Epidemiology of tuberculosis meningitis in an area with a high prevalence of HIV-infection

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DECLARATION

I, Shaakira Chaya declare that this research report is my own work. It is being submitted for the degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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The ……..day of ……., 2014

DEDICATION

I dedicate this work to the most wonderful parents, Haroon and Yasmin Chaya, who always stood behind me. Thanks for all you did. You are always missed.
I also dedicate it to my brother, Riyaad, who has been supportive, understanding and encouraging in my many, many moments of crises; and to all my family and friends who have been supportive.
I will remain grateful for everything you have done and continue to do.

"With time and patience the mulberry leaf becomes a silk gown"
Chinese Proverb

ABSTRACT

Introduction

Mycobacterium tuberculosis meningitis (TBM) is a severe manifestation of extra-pulmonary tuberculosis (EPTB) in children, particularly under 5 years of age. Children are vulnerable to EPTB as they are immunologically immature and unable to contain *Mycobacterium tuberculosis* (MTB) infection in the lung. Common neurological sequelae of TBM include focal motor deficits, vision loss and hydrocephalus. Early stage diagnosis and timeous anti-tuberculosis treatment decreases the case fatality rate of TBM.

Objective

To characterise the burden, clinical presentation, laboratory markers and short-term outcome of TBM in HIV-infected and HIV-uninfected children.

Methods

The electronic databases of admission of children at Chris Hani Baragwanath Academic Hospital (CHBAH), between January 2006 and December 2011 with a diagnosis of TBM were reviewed. Individual patient records were retrospectively reviewed for clinical and laboratory data. In addition, admissions from the neurosurgery wards were also reviewed. In patients whose medical records were unavailable, laboratory data was used.

Results

The overall incidence of TBM in 2006 was 6.96 per 100 000 (95% Confidence Interval [95%CI]: 4.46-10.36), peaked at 9.87 per 100 000 (95% CI: 6.91-13.67) in 2009 and subsequently declined to 3.18 per 100 000 by 2011 (95% CI: 1.64-5.56). There was a 38.6% (95% CI: 10.0-58.0; p=0.011) reduction in the overall incidence of TBM when comparing the period 2006-2009 with the period 2010-2011. This decline was particularly evident in HIV-infected children (49.6% reduction; 95%CI: 1.05-74.35; p=0.042).

There were no differences in the clinical symptoms of TBM or tuberculosis between HIV-infected and -uninfected children. Previous history of TB was significantly higher in HIV-infected children compared to HIV-uninfected children (OR 4.63; 1.40-15.22; p=0.011). Tuberculin skin test positive-reactivity (OR 0.09; 0.02-0.43; p=0.002) and sputum culture positivity (OR 0.29; 0.10-0.86; p=0.025) were less common in HIV-infected compared to -uninfected children. Cerebrospinal fluid cytology and biochemistry results were similar between HIV-infected compared to -uninfected children. Morbidity (22.7% in HIV-infected vs. 33.0% in -uninfected) and mortality (6.4% in HIV-infected vs. 6.9% in -uninfected) were similar between HIV-infected and -uninfected children.

Conclusion

The incidence of TBM has decreased over the study period 2006 to 2011.This decrease was temporally associated with an increase in the uptake of antiretroviral treatment in HIV-infected individuals.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ADA	Adenosine deaminase
AFB	Acid fast bacilli
AZT	Zidovudine
BCG	Bacille Calmette-Guérin
CFR	Case fatality rate
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CT	Computed Tomography
CXR	Chest X-Ray
EPTB	Extra-pulmonary tuberculosis
FTC	Emtracitabine
HAART	Highly Active Antiretroviral Treatment
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
INH	Isoniazid
LP	Lumbar puncture
MTB	Mycobacterium Tuberculosis
PMTCT	Prevention of Mother to Child Transmission
PPD	Purified protein derivative
PTB	Pulmonary tuberculosis

