EXTRAPULMONARY TUBERCULOSIS AT KING EDWARD VIII HOSPITAL: A RETROSPECTIVE DESCRIPTIVE STUDY

By

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As the candidate's supervisor I have/have not approved this thesis for submission.

Signed: _____ Name: _____ Date: ____

Declaration

I, Strinivasen Gounden declare that

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(ii) This dissertation has not been submitted for any degree or examination at any other university.

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Dedication

Dedicated to my wife, Yejna Narain and children Maheera and Kaveera.

Thank you for your everlasting love and support.

Acknowledgements

I am grateful to my supervisor Prof. NP Magula for her expert advice and encouragement throughout this project. I am truly grateful for her continuous assistance, guidance and dedication.

I thank King Edward VIII Hospital staff for their assistance in completing this project, in particular, the admin staff of the records department.

To my family and friends, I thank you for your everlasting support and prayers. In particular, to my wife, Dr Yejna Narain, I am forever grateful for your love, support and encouragement. To my wonderful little girls, Maheera and Kaveera, thank you for your love and understanding. To my parents and parents-in-law, thank you for always guiding me to the right path.

I would also like to thank my colleagues, Dr Dhiren Sadhabiriss and Dr Rubeshan Perumal, for assisting me in those endless nights with my statistics and editing. Your words of motivation and encouragement have finally borne fruit.

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Abstract

Background

Globally, South Africa remains one of the top twenty high tuberculosis(TB) burden countries. In addition, South Africa has the highest burden of tuberculosis/Human Immunodeficiency virus (TB/HIV) co-infection in the world, with the province of KwaZulu-Natal representing the global epicenter of TB/HIV. With the scaling up of one of the world's largest antiretroviral therapy programs, it was envisioned that the burden of tuberculosis would be reduced. While significant progress has been made to improve the diagnosis of pulmonary tuberculosis, the diagnosis of extrapulmonary TB(EPTB) remains a significant challenge in resource constrained settings. This study describes the profile of patients with EPTB at a tertiary hospital in a TB/HIV hyperendemic setting in Durban, South Africa.

Methods

A retrospective chart review was conducted, and included all adult patients diagnosed with EPTB at a tertiary hospital in Durban, South Africa, between 1 January 2016 and 31 March 2016. Data was extracted from the facility TB register, as well as patient clinical records. All data was analysed using SPSS software (SPSS 23.0, Armonk NY: IBM Corp). For all statistical comparisons, a 5% level of significance was used; correspondingly 95% confidence intervals were used to describe effect size. All data was assessed for normality, and non-parametric tests were used where necessary. Medians and inter-quartile ranges were used for data not amenable to parametric description. Pearson's Chi-square test was utilised for comparison between subgroups. All p values were 2-tailed and considered significant below 0.05. Significant findings were analysed for association using Phi and Kramers V test for symmetric measures.

Results

There were 188 new cases of TB during the study period, with 80 patients diagnosed with EPTB. The mean age of patients was 34.73 years (SD ±9.44). Forty two (52.5%) patients were female, while 76(96%) were black African. The most common risk factor for EPTB was HIV co-infection (88.8%). The median CD4 cell count was 68 (IQR 32-165) cells/mm^3. Pleural (36.3%), lymph node (28.7%) and abdominal(27.5%) involvement were the most common sites of extrapulmonary disease Eleven of the 80 patients (13.8%) presented with EPTB involving more than one anatomical system. Weight loss, fever, night sweats and cough were amongst the most common symptoms reported. Signs varied according to the site of infection. Non-specific symptoms were common. In the majority of cases, more than one diagnostic method was used to confirm the presence of TB in distant organs.

Conclusion

A high index of suspicion is required when assessing a patient with known risk factors for EPTB. Immunosuppression remains the most significant risk factor for the development of EPTB. In our setting, HIV co-infection remains the most common risk factor. Advancements in Xpert MTB/Rif and computer tomography have greatly assisted in rapidly diagnosing EPTB. Despite improved access to antiretroviral therapy over the past years, advanced HIV disease remains a significant challenge to eradicating TB.

Keywords: HIV; Extrapulmonary TB; Risk factors for extrapulmonary TB; Signs and symptoms of extrapulmonary TB

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Part 1: The Review of Literature

1. Introduction

The World Health Organization declared tuberculosis a global health emergency in 1993[1]. In South Africa, tuberculosis(TB) remains a major cause of mortality[2]. TB is caused by one of several mycobacterial species that belong to the Mycobacterium tuberculosis complex. The human pathogens are *Mycobacterium tuberculosis*, *Mycobacterium africanum*, and *Mycobacterium bovis*. TB describes many clinical illnesses caused by *Mycobacterium tuberculosis*[1]. *M. tuberculosis* is an aerobic, non-motile, non-spore forming bacillus. Spinal tuberculosis can be dated back to the time of the Egyptians. The cell wall consists of high-molecular-weight lipids. Unlike other bacteria, the growth time is 15 to 20 hours, with visible growth taking 3 to 8 weeks on solid media[1]. Humans remain the only reservoir, but other animals can be infected. Tuberculosis becomes aerosolized by coughing and remains suspended in the air for long periods of time. The air in the room may remain infectious for up to 30 minutes after the absence of the infected individual. *M. tuberculosis* can be destroyed by ultraviolet light[1]. Approximately 3% to 4% of infected individuals develop active infection in the first year[1]. The current HIV epidemic has seen these numbers rise drastically[1].

TB is the most common HIV related disease in the world[3]. The presentation of the disease may be related to the level of immunosuppression of the patient. Extra-pulmonary involvement has been associated with advanced HIV infection. Overall pulmonary TB is more common than extra pulmonary TB in all HIV infected patients[3]. Since 2004, anti-retroviral drugs have been freely available in the public sector. With more than a decade of antiretroviral treatment more than 3 million people now have access to life saving medication[4].

2. Immunopathogenesis

Tuberculosis requires a cellular immune response for control. Despite abundant antibody production during infection, the role of antibodies in host defense is still unclear. Inoculate multiply freely in the alveolar space and alveolar macrophages. Interactions with compliment receptors, mannose receptors and Fc receptors are responsible for the entry into the macrophage. Mycobacterial urease limits the effect of bacteriocidic enzymes within the macrophage[1]. The organism remains within a phagosome inside the macrophage thereby not eliciting a strong CD8+ cytotoxic T-cell response. Replication proceeds for weeks within the primary focus and the lymphohematogenous metastatic foci[1]. The adaptive immunity leads to the activation of localized T-helper1(Th1) along with several cytokines (TNF-α, IL-1 β, and IL-12), lymphocytes, monocytes, natural killer T cells, and B lymphocytes that form granulomas and kills almost all MTB preventing the growth of those that remain viable. Extrapulmonary spread may be facilitated by inducing epithelial transcytosis[1].

3. Epidemiology

Globally in 2013, nine million people developed TB, with 1.5 million tuberculosis-related deaths reported in the same period. In the same year, there were 328 826 case reports of TB in South Africa, 37 709 of these cases were extrapulmonary TB(EPTB)[5]. Between 2008-2010 TB has remained the leading cause of death in the country [2]. KwaZulu-Natal has the highest prevalence of TB disease in the country(1076 cases per 100 000)[6]. Classically, young children and the elderly carried a disproportionately increased risk of EPTB [7]. However, the high burden of HIV in the young adult population in our setting, has seen an increased burden of tuberculosis in this group. Low socio-economic status has been shown to confer an increased risk of extra-pulmonary infection[8].

4. Risk Factors

Many risk factors for development of EPTB have been identified. One of the key risk factors is a compromised immune system, particularly a weaker cellular immunity[9], diabetes, smoking, malignancies, low body weight, chronic kidney disease and use of immunosuppressive drugs.[10]

a) Diabetes

Diabetes affects both the innate and adaptive immunity. A reduction in the phagocytosis of *M. tuberculosis* by monocyes is implicated as the cause of the weakened immune system. Diabetic patients are 3 times more susceptible to TB as compared to normal individuals[11]. Diabetic patients remain infected for longer periods and have increased risk of reactivation[11]. In diabetes the immunological responses are weakened and diabetic patients with EPTB, have an increased risk of death when compared to patients without EPTB[12]. Patients with EPTB and diabetes have been shown to have a higher risk of cavitation than non-diabetics [13].

b) Smoking

Smoking is an independent risk factor for TB. It causes a twofold increase in latent and active TB. Treatment failure and increased levels of recurrence have been noted in smokers[14]. Smoking has also been associated with increased mortality in patients with TB [15]. In a study by Brunet et al, it was found that in Cape Town, 56% of patients with active TB were current smokers[16]. The exact pathophysiology is hypothesized to be multifactorial. Smoking may irreversibly inhibit nitric oxide synthase, which is needed to inhibit the replication of *M. tuberculosis*. Smoking increases the availability of iron in the lower respiratory tract thereby increasing toxic radicals which inhibit macrophage function[14]. Smoking cessation programs need to be targeted at the entire population and not only at patients diagnosed with TB. Current TB treatment programs need to emphasize the importance of smoking cessation since it increases the risk of poor treatment outcome[14].

c) Malignancy

The relationship between TB and malignancy is complicated. Recent studies in the field of cancer research have shown the link between chronic inflammation and cancer development. Pyothorax-associated lymphoma has been associated with TB infection at scar sites within the peripheral lung tissue[17]. A study by Kung et al, however noted the association between cancer and TB infection as merely co-incidental[17]. Undoubtedly, the association between therapeutics used for treatment of malignancies and TB development exists. The immunosuppression from the cytotoxic chemotherapy drugs provide the ideal setting for reactivation of latent infection, or the acquisition of a primary infection. Drugs used for T-cell immunosuppression, is associated with mycobacterial infection[17]. Leukemia and lymphoma have also been implicated as risk factors for TB reactivation[17]. TB and malignancy have been documented to exist concurrently in patients. This provides a diagnostic and therapeutic challenge. Mimicry of TB and malignancy leads to delayed diagnosis and could hamper eventual outcomes.[17]

5. Sites of Disease

EPTB is defined as TB of organs other than the lungs. It usually is due to lympho-haematogenous spread. Sites of infection include the pleura, central nervous system, lymphatic system, genitourinary system and muscular skeletal system[8]. In a composite of multiple studies of EPTB, lymph node and pleural involvement were the most common[18].

Patients with pleural TB may have underlying pulmonary TB[19]. The clinical manifestations are similar to pulmonary involvement. It was previously thought that TB pleural effusions developed due to delayed hypersensitivity reactions[20]. Recent studies have shown positive culture growth from both pleural fluid and pleural tissue[20]. The infection within the pleural space most likely increase pleural fluid formation and decreases pleural fluid removal[21]. There is a lymphocyte driven immune reaction which is accompanied by granuloma formation and release of adenosine deaminase (ADA)[20]. Measuring ADA levels has been shown to be useful in assisting in the diagnosis of pleural TB. Common complications of pleural TB are chronic TB empyema and pleural fibrosis. Pleural fibrosis has been associated with significant morbidity[22].

TB lymphadenopathy dates back thousands of years. It is still unclear whether the port of entry of the infection is primarily in the lungs or through the tonsil and adenoid glands[23]. The clinical manifestation is variable. The infected glands have characteristic features, i.e. caseation, matting and adherence to surrounding tissue[23]. Bacterial culture is the gold standard for diagnosis, but is limited by the slow growth of the organism. Fine needle aspirate sent for molecular diagnostic tests for TB, have shown to perform well in the diagnosis of TB lymphadenopathy[24]. The advantage of molecular testing is the rapid time to diagnosis and the ability to detect rifampicin resistance.

TB involving the central nervous system is a devastating disease with a high mortality and morbidity[25]. There are different clinical and pathological manifestations of cerebral TB. The most common is TB meningitis, followed by tuberculoma, tuberculous abscess, tuberculous encephalopathy, tuberculous encephalitis and tuberculous arteritis[25]. There is significant brain tissue damage due to the excessive cellular mediated immune response and related excessive inflammation[25].

6. Diagnosis

Extra-pulmonary tuberculosis, especially invasive disease, presents more commonly in patients with HIV co-infection. In HIV infected patients with CD4 counts less than 250 cells/mm^3, the rate of progression of disease is markedly different when compared to patients with higher CD4 cell counts[26]. Symptoms may be subacute or acute with rapid progression and death.[27]. The clinical manifestations are variable, but often reflect the organs involved[26]. The diagnosis of extra pulmonary tuberculosis remains challenging due to the protean presentation of the disease[28]. A high index of suspicion is always required when assessing a patient with possible EPTB. Fever, weight loss, anorexia and malaise have been documented in multiple studies as the most common symptoms reported by patients.[26]

Laboratory and radiological advancements have assisted in improving the diagnosis of extra pulmonary TB. The increasing use of Adenosine deaminase(ADA) levels in body fluids, and nucleic acid amplification techniques on body fluid and tissue samples has improved our diagnostic armamentarium. These have assisted greatly in improving the diagnosis of EPTB especially in sputum, urine, blood and cerebrospinal fluid.

Xpert MTB/Rif has a high specificity(97.3%) but sensitivity varies according to the type of tissue tested.[29]

Table 1. Xpert MTB/Rif* sensitivity and specificity of non-pulmonary specimens.[29-31]

Specimen type	Sensitivity	Specificity
Stool	100	91.7
Lymph node specimens	87.5	73.3
Biopsy tissue	86.6 (79-94)	95.5
Pus	85.1 (75-95)	94.6
Cerebrospinal Fluid	84.6 (65-104)	98.3
Urine	84.6 (65-104)	98.3
Gastric Aspirate	77.6 (67-88)	97.6
Pleural Fluid	33.3 (9-57)	99

* Xpert MTB/Rif – Nucleic acid amplification test for *Mycobacterium tuberculosis*/Rifampicin resistance.

In a recent study by Naidoo et al. the introduction of Xpert MDR/rif, in the South Africa context has had a substantial increase in the cost of diagnosis of TB, without the expected increase in diagnostic efficiency.[32]. The use of Xpert MTB/Rif has not had economic and diagnostic benefits as predicted by Meyer-Rath et al in 2012 [33]. Use of Xpert MDR/Rif can be used by non-laboratory technicians in decentralized models, thereby reducing the costs associated with transportation of specimens and results. It also allows for rapid turnaround of results [34]. Cox et al. found that in Khayelitsha, the use of decentralized Xpert MDR/rif, led to an improved case detection of rifampicin resistant TB. It also showed a decreasing time to treatment of 8 days ($p < 0.0001$) [35]. Xpert MTB/Rif has been shown to be useful in rapidly diagnosing lymph node TB.[31]. In a high TB endemic area, Xpert MTB/Rif on cerebrospinal fluid is a good rule-in test in HIV infected patients with TM meningitis.[36]

ADA has been shown to be useful in diagnosis of pleural effusions secondary to tuberculosis[37]. Although pleural biopsy is the most effective in diagnosis of TB pleural effusions, ADA remains a reliable and inexpensive tool in a high endemic area. [38] In a recent meta-analysis of Indian studies involving the use of ADA in tuberculous pleural effusions, ADA was shown to have good diagnostic accuracy in Indian patients. [39]. Serum ADA has been found to be elevated in patients with TB, and has been suggested as an alternate rule-out test in resource restricted areas[40].

Abdominal ultrasound is a useful tool in the diagnosis of EPTB, especially TB of the abdomen. A combination of ultra-sonographic findings suggests abdominal TB, especially the presence of multiple abdominal lymphadenopathy[41]. Abdominal Ultrasound findings in patients with active TB at G F Jooste Hospital, Cape Town[42] showed a significant correlation($p<0.05$) with abdominal lymphadenopathy greater than 1 cm; ascites; splenic granulomas; and combinations of the above. Computer tomography has been shown to significantly increase the detection of abdominal TB in inconclusive cases.[43] Computer tomography is not freely available at all hospitals.

Due to the variability of presentation of EPTB, a standardized diagnostic algorithm is difficult. Clinicians require a high index of suspicion. A thorough history and examination will assist in assessing a patient's risk profile for EPTB. Further confirmatory tests will be dependent on the signs and symptoms experienced by the patients.

The study aims to describe the profile of patients with EPTB diagnosed at a tertiary facility in a setting of TB-HIV hyperendemicity.

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Part 2: Manuscript.

Extrapulmonary tuberculosis in the setting of HIV hyperendemicity at a tertiary hospital in Durban, South Africa

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Abstract

Background

Globally, South Africa remains one of the top twenty high tuberculosis(TB) burden countries. In addition, South Africa has the highest burden of tuberculosis/Human Immunodeficiency virus (TB/HIV) co-infection in the world, with the province of KwaZulu-Natal representing the global epicenter of TB/HIV. With the scaling up of one of the world's largest antiretroviral therapy programs, it was envisioned that the burden of tuberculosis would be reduced. While significant progress has been made to improve the diagnosis of pulmonary tuberculosis, the diagnosis of extrapulmonary TB(EPTB) remains a significant challenge in resource constrained settings. The entity of EPTB comprises a heterogeneous group of diseases, described primarily by the anatomical site of involvement. A diagnostic approach has not been well established, and there is a strong reliance on radiological and laboratory services to secure a diagnosis. Innovations in pulmonary TB diagnostics have incidentally contributed to the improved diagnosis of EPTB, however there is a paucity of evidence in this group. The burden of EPTB is high in this setting, and contributes to excess morbidity and mortality from a curable infectious disease. This study describes the profile of patients with EPTB at a tertiary hospital in a TB/HIV hyperendemic setting in Durban, South Africa.

Methods

A retrospective chart review was conducted, and included all adult patients diagnosed with EPTB at a tertiary hospital in Durban, South Africa, between 1 January 2016 and 31 March 2016. Data was extracted from the facility TB register, as well as patient clinical records. All data was analysed using SPSS software (SPSS 23.0, Armonk NY: IBM Corp). For all statistical comparisons, a 5% level of significance was used; correspondingly 95% confidence intervals were used to describe effect size. All data was assessed for normality, and non-parametric tests were used where necessary. Medians and inter-quartile ranges were used for data not amenable to parametric description. Pearson's Chi-square test was utilised for comparison between subgroups. All p values were 2-tailed and considered significant below 0.05. Significant findings were analysed for association using Phi and Kramers V test for symmetric measures.

Results

There were 188 new cases of TB during the study period, with 80 patients diagnosed with EPTB.The mean age of patients was 34.73 years (SD ±9.44). Forty two (52.5%) patients were female, while 76(96%) were black African. The most common risk factor for EPTB was HIV co-infection (88.8%). The median CD4 cell count was 68 (IQR 32-165) cells/mm^3. Pleural (36.3%), lymph node (28.7%) and abdominal(27.5%) involvement were the most common sites of extrapulmonary disease Eleven of the 80 patients (13.8%) presented with EPTB involving more than one anatomical system. Weight loss, fever, night sweats and cough were amongst the most common symptoms reported. Signs varied according to the site of infection. Non-specific symptoms were common. Tachycardia (48.75%) was the most common sign noted. A microbiologically confirmed diagnosis was made in only 65% of cases. In the majority of cases, more than one diagnostic method was used to confirm the presence of TB in distant organs.

Conclusion

A high index of suspicion is required when assessing a patient with known risk factors for EPTB. Immunosuppression remains the most significant risk factor for the development of EPTB. In our setting, HIV co-infection remains the most common risk factor. Advancements in Xpert MTB/Rif and computer tomography have greatly assisted in rapidly diagnosing EPTB. Despite improved access to antiretroviral therapy over the past years, advanced HIV disease remains a significant challenge to eradicating TB.

Keywords: HIV; Extrapulmonary TB; Risk factors for extrapulmonary TB; Signs and symptoms of extrapulmonary TB

Extrapulmonary tuberculosis at a tertiary hospital in the setting of TB-HIV hyperendemicity in Durban, South Africa

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Tuberculosis(TB) describes many clinical illnesses caused by *Mycobacterium tuberculosis*[1]. Globally in 2013, nine million people developed TB, with 1.5 million tuberculosis-related deaths reported in the same period. In the same year, there were 328 826 cases reported of TB in South Africa, 37 709 of these cases were extrapulmonary TB(EPTB)[5]. Between 2008 and 2010 TB has remained the leading cause of death in the country [2]. KwaZulu-Natal has the highest prevalence of TB in the country with1076 notified cases per 100 000 population[6].

Tuberculosis is the most common Human Immunodeficiency Virus(HIV) associated disease, and represents the leading cause of death in these patients globally. The presentation of the disease may be related to the level of immunosuppression of the patient. Extrapulmonary involvement in particular has been associated with advanced HIV infection. Overall pulmonary TB is more common than EPTB in people living with HIV[3]. Since 2004, anti-retroviral drugs have been freely available in the South African public health sector. After more than a decade of rolling out the largest antiretroviral program in the world, more than 2.4 million people now have access to ART in this country. With the newly introduced test and treat strategy, these numbers are expected to increase to include all of the nearly seven million South Africans living with HIV.

Many risk factors for development of EPTB have been identified. One of the key risk factors is a compromised host immunity, particularly a weak cellular immunity[9]. Diabetes, smoking, malignancies, low body weight, chronic kidney disease and use of immunosuppressive drugs represent secondary causes of immunosuppression and have also been associated with an increased risk of developing EPTB.[10] Diabetic patients with EPTB, had an increased risk of death when compared to non-diabetic patients with EPTB(23.8% vs 9.8%,$p<0.01$) [12]. Classically, young children and the elderly carried a disproportionately increased risk of EPTB [7]. However, the high burden of HIV in the young adult population in our setting has seen an increased burden of tuberculosis in this group. Low socio-economic status has been shown to confer an increased risk of extrapulmonary infection[8].

EPTB, especially invasive disease, presents more commonly in patients with HIV co-infection. In patients with advanced HIV with CD4 cell counts of less than 250 cells/mm^3, disease progression is markedly different as compared to patients with higher CD4 cell counts [26]. Symptoms may be subacute or acute with rapid progression and death.[27]. The clinical manifestations are variable, but often reflect the organs involved [26]. Common sites of infection include the pleura, central nervous system, lymphatic system, genitourinary system and muscular skeletal system[8]. The diagnosis of EPTB remains challenging due to the protean presentation of the disease and a high index of suspicion is required when assessing a patient with possible EPTB.[28]. Fever, weight loss, anorexia and malaise have been documented as the most common symptoms reported by patients with EPTB regardless of the site of disease[26].

EPTB poses a significant diagnostic dilemma. Due to the high variability in the clinical presentation of the disease, clinicians are reliant on a high index of suspicion and on laboratory and radiological

tests to establish the diagnosis. The gold standard for the diagnosis of tuberculosis is culture of the organism. In EPTB, the long turn-around time associated with culture creates an unacceptable delay in diagnosis and treatment, and contributes to the increased mortality in this group. Liquid culture medium, however, has a shorter turn-around time as compared to conventional solid medium[44]. In a resource limited environment like South Africa, the need for expensive and sophisticated laboratory equipment presents another challenge to the routine use of culture. Traditional microscopy has been shown to be cost effective and time efficient as compared to culture. Even with recent improvements in microscopy, its use has been limited in EPTB.

During the last decade, scientific advancements have greatly assisted in improving diagnosis of EPTB. The increasing use of Adenosine Deaminase(ADA) levels and nucleic acid amplification techniques like Xpert MDR/Rif on body fluid and tissue samples have improved our diagnostic armamentarium. These have assisted greatly in improving the diagnosis of EPTB in sputum, urine, blood and cerebrospinal fluid following numerous validation studies globally [26, 45].

Xpert MTB/Rif has a high specificity regardless of the tissue sampled, however sensitivity varies significantly[29] Compromised sensitivity limits the role of this test in ruling out disease. In a recent study by Naidoo et al. the introduction of Xpert MDR/Rif, in the South African context has substantially increased the cost of diagnosis of TB, without the expected increase in diagnostic efficiency that was predicted by an earlier population level decision model[32, 33]. Nonetheless, the use of Xpert MDR/Rif can be used by non-laboratory technicians in decentralized models, thereby reducing the costs associated with transportation of specimens and results and theoretically reducing diagnostic delay [34]. Cox et al. found that in Khayelitsha, the use of decentralized Xpert MDR/Rif, led to the improved case detection of rifampicin resistant TB and a significantly reduced time to treatment of drug resistant TB [35]. The role of Xpert MTB/Rif in diagnosing TB in specimens other than sputum has revealed its potential utility in diagnosing EPTB. Xpert MTB/rif has a sensitivity between 77.6% to 100% depending on the specimen involved. The specificity ranges between 73.3 to 98.3%.[29-31]

ADA is an enzyme which catalyzes the conversion of adenosine to inosine and plays an important role in the differentiation of lymphoid cells. Diseases which stimulate cellular immunity increase ADA levels[46]. Lymphocyte predominant pleural effusions with elevated ADA suggest pleural TB or malignancies. ADA levels over 40U/I with more than 50% lymphocyte proportion suggest pleural TB(sensitivity 90.7% (CI 87.3-94.1%) specificity of 97.7% (CI 95.9-99.5%))[47].

ADA has been shown to be useful in diagnosis of tuberculous pleural effusions[37]. Although pleural biopsy is the most effective method of diagnosis of TB pleural effusions, ADA remains a reliable and inexpensive tool in a high prevalence setting. [38] In a recent meta-analysis of the role of ADA in the diagnosis of tuberculous pleural effusions, ADA was demonstrated to have good diagnostic accuracy [39]. Serum ADA has been found to be elevated in patients with TB, and has been suggested as an alternate rule-out test in resource constrained settings[40]. False positives are a concern with the use of ADA due to the low yield of positive TB culture on pleural fluid[48]. Suggested cut off values for ADA: Ascites>39U/L; Cerebrospinal fluid >6 U/L and in Pleural fluid >40 U/L [39, 49, 50].

Computer tomography has been shown to significantly increase the detection of abdominal TB in inconclusive cases, however its utility is limited by the scarcity of CT facilities in our setting [43]. Abdominal ultrasound is a useful tool in the diagnosis of abdominal tuberculosis and is the most widely used screening study for suspected abdominal involvement. A variety of ultrasonographic features may suggest active TB, most commonly the presence of multiple abdominal lymphadenopathy [41]. Patel et. al. used ultrasound to assess patients' abdomen with active pulmonary TB at a South African hospital. Abdominal lymphadenopathy over 1 cm; Ascites, splenic lesions with lymphadenopathy and/or ascites, were significant findings($p<0.05$) in patients with active pulmonary tuberculosis.[42]

Due to the variability of presentation of EPTB, a standardized diagnostic algorithm remains elusive. A high index of suspicion in the appropriate clinical setting is necessary to direct further investigation.

The study aims to describe the profile of patients with EPTB diagnosed at a tertiary facility in a setting of TB-HIV hyperendemicity.

Methods

A retrospective chart review of all adult patients diagnosed with EPTB at a tertiary hospital in Durban, KwaZulu-Natal between 1 January 2016 and 31 March 2016 was conducted. All adult cases were identified from the facility TB register, and clinical data was extracted from the associated clinical records. EPTB was defined as any organ system involvement outside the lungs. Patients who had both pulmonary and EPTB, were classified as EPTB. All data was analysed using SPSS software (SPSS 23.0, Armonk NY: IBM Corp). For all statistical comparisons, a 5% level of significance was used; correspondingly 95% confidence intervals were used to describe effect size. All data was assessed for normality, and non-parametric tests were used where necessary. Medians and inter-quartile ranges were used for data not amenable to parametric description. Pearson's Chi-square test was utilised for comparison between subgroups. All p values were 2-tailed and considered significant below 0.05. Significant findings were analysed for association using Phi and Kramers V test for symmetric measures. The study was conducted under the oversight of the UKZN Biomedical Research Ethics Committee (BREC no: BE283/16). The necessary permissions were obtained from health system gatekeepers.

Results

During the period under review, 188 new cases of TB were notified, and included 80 cases of EPTB(42.6%) (Table 1).

The mean age of patients was 34.73 (SD ±9.44) years. HIV co-infected males were younger than those who were HIV uninfected, with a mean age of 35.5(SD± 7.0) and 41 (SD±13.8) respectively. This is in contrast to the HIV infected females and HIV uninfected females with a mean age of 33.3(SD±10) and 28(SD±5.6) respectively. Forty two (52.5%) patients were female, and the majority(96%) of patients were black African. Patients were predominantly from local urban areas. Fifty seven (71.2%) patients were unemployed, while five patients were employed in high risk working environments i.e. 1 in a health facility, and 4 in correctional services facilities. The most common risk factor for EPTB was HIV co-infection (88.8%). The median CD4 cell count was 68(IQR 32-165). Less than half (42.5%) of patients were on antiretroviral therapy prior to the diagnosis of EPTB. Tobacco use was reported in 44(55%) patients. Smoking and alcohol use was more common in males (63.2%). Chronic kidney disease, diabetes mellitus, malignancy and immunosuppressive therapy contributed minimally to the overall risk factors for EPTB.

Pleural (36.3%), lymph node(28.8%) and abdominal(27.5%) involvement were the most common. Fifteen (18.8%) patients had tuberculosis diagnosed at more than one anatomical site. Although the site of involvement between males and females were fairly evenly distributed in the HIV infected population, the presence of pleural involvement in females(40%) was more frequent than that in males(29%). In the HIV uninfected/unknown population, abdominal involvement was the only site that was involved in females.

In the subgroup of patients who had confirmed pulmonary and other site infection, 4 of the 5 patients were infected with HIV. Abdominal involvement was the most common in this subgroup (3 of the 5 patients). The remaining 2 patients had pericardial and pleural involvement.

Table 1. Distribution of extrapulmonary tuberculosis at KEH during the study period

Characteristic	Total Population N=80		HIV Positive n=71		HIV Negative/Unknown n=9	
	Male n=38	Female n=42	Male n=31	Female n=40	Male n=7	Female n=2
Age	36.6±8.7	33.1±9.9	35.5±7.0	33.3±10.0	41.0±13.8	28±5.6
< 30 year	7 (18.4)	15 (35.7)	6 (19.4)	14 (35)	1 (14.3)	1 (50)
30 to 39	19 (50)	20 (47.6)	17 (54.8)	19 (47.5)	2 (28.6)	1 (50)
40 to 49	9 (23.7)	3 (7.2)	7 (22.6)	3 (7.5)	2 (28.6)	0 (0)
50 or older	3 (7.9)	4 (9.5)	1 (3.2)	4 (10)	2 (28.6)	0 (0)
Ethnicity						
Black African	34 (89.5)	42 (100)	30 (96.8)	40 (100)	4 (57.1)	2 (100)
Asian	3 (7.9)	0 (0)	1 (3.2)	0 (0)	2 (28.6)	0 (0)
Caucasian	1 (2.6)	0 (0)	0 (0)	0 (0)	1 (14.3)	0 (0)
Living economy						
Rural	5 (13.2)	4 (9.5)	5 (16.1)	4 (10)	0 (0)	0 (0)
Urban	33 (86.8)	38 (90.5)	26 (83.9)	36 (90)	7 (100)	2 (100)
Employment status/High risk environment						
Unemployed	25 (65.8)	32 (76.2)	21 (67.7)	31 (77.5)	4 (57.1)	1 (50)
Private sector	10 (26.3)	8 (19)	7 (22.6)	7 (17.5)	3 (42.9)	1 (50)
Correctional service	3 (7.9)	1 (2.4)	3 (9.7)	1 (2.5)	0 (0)	0 (0)
Health Care facility	0 (0)	1 (2.4)	0 (0)	1 (2.5)	0 (0)	0 (0)
Past TB infection	8 (21.1)	8 (19)	8 (25.8)	8 (20)	0 (0)	0 (0)
Risk factors for TB						
HIV Co-infection	31 (81.6)	40 (95.2)	31 (100)	40 (100)	-	-
CKD	3 (7.9)	4 (9.5)	1 (3.2)	4 (10)	2 (28.6)	0 (0)
Alcohol /smoking	24 (63.2)	12 (28.6)	19 (61.3)	11 (27.5)	5 (71.4)	1 (100)
Diabetes Mellitus	4 (10.5)	1 (2.4)	1 (3.2)	1 (2.5)	3 (42.9)	0 (0)
Immunosuppressant	2 (5.3)	1 (2.4)	2 (6.5)	1 (2.5)	0 (0)	0 (0)
Low body weight	15 (39.5)	15 (35.7)	14 (45.2)	15 (37.5)	1 (14.3)	0 (0)
Head/Neck Malignancy	1 (2.6)	2 (4.8)	0 (0)	2 (5.0)	1 (14.3)	0 (0)
Mean CD4 cell count#	102.9	120.7	102.9	120.7	-	-
ART Naïve	15 (48.4)	22 (55)	15 (48.4)	22 (55)	-	-
Sites of EPTB						
Only 1 EPTB site	30 (78.9)	35 (83.3)	24 (77.4)	33 (82.5)	6 (85.7)	2 (100)
>1 EPTB site	8 (21.1)	7 (16.7)	7 (22.6)	7 (17.5)	1 (14.3)	0 (0)
Pulmonary*	3 (7.9)	2 (4.8)	2 (6.5)	2 (5.0)	1 (14.3)	0 (0)
Lymph Node	12 (31.6)	11 (26.2)	12 (38.7)	11 (27.5)	0 (0)	0 (0)
Pleura	13 (34.2)	16 (38.1)	9 (29)	16 (40)	4 (57.1)	0 (0)
Bone	1 (2.6)	1 (2.4)	0 (0)	1 (2.5)	1 (14.3)	0 (0)
Abdomen	11 (28.9)	11 (26.2)	9 (29)	9 (22.5)	2 (28.6)	2 (100)
CXR-Miliary Pattern	0 (0)	2 (4.8)	0 (0)	2 (5.0)	0 (0)	0 (0)
Pericardium	3 (7.9)	1 (2.4)	3 (9.7)	1 (2.5)	0 (0)	0 (0)
Meninges	4 (10.5)	4 (9.5)	4 (12.9)	4 (10)	0 (0)	0 (0)
Blood/Bone Marrow	1 (2.6)	1 (2.4)	1 (3.2)	1 (2.5)	0 (0)	0 (0)

Data expressed as n(%) or as Mean ± Standard deviation;

cells/mm^3;

KEH - King Edward VIII Hospital; HIV- Human Immunodeficiency Virus; TB- Tuberculosis; EPTB- Extra-Pulmonary Tuberculosis; CKD- Chronic Kidney Disease; ART- Anti-Retroviral therapy; CXR- Chest X-Ray * - Patients had confirmed pulmonary TB and another site of TB infection

Table 2. Distribution of sites of EPTB in the HIV co-infected subpopulation (n=71) according to CD 4 cell count range and gender.

Characteristic n (%)	CD_4 cell count less than 100		CD_4 cell count more than 100	
	Male	Female	Male	Female
Age	35.5±6.2	35.4±11.5	35.5±8.7	29.9±5.8
Only 1 extrapulmonary	15 (71.4)	19 (76.0)	9 (90.0)	14 (93.3)
> 1 extrapulmonary site	6(28.6)*	6 (24.0)*	1 (10.0)	1 (6.7)
Pulmonary**	1 (4.8)	2 (8.0)	1 (10.0)	0 (0.0)
Lymph Node	10 (47.6)	6 (24.0)	2 (20.0)	5 (33.3)
Pleura	5 (23.8)	10 (40.0)	4 (40.0)	6 (40.0)
Bone	0 (0)	0 (0.0)	0 (0.0)	1 (6.7)
Abdomen	8 (38.1)	7 (28.0)	1 (10.0)	2 (13.3)
CXR with miliary Pattern	0 (0)	0 (0)	0 (0.0)	2 (13.3)
Pericardial	1 (4.8)	1 (4.0)	2 (20.0)	0 (0.0)
Meninges	3 (14.3)	4 (16.0)	1 (10.0)	0 (0.0)
Blood or Bone Marrow	0 (0)	1 (4.0)	0 (0.0)	0 (0.0)

SD- Standard Deviation; EPTB- Extrapulmonary tuberculosis; CXR-Chest X-ray.
*- Indicates p value <0.05 when compared to group of patients with CD4 cell counts greater than 100 cells/mm^3 .using independent student T-test.
** - Patients had confirmed pulmonary TB and another site of TB infection
Data expressed as n(%) or as Mean ± Standard deviation

Patients with CD4 cell count less than 100 cells/mm^3 presented more frequently with EPTB involving more than one site ($p < 0.05$) as compared to patients with CD4 cell counts with at least 100 cells/mm^3.(Table 2)

On analysis of symptoms (Table 3) with which patients presented, constitutional symptoms were the most frequent. Unintentional weight loss and fever were the predominant symptom regardless of site of disease. Night sweats was present in almost half of the cases regardless of the system affected except for the patients with bone involvement. Using the Pearson Chi Square test to evaluate for differences between symptoms in patients affected by TB at the different sites and those unaffected we found that even though weight loss and fever were the most frequent complaints, it demonstrated poor correlation with any specific disease site and there was no significant difference when these symptoms were compared to patients with the different sites of TB. A few symptoms were specific for the system affected. Lymphadenopathy was only reported with TB lymphadenitis, as was joint symptoms limited to bone involvement. Respiratory symptoms like cough, dyspnoea and chest pain were predominant in pulmonary and pleural involvement but also in TB of other sites except bone involvement. Headache, photophobia and confusion were significant symptoms in TB meningitis. In patients with abdominal involvement, weight loss was significantly more frequent (86.4%). As expected, other gastrointestinal symptoms like abdominal pain, diarrhoea and vomiting were also statistically significant in patients with abdominal TB.

As expected the clinical signs correlated to the site of EPTB (Table 4). Significantly, all cases of TB involving the lymphatic system had lymphadenopathy. Similarly, all cases of meningeal involvement

had meningism. Hypotension was a significant feature in pulmonary involvement. Peritonitis, ascites and hepatomegaly also showed significance in cases of TB abdomen.

Table 3. Reported symptoms according to the sites of tuberculosis infection

Symptom N(%)	Pulmonary n=5	Lymphatic n=23	Pleura n=29	Bone n=2	Abdomen n=22	Miliary Pattern on CXR n=2	Pericardium n=4	Meningeal n= 8	Blood/bone marrow n=2	Cohort N=80
Weight Loss	5 (100)	17 (73.9)	17 (58.6)	1 (50)	19 (86.4)*	1 (50)	2 (50)	3 (37.5)	1 (50)	53 (66.3)
Fever	3 (60)	16 (69.6)	16 (55.2)	1 (50)	9 (40.9)	1 (50)	3 (75)	6 (75)	2 (100)	46 (57.5)
Night Sweats	2 (40)	15 (65.2)	15 (51.7)	0 (0)	8 (36.4)	1 (50)	3 (75)	1 (12.5)*	0 (0)	39 (48.8)
LN enlargement	0 (0)	15 (65.2)*	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	15 (18.8)
Cough	4 (80)*	5 (21.7)	17 (58.6)*	0 (0)	4 (18.2)	2 (100)	3 (75)	2 (25)	1 (50)	29 (36.3)
Haemoptysis	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.5)
Dyspnoea	1 (20)	1 (4.3)	13 (44.8)*	0 (0)	1 (4.5)	2 (100)*	4 (100)*	2 (25)	0 (0)	19 (23.8)
Chest Pain	1 (20)	0 (0)	12 (41.4)*	0 (0)	0 (0)	1 (50)	3 (75)*	0 (0)	0 (0)	16 (20)
Body swelling	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	1 (1.3)
Headache	1 (20)	1 (4.3)	2 (6.9)	0 (0)	2 (9.1)	0 (0)	1 (25)	7 (87.5)*	1 (50)	8 (10)
Photophobia	0 (0)	0 (0)	2 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)	5 (62.5)*	1 (50)	5 (6.3)
Confusion	1 (20)	1 (4.3)	1 (3.4)	0 (0)	2 (9.1)	0 (0)	0 (0)	4 (50)*	1 (50)	5 (6.3)
Paraparesis	0 (0)	0 (0)	0 (0)	1 (50)*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Abdominal Pain	2 (40)	2 (8.7)	0 (0)	0 (0)	16 (72.7)*	1 (50)	0 (0)	0 (0)	1 (50)	17 (21.3)
Diarrhoea	0 (0)	4 (17.4)	1 (3.4)	0 (0)	11 (50)*	0 (0)	0 (0)	1 (12.5)	1 (50)	13 (16.3)
Vomiting	0 (0)	1 (4.3)	1 (3.4)	0 (0)	8 (36.4)*	0 (0)	0 (0)	1 (12.5)	0 (0)	9 (11.3)
Joint Pain/swelling	0 (0)	0 (0)	0 (0)	1 (50)*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)

CXR – Chest X-ray; LN – Lymph Node

*- Indicates p value <.05 comparison between symptoms in patients affected by TB at the different sites using Pearson Chi Square test

Data expressed as n(%) or as Mean ± Standard deviation

Table 4. Clinical signs according to site of tuberculosis infection

Sign N(%)	Pulmonary n=5	Lymphatic n=23	Pleura n=29	Bone n=2	Abdomen n=22	Miliary Pattern on CXR n=2	Pericardium n=4	Meningeal n= 8	Blood/Bone Marrow n=2	Cohort N=80
Tachycardia	4 (80)	12 (52.2)	8 (27.6)	2 (100)	13 (59.1)	2 (100)	4 (100)	5 (62.5)	1 (50)	39 (48.8)
Hypotension	4 (80)*	2 (8.7)	0 (0)	2 (100)	3 (13.6)	1 (50)	1 (25)	1 (12.5)	1 (50)	8 (10)
Pyrexia	1 (20)	5 (21.7)	6 (20.7)	1 (50)	10 (45.5)	2 (100)	1 (25)	4 (50)	1 (50)	25 (31.3)
Lymphadenopathy	2 (40)	23 (100)*	5 (17.2)	0 (0)	8 (36.4)	1 (50)	1 (25)	2 (25)	0 (0)	33 (41.3)
Anaemia	2 (40)	5 (21.7)	11 (37.9)	0 (0)	5 (22.7)	1 (50)	4 (100)	5 (62.5)	1 (50)	25 (31.3)
Clubbing	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Anasarca	0 (0)	0 (0)	1 (3.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Meningism	1 (20)	1 (4.3)	2 (6.9)	0 (0)	2 (9.1)	0 (0)	1 (25)	8 (100)*	1 (50)	9 (11.3)
Consolidation	1 (20)	1 (4.3)	1 (3.4)	0 (0)	2 (9.1)	0 (0)	0 (0)	1 (12.5)	1 (50)	5 (6.3)
Pleural Effusion	0 (0)	1 (4.3)	28 (96.6)*	0 (0)	1 (4.5)	0 (0)	1 (25)	3 (37.5)	1 (50)	29 (36.3)
Peritonitis	0 (0)	0 (0)	0 (0)	0 (0)	6 (27.3)*	0 (0)	0 (0)	0 (0)	0 (0)	6 (7.5)
Abdominal Mass	1 (20)	0 (0)	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Ascites	0 (0)	0 (0)	1 (3.4)	0 (0)	4 (18.2)*	1 (50)	0 (0)	0 (0)	0 (0)	5 (6.3)
Hepatomegaly	0 (0)	2 (8.7)	0 (0)	0 (0)	7 (31.8)*	0 (0)	0 (0)	1 (12.5)	0 (0)	7 (8.8)
Gibbus	0 (0)	0 (0)	0 (0)	1 (50)*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Arthritis	0 (0)	0 (0)	0 (0)	1 (50)*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)

CXR – Chest X-Ray

*- Indicates p value <.05 comparison between signs in patients affected by TB at the different sites using Fishers Exact test

Data expressed as n(%) or as Mean ± Standard deviation

A microbiologically proven diagnosis was made in only 52(65%) cases of EPTB. In 53(66.25%) cases the diagnosis was made using a combination of microbiological, radiological and chemistry data. (Table 5)

Table 5. Special investigations used to diagnose EPTB at King Edward VIII Hospital

Method of diagnosis	*n (%)*
Radiology*	28 (35)
Microbiology**	52 (65)
Chemistry***	38 (47.5)
Microbiology plus Chemistry	32 (40)
Microbiology plus Radiology	17 (21.25)
Radiology plus Chemistry	4 (5)

Data expressed as n(%), n = number of tests conducted

*- Includes Chest X-rays, Computer tomography, ultrasound.

**- Includes microscopy, culture and Xpert MDR/Rif.

***- Includes exudative pleural fluid and ascites assessment by lights criteria; Adenosine Deaminase levels

Cerebrospinal fluid and pleural fluid were sent for Xpert MTB/Rif assessment. A positive Xpert result supported the diagnosis of TB meningitis in five (62.5%) out of the eight cases. In cases of pleural tuberculosis, Xpert MTB/Rif supported the diagnosis in one (3.8%) of twenty seven cases.

The diagnosis of TB pleura was supported by the presence of an exudative pleural effusion with an elevated pleural fluid ADA in most cases. The median total protein on pleural fluid assessment was 57 mmol/L (IQR 52-62 mmol/L); median LDH 985 mmol/L (IQR 637-1252 mmol/L) and median ADA was 45 U/L (IQR 40-57 U/L).

Discussion

This study provides a description of patients with EPTB in a high TB/HIV burden setting more than a decade into widespread access to ART. Patients with EPTB in this setting were predominantly young (34.73 ±9.44) years and female (52.5%). This is different to settings of low HIV prevalence, where TB continues to predominantly affect the children and the elderly. The increased burden of TB among young adults is consistent with the findings in other high HIV burden settings, where the HIV has been described as the key driver of TB[51, 52]. Similarly, the significant representation of women in this group of patients with EPTB is likely a result of the high HIV prevalence in young women in our setting[53, 54]. Studies in Turkey and Brazil have confirmed similar prevalence rates amongst women, as a result of their disproportionate burden of HIV due to biological and systematic factors[8, 55].

The vast majority of patients were black African(96%) and unemployed (71.2%) reflecting both the contribution of poor socio-economic circumstances to the risk of TB, and the differential utilisation of public hospitals along racial lines in our setting. According to South African Census 2011, the drainage area of our facility consists of 76% black Africans, however black African patients make up more than 90% of hospital inpatients[2]. The majority of these patients live either in peri-urban informal settlements or underdeveloped urban living areas created during the period of racial segregation. The living conditions in these areas have been demonstrated to promote the spread of tuberculosis[56, 57].

HIV co-infection was the leading risk factor (88.8%) for EPTB in this study. Despite the widespread availability of ART in our health district, and the availability of HIV services at this facility, only 42.5% of patients with HIV were on ART. Importantly, all patients with HIV had a CD4 cell count below the threshold for initiation of ART at the time of the study. The mean CD4 cell count in patients with HIV was 68 cells/mm^3 overall and 106 cells/mm^3 (IQR 60-188) in those patients on ART. This was in keeping with well-established association between EPTB and low CD4 cell counts. The presentation of patients with advanced untreated HIV is concerning in this setting, where the threshold for initiation of ART was 500cell/mm^3 at the time of the study[58]. It remains uncertain whether the move to universal ART access together with a test and treat strategy will result in the anticipated reduction in the burden of TB[59, 60].

In keeping with epidemiological data from other settings, involvement of the lymph nodes (23%), pleura (29%) and abdomen (22%) constituted the majority of extrapulmonary sites of disease. With the paucity of screening studies for EPTB and the absence of an adequate diagnostic tool, it is difficult to evaluate the true prevalence of disease or accurately assess the anatomical distribution of disease. This diagnostic bias is unlikely to be resolved without the development of improved diagnostic tools. Tuberculosis involving the haematological system often requires an invasive diagnostic strategy including a bone marrow biopsy which may not be easily available or accessible in resource limited settings. The diagnosis of bone marrow involvement is often made presumptively in patients with haematological abnormalities in the presence of proven TB at other sites. Similarly for sites of TB involvement other sites which require an invasive diagnostic strategy, there is often under-reporting in favour of sites of involvement requiring a less invasive diagnostic strategy. The diagnosis of EPTB is often secured by identifying involvement of one extrapulmonary site, which favours the diagnosis of disease at sites that are most amenable to low technology, minimally invasive diagnostic modalities. Only 15 patients (18.75%) had the diagnosis of TB confirmed at more than one anatomical site in this study. A CD4 cell count of less than 100 cells/mm^3 demonstrated a significantly higher frequency of more than 1 site of EPTB. Karstaedt et al. performed a retrospective study of EPTB at a regional hospital in Johannesburg, South Africa, in the pre-ART era which revealed a similar predominance of pleural TB(39.1%) and lymph node TB(31%) among their patients. Significantly, in that study abdominal TB comprised only 2.9% of all cases of EPTB, as compared to 22% in our study[18]. The inclusion of only culture-confirmed disease in that study, systematically excluded sites of involvement, such as abdominal TB, in which the diagnosis is often made using other diagnostic modalities. By including cases of EPTB based on the clinical diagnosis of EPTB, we provide a description of EPTB that more closely reflects our clinical encounter with the disease. We recognize the inherent limitation of this approach in possibly including patients who were misdiagnosed with EPTB. This is a real concern in our setting where a retrospective study showed that 18 out 21 patients diagnosed with lymphoma over a 4 month period were incorrectly diagnosed with TB in the preceding 12 months[61].

Large retrospective series have been unable to make proper comparisons between features of EPTB because of the protean manifestations of the disease. In this study, weight loss (66.3%), fever (57.5%), night sweats (48.8%) and cough (29%) were the most common symptoms across all sites of disease. A study in Brazil found similar non-specific symptoms in their patients with EPTB. The differential diagnosis for the presence of these constitutional symptoms is broad, but remains an important signal for the diagnosis of TB in a high TB/HIV burden setting. While symptoms and signs are useful in directing further investigation to establish the site of disease, there is a paucity of data on discriminatory clinical features which may be used to support the diagnosis of TB over other pathologies at these sites.

A microbiologically confirmed diagnosis was made in only 65% of cases in this study. In the remaining cases, other laboratory data together with radiological studies were necessary to establish the diagnosis of EPTB. This reliance on laboratory and radiological data adds complexity to the diagnosis of the disease in resource limited settings where such facilities are not widely available or accessible. Due to the

retrospective design of the study, some of the microbiological confirmation was not available to the attending clinician at the time of initiating therapy for EPTB. The clinician's decision to initiate treatment was based on the presentation of the patient and a high index of suspicion given the high prevalence in our study community. It is important to note that 35% of patients were started on therapy without ever having a confirmed microbiological diagnosis. The outcome of these patients are not known because it lies outside the parameters of this study. This does however highlight the possibility of other illnesses as causes of the patient's initial presentation[57].

Use of Xpert MDR/Rif technology has improved the diagnosis of pulmonary TB. Its role in the diagnosis of EPTB is still being evaluated, but the available data is promising. Pleural and cerebrospinal fluid specimens are consistently sent for Xpert MDR/Rif analysis at our facility. Xpert MDR/Rif testing of cerebrospinal fluid has been shown to be a useful rule-in test in other studies in our setting. Our experience with the use of Xpert MDR/Rif in pleural fluid analysis is similar to the available data which suggests a limited role for the test in pleural fluid. In contrast, evaluation of pleural fluid ADA has emerged as a useful diagnostic study for suspected pleural involvement. In a high TB burden setting, pleural biopsy for suspected pleural TB has been largely obviated by the use of ADA[62].

Limitations of study

This study is limited by its retrospective design. The sample size is small and the study period is only three months. Long term follow up and outcomes of the patients are not known as they lie outside the study design parameters. The diagnosis of EPTB was not always confirmed by gold standard i.e. culture. This allows for the possibility of alternate diagnosis.

Conclusion

Extrapulmonary TB is a common disease entity in this high TB/HIV burden setting. The clinical entity comprises a heterogeneous group of diseases described primarily by the anatomical site of involvement. In patients with a CD4 cell count of less than 100 cells/mm^3, clinicians should have a high index of suspicion of more than one organ system involvement. The clinical presentation of EPTB is protean, and establishing the diagnosis presents significant challenges in resource limited settings. The current screening tools used in primary health care facilities and hospitals in South Africa only screen for pulmonary, lymphatic and pleural involvement[63]. A new fully integrated screening tool is needed in our setting given the high prevalence of abdominal TB and TB meningitis. Hopefully with a screening tool that is able to include both pulmonary and EPTB involvement, in high risk groups, early detection and treatment will lead to a decrease in morbidity and mortality. Screening tools may still miss EPTB, because of the varying presentation of the disease. The lack of diagnostic tools contributes to this dilemma, and future developments in molecular based technology may improve our ability to appropriately diagnose the disease. The use of available technologies requires greater evidence based direction. Due to the reliance on non-microbiological diagnostic tests for EPTB, the doubt of the diagnosis of EPTB is always a possibility. HIV continues to fuel the TB epidemic in this part of the world, and it remains to be seen whether the increased public health efforts in HIV will translate to a reduced burden of TB. Addressing the burden of EPTB will require our ongoing high index of suspicion and the use of innovative screening and diagnostic strategies.

Conflict of interest

I declare that I have no financial or personal relationship(s) which may have inappropriately influenced me in writing this paper.

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Appendix 1: Study Protocol

Extra-pulmonary tuberculosis at King Edward VIII Hospital: A retrospective descriptive study

ABSTRACT

Background

Extra-pulmonary TB has the highest prevalence in KwaZulu Natal according to the 2006 South African statistics. With freely available anti-retroviral drugs, it is believed that this prevalence may have decreased. The diagnosis of extra-pulmonary TB has also posed a challenge, but with the development of better imaging and laboratory testing it is believed that there is improvement in the ability to diagnose extra-pulmonary TB. This study will look at the profile of patients with extra-pulmonary TB at King Edward VIII hospital. It will also correlate the presenting symptoms with the site of clinical manifestation of the disease. The aim is to describe the common pattern of clinical presentations of extra pulmonary TB. The study will hopefully assist clinicians to identify patients with extra-pulmonary TB earlier and thus initiate treatment sooner.

Objectives:

- Determine the incidence of extra-pulmonary TB at King Edward VIII hospital
- Determine the most common site of extra-pulmonary tuberculosis
- Determine the symptoms correlating with the site of extra-pulmonary TB tuberculosis
- Determine the signs correlating with the site of extra-pulmonary TB tuberculosis
- Determine the immune status of patients with extra-pulmonary TB, and determine if there is correlation with CD4 levels.

Study Design:

- Retrospective descriptive study

Setting/Participants:

- Single site: King Edward VIII Hospital
- Patients registered on the TB register at King Edward VIII Hospital
- Age: 12 years and older
- Period of study: 1st January 2016 to 31st March 2016

Study Interventions and Measures:

- Review of TB register
- Review of medical records
- Measures: As per data collection sheet attached

1 BACKGROUND INFORMATION AND RATIONALE

In South Africa, tuberculosis (TB) remains the major cause of mortality (Statistics South Africa, 2013). Globally in 2013, nine million people developed tuberculosis, of which 1.5 million demised. In the same year, there were 328 826 case reports of tuberculosis in South Africa, 37 709 of these cases were extra-pulmonary tubeculosis. (World Health Organization, 2014)

Tuberculosis describes many clinical illnesses caused by *Mycobacterium tuberculosis. M. tuberculosis* is an aerobic, non-spore-forming, non-motile bacillus.

It has a high cell wall content of high-molecular-weight lipids. It is slow growing and generation time is 15 to 20 hours. Visible growth on solid media takes from 3 to 8 weeks. Infections with *M. tuberculosis* are due to inhalation of droplet nuclei. Droplet nuclei remain suspended for long periods in the air, approximately 30 minutes after being occupied by a person with pulmonary tuberculosis. (Mandell, Bennet, & Dolin, 2010).

Extra pulmonary tuberculosis is most common in immunocompromised patients. Common sites of infection include pleura, central nervous system, lymphatic system, genitourinary system and muscular skeletal system. Diagnosis of extra pulmonary tuberculosis remains elusive and difficult. (Marjorie & Holenarasipur, 2005). Microbiological and radiographic advancements have assisted in improving diagnosis of extra pulmonary TB.

KwaZulu Natal has the highest prevalence of TB cases in the country. Of all the cases of reported TB in KwaZulu Natal in 2006, 16 424(15.7%) cases were of miliary tuberculosis.

TB is the most common HIV related disease in the world. The presentation of the disease may be related to the level of immunosuppression of the patient. Extra pulmonary TB is generally noticed at a fairly advanced stage. In the early days of the HIV epidemic, studies suggested that extra pulmonary TB was more common in HIV infected individuals. Overall pulmonary TB is more common than extra pulmonary TB in all HIV infected patients (Chakraborty & Chakraborthy, 2000). Since 2004, anti-retroviral drugs have been freely available in the public sector. With more than a decade of antiretroviral treatment more than 2.4 million people now have access to life saving medication.

Extra-pulmonary tuberculosis, especially invasive disease, presents more commonly in patients with HIV infection. The rate of progression is markedly different among individuals with advanced HIV and CD4 counts of less than 250 cells/mm^3. Symptoms may be subacute or acute with rapid progression and death. (Martinson & Hoffmann, 2011). This study will describe the CD4 level and the manifestation of extra-pulmonary tuberculosis. It is expected that the patients in the study should have CD4 levels lower than 250 cells/mm^3.

This study aims to describe the prevalence of extra-pulmonary TB at King Edward VIII hospital in the setting of a decade of anti-retroviral drugs. The study will describe the common sites of extra-pulmonary tuberculosis. It aims to correlate the signs and symptoms with the site of tuberculosis infections.

The study will benefit local clinicians in assisting with earlier diagnosis and treatment of a life threatening illness.

2 STUDY OBJECTIVES

2.1 Aim of study

- Describe the profile of extra-pulmonary tuberculosis at King Edward VIII hospital

2.2 Specific objectives

- Determine the incidence of extra-pulmonary tuberculosis at King Edward VIII hospital
- Assess the most common site of extra-pulmonary tuberculosis at King Edward VIII hospital
- Determine the common presenting complaint correlating with the site of extra-pulmonary tuberculosis
- Determine the common presenting signs correlating with the site of extra-pulmonary tuberculosis

- Assess the immune status of patients with extra-pulmonary TB, and determine if there is correlation with CD4 levels.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This study is a retrospective descriptive study of patients diagnosed with extra-pulmonary TB

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Date Range of Study

The study will enroll all patients 18 years and older diagnosed with extra-pulmonary TB from the 1st of January 2016 to 31st March 2016. The source of the enrollment will be the TB register at King Edward VIII Hospital. Data will be correlated with information from patient's clinical records

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at a single site i.e. King Edward VIII hospital

The number of patients enrolled in the study will be determined by the number of patients found to have extra pulmonary TB over the study period. The estimated number of patients diagnosed with tuberculosis at King Edward VIII Hospital during the study period were 287. This estimation was taken from the admission books of the medical wards.

3.3 Study Population

3.3.1 Inclusion Criteria

Patients diagnosed with extra pulmonary TB

Patients 12 years old and over at time of diagnosis

Both Males and females will be enrolled in the study

Inpatients and outpatients will be included

3.3.2 Exclusion Criteria

Patients not registered on the TB register

Patients with pulmonary TB only

3.3.3 Study Procedures

The study procedures are limited to review of existing medical records.

3.4 Data Sources

3.4.1 Case ascertainment

Each case will be filled onto a data sheet to extract relevant information. The data sheet is attached. (Annexure A). Each patient will be given a study number that will be used to identify the patients record. This will also ensure patient confidentiality during the data analysis.

All patients with extra-pulmonary TB will be enrolled once into the study. Patients who have both pulmonary and an extra-pulmonary site, will be classified as extra-pulmonary TB.

3.4.2 Data sources

The data will be sourced from the TB register and the patient's hospital file.

3.5 Data Elements to Abstracted

3.5.1 Demographics

- Age
- Race
- Sex
- Hospital number

3.5.2 Site of disease

- CNS manifestation as in TB Meningitis
- Lymph node manifestation as in TB lymphadenitis
- Pleural space manifested as TB pleural effusion
- Bone manifested TB spine/TB bone
- Abdomen manifested as TB abdomen
- Miliary TB on Chest X-ray
- TB at other sites, other than pulmonary TB

3.5.3 Clinical Symptoms

- Headache
- Photophobia
- Enlargement of lymph nodes
- Abdominal pain
- Diarrhea
- Vomiting
- Weight loss
- Fever
- Cough – Productive versus non productive
- Hemoptysis
- Bone pain

- Para paresis

3.5.4 Signs

- **Meningism**
- **Lymphadenopathy**
- **Hepatomegaly**
- Consolidation
- Peritonitis
- Clinical features of pleural effusion e.g. Stoney dullness
- Gibbus
- Other signs

3.6 Limitations of study

The study will enroll patients from the TB register at King Edward VIII hospital. There are many problems with data collection of TB statistics in South Africa. In a study in Dr. JS Mooka district, missing information, incorrect filling and coding of patients were highlighted as a major source of inaccurate data reporting. (Matji, January 2007) This will be a major limitation of this study. To overcome some of the data recording errors, patient's clinical files will be reviewed to correlate with the information filled into the TB register.

4 STATISTICAL CONSIDERATIONS

4.1 Primary and Secondary Endpoints

Site of Extra-pulmonary TB

Clinical manifestations of patients diagnosed with extra-pulmonary TB

4.2 Measures to Avoid Bias

The data will be analyzed by a trained statistician.

King Edward VIII is an academic/training hospital. The diagnosis is determined by trained/experienced clinicians.

4.3 Statistical Methods

The study is purely descriptive and the data will be summarized using descriptive measures. Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

Student's t-test will be used for univariate analysis of normally distributed continuous variables. Chi-squared test will be used for categorical variables.

4.4 Sample Size

Determined by the number of patients diagnosed with extra pulmonary TB during the study period. The estimated number of patients diagnosed with tuberculosis at King Edward VIII Hospital during the study period were 287. This estimation was taken from the admission books of the medical wards.

5 STUDY ADMINISTRATION

5.1 Data Collection and Management

Patients names will not be recorded on the data sheet. Each data sheet will include a study number for the participant. This will ensure confidentiality is maintained. Once the data sheets are completed using the TB register, the patients file will be traced using the patient's hospital number. The data from both the TB register and the patients file will be correlated to asses if the register was filled in correctly. The rest of the data sheet will be completed on the hospital premises. No files will leave the hospital. All data and records generated during this study will be kept confidential. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Patients with extra-pulmonary TB will be enrolled once into the study if they have more than one site of disease. Patients who have both pulmonary and an extra-pulmonary site, will be classified as extra-pulmonary TB.

5.2 Ethical Considerations

The study team will apply for expedited ethical permission from BREC. The study is purely a descriptive study and will not impact on the current or future care of the patient. The study will only review the TB register and patient's charts. There will be no contact with patients directly. The benefit of the study is that it may improve clinician's ability to diagnose or suspect extra-pulmonary TB earlier.

5.3 Informed Consent/Assent and HIPAA Authorization

The study team will request consent from the hospital manager to use medical records. Informed consent from patients is not required since there is no interaction with patients. The study is purely a descriptive study.

6 REFERENCES

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Appendix 2: The Guidelines for Authorship for the Journal selected for submission of the manuscript: South African Family Practice

Title page: All articles must have a title page with the following information and in this particular order: Title of the article; surname, initials, qualifications and affiliation of each author; The name, postal address, e-mail address and telephonic contact details of the corresponding author; at least 5 keywords. Please do not use capital letters only for headings and names, but stick to the normal use of capital letters.

Abstract. All articles should include an abstract. The structured abstract for an Original Research article should be between 200 and 250 words and should consist of four paragraphs labelled "Background, Methods, Results, and Conclusions".

Only the abstract of Original Research articles will be published in print, and the abstract with the full article will be published online. It should briefly describe the problem or issue being addressed in the study, how the study was performed, the major results, and what the authors conclude from these results.

The abstracts for other types of articles should also be no longer than 250 words and need not follow the structured abstract format.

Keywords. All articles should include keywords. Up to five words or short phrases should be used. Use terms from the Medical Subject Headings (MeSH) of Index Medicus when available and appropriate. Key words are used to index the article and may be published with the abstract.

Acknowledgements. In a separate section, acknowledge any financial support received or possible conflict of interest. This section may also be used to acknowledge substantial contributions to the research or preparation of the manuscript made by persons other than the authors.

References. Cite references in numerical order in the text, in **superscript** format. Do not use brackets. In the References section, references must be numbered consecutively in the order in which they are cited, not alphabetically.
Abbreviations for **journal titles** should follow *Index Medicus* format. Authors are responsible for the accuracy of all references. Personal communications and unpublished data should not be referenced. If essential, such material should be incorporated in the appropriate place in the text. List all authors when there are six or fewer; when there are seven or more, list the first three, then ";et al.";

When citing URLs to web documents, place in the reference list, and use following format: Authors of document (if available). Title of document (if available). URL. (Accessed [date]).

The following are sample references:

1. London L, Baillie R. Notification of Pesticide Poisoning: Knowledge, Attitudes and Practices of Doctors in the Rural Western Cape. S A Fam Pract 1999;20(1):117-20.
2. FDA Talk Paper: http://www.fda.gov/bbs/topics/ANSWERS/2002/ANS01151.html (Accessed 04/10/2002).

Tables. Tables should be self-explanatory, clearly organised, and supplemental to the text of the manuscript. Each table should include a clear descriptive title on top and numbered in Roman numerals (I, II, etc) in order of its appearance as called out in text. Tables must me inserted in the correct position in the text. Authors should place explanatory matter in footnotes, not in the heading. Explain in footnotes all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *,†,‡,§,‖,,**,††,‡‡

Figures. All figures must be inserted in the appropriate position of the electronic document. Symbols, lettering, and numbering (in Arabic numerals e.g. 1, 2, etc. in order of appearance in the text) should be placed below the figure, clear and large enough to remain legible after the figure has been reduced. Figures must have clear descriptive titles.

Photographs and images: If photographs of patients are used, either the subject should not be identifiable or use of the picture should be authorised by an enclosed written permission from the subject. The position of photographs and images should be clearly indicated in the text. Electronic images should be saved as either jpeg or gif files. All photographs should be scanned at a high resolution (300dpi, print optimised). Provision is made to upload individual images on the website as *supplementary files*. Please number the images appropriately.

Permission. Permission should be obtained from the author and publisher for the use of quotes, illustrations, tables, and other materials taken from previously published works, which are not in the public domain. The author is responsible for the payment of any copyright fee(s) if these have not been waived. The letters of permission should accompany the manuscript. The original source(s) should be mentioned in the figure legend or as a footnote to a table.

Review and action. Manuscripts are initially examined by the editorial staff and are usually sent to independent reviewers who are not informed of the identity of the author(s). When publication in its original form is not recommended, the reviewers' comments (without the identity of the reviewer being disclosed) may be passed to the first author and may include suggested revisions. Manuscripts not approved for publication will not be returned.

Ethical considerations. Papers based on original research must adhere to the Declaration of Helsinki on ";Ethical Principles for Medical Research Involving Human Subjects"; and must specify from which recognised ethics committee approval for the research was obtained.

Conflict of interest. Authors must declare all financial contributions to their work or other forms of conflict of interest, which may prevent them from executing and publishing unbiased research. [Conflict of interest exists when an author (or the author's institution), has financial or personal relationships with other persons or organizations that inappropriately influence (bias) his or her opinions or actions.]*

**Modified from: Davidoff F, et al. Sponsorship, Authorship, and Accountability. (Editorial) JAMA 2001: 286(10)*

The following declaration may be used if appropriate: ";I declare that I have no financial or personal relationship(s) which may have inappropriately influenced me in writing this paper.";

Appendix 3: Ethical approvals

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

06 July 2016

Dr S Gounden (203500311)
Discipline of Medicine
School of Clinical Medicine
s23050994@gmail.com

Protocol: Extra-pulmonary tuberculosis at King Edward VIII Hospital: A retrospective descriptive study.
Degree: MMed
***BREC reference* number:** BE283/16

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 05 May 2016.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 01 July 2016 to queries raised on 31 May 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval.**

This approval is valid for one year from **06 July 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **16 August 2016.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: magulan@ukzn.ac.za
cc postgraduate administration: konar@ukzn.ac.za

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
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DIRECTORATE:
Health Research & Knowledge Management

HRKM Ref: 189/16
NHRD Ref: KZ_2016RP49_798

Date: June 2016

Dear Dr S. Gounden
UKZN

Approval of research

1. The research proposal titled '**Extra-pulmonary tuberculosis at King Edward VIII Hospital: A retrospective descriptive study**' was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 21/06/16

health
Department:
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Ref.: KE 2/7/1/(30/2016
Enq.: Mrs. R. Sibiya
Research Programming

10 June 2016

Dr. S. Gounden
Discipline of Medicine
Nelson R. Mandela School of Medicine
UNIVERSITY OF KWAZULU-NATAL

Dear Dr. Gounden

Protocol: "Extra-pulmonary tuberculosis at King Edward VIII Hospital: A retrospective descriptive study.
Degree: MMed; BRC Ref. No. BE283/16

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

SUPPORTED/~~NOT SUPPORTED~~

DR. SA MOODLEY
ACTING SENIOR MEDICAL MANAGER

09/06/16
DATE

Fighting Disease, Fighting Poverty, Giving Hope