records of 1685 prisoners were reviewed for 2007 and of 1209 prisoners for 2008. An analysis of tuberculosis cases over the 2-year period was also undertaken.

Results and discussion

Eighteen major diseases were found among the inmates (see Table 1). In 2007, upper respiratory infection accounted for 17.5% of the diseases with malaria accounting for 17%. In 2008, malaria had risen to 23% with upper respiratory infection at 16%. These high rates of morbidity could be attributed to overcrowding in the prison. According to the prison authorities, the building was meant for 150 inmates but they are taking more than 500.

Anaemia constituted 3% of the disease in 2007 and 5% in 2008. The probable reason for this could be hookworm infestation or poor nutrition. There is a need for stool examination among the inmates to rule out worm infestation. The findings of the stool examinations will assist us in unveiling the causes of stomach disorders, which stand at 6.2%. It is appropriate to find out the source of schistosomiasis among the inmates to see if they brought it in or not, as they may be bathing in some rivers when they go out.

Tuberculosis, which constituted 6% of the disease morbidity in 2007, fell to 3% in 2008 (see Table 2).

Table 1 Diseases diagnosed among inmates in Tarkwa Prison in 2007 and 2008

Disease	Number of cases	
	2007	2008
Upper respiratory tract infection	296	193
Malaria	288	283
Rheumatism and joint pains	164	137
Diarrhoeal diseases	131	41
All other diseases	129	77
Diseases of the skin	128	126
Stomach disorders	105	77
Chicken pox	96	82
Ear infection	75	40
Accidents and wounds	53	36
Diseases of the oral cavity	51	11
Anaemia	50	64
Acute eye infection	40	21
Intestinal worms	33	28
Heart disease	15 25	
Schistosomiasis	11	13
Pneumonia	10	–
Tuberculosis	10	4
Total	**1685**	**1209**

Table 2 Tuberculosis cases in Tarkwa prison 2007 and 2008

Year		Status
Outcome		
2007	TB Positive	Cured
	HIV positive/TB negative	Completed treatment
	HIV/AIDS	Died
	Relapse	Cured
	TB positive	Cured
	TB positiveHIV/AIDS	Died
	X-ray negative	Completed treatment
	TB positive	Cured
	TB positive	Cured
	TB positive	Cured
2008	TB positive	Cured
	Defaulter (2006; old drug)	Still on treatment
	TB Positive	Died
	Defaulter (old drug)	Still on treatment

Conclusion

Malaria and upper respiratory tract infections were the commonest diseases among the inmates of Tarkwa prison during 2007 and 2008.

The following recommendations are made:

- overcrowding in the prison should be addressed;
- public health, local government, prisons, and human rights authorities must take corrective actions to redeem the health of the inmates;
- a similar study should be carried out in other prisons for comparison;
- other studies of the health of prison inmates and staff should be undertaken;
- pneumonias must be looked at thoroughly to exclude tuberculosis.

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