RMPRU	Respiratory and Meningeal Pathogens Research Unit
TB	Mycobacterium tuberculosis
TBM	Tuberculosis meningitis
TNF	Tenofovir
WCC	White Cell Count

1.0 INTRODUCTION

1.1 Background

An estimated one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB) (1). The burden of tuberculosis (TB) mainly affects low-income countries particularly in Asia and Sub-Saharan Africa (2, 3). Of all childhood TB cases, 75% occur among the 22 highest burdened countries, South Africa being one of them (4-6). Paediatric TB cases in South Africa account for approximately 15% of all TB cases in the country (6). Human Immunodeficiency Virus (HIV) infection has contributed significantly to the increased incidence of TB in countries where both HIV and TB are endemic. In 2012, approximately 1.1 million (13%) of the 8.6 million TB cases were HIV-infected, 75% of whom lived in Africa. HIV-infected children have increased risk of developing TB, as well as more rapid TB progression, suboptimal treatment response and an increased risk of recurrence (7). CD4+ cell count depletion enhances susceptibility to TB (8, 9); whilst TB co-infection may promote HIV viral replication which, in turn, further decreases CD4+ cells counts (10). The risk of developing extra-pulmonary tuberculosis (EPTB) is increased 17-fold in HIV-infected individuals, especially in those with low CD4+ counts (11-14).

Tuberculosis meningitis (TBM) is a severe manifestation of EPTB in children (11, 15), particularly those under 5 years of age (16). Children may be vulnerable to disseminated TB, possibly due to an immature immune system, predisposing to being unable to contain the MTB infection to the lung (17). MTB reaches the central nervous system (CNS) through the haematogenous route. Granulomas are produced in the

meninges and adjacent brain parenchyma (Rich focus). Tuberculosis meningitis develops when the Rich focus ruptures and its contents spill into the subarachnoid space, (12) possibly due to a decrease in host-immunity (17). Following rupture, thick gelatinous exudates form in the basilar region of the brain which may block CSF flow resulting in hydrocephalus. Intracranial vessels may also get trapped within the exudates causing cerebral infarction and/or cranial nerve entrapment leading to cranial nerve palsies. The resulting inflammation presents clinically with varying degrees of encephalopathy (12). Common neurological sequelae of TBM include hemiplegia, quadriplegia, cognitive impairment, seizures and cranial nerve palsies in 25%-56% of survivors (9, 18-20).Mortality is about 30% (21). Early diagnosis and treatment have been proven to significantly reduce the risk of developing complications and mortality (19, 22). Signs of TBM are often nonspecific and similar to other bacterial causes of meningitis.

Signs and symptoms suggestive of TBM are divided into 3 stages. Stage 1 is non-specific features such as personality change, irritability, anorexia, listlessness and fever as a result of meningeal inflammation. Stage 2 presents 1 to 2 weeks later with features of increased intracranial pressure and neurological signs and symptoms such as drowsiness, neck stiffness, cranial nerve palsies, vomiting and focal or generalized convulsions (23). Stage 3 is associated with severe neurological deficits such as coma, autonomic instability and a rising fever (17). The long-term neurological morbidity in children with TBM is reported to correlate to the clinical stage of the disease at presentation and the timeliness of anti-TB therapy. The majority (11-61%) of children

with stage 1 TBM have a normal outcome, whereas stage 3 is associated with high case fatality rates (7-57%) and severe neurological sequelae (34-94%) among survivors (24, 24). Motor deficits are observed in 10-25% of children (17, 25). Furthermore, risk factors associated with poor outcome include a young age (<12 months), malnutrition, and an elevated intracranial pressure (17).

The clinical diagnosis of TB requires a high index of suspicion in children. Signs and symptoms of TB may include low grade fever, a chronic cough and poor weight gain (23). These clinical features are supported by a reactive tuberculin skin test (TST) and radiological evidence suggestive of TB**.** The tuberculin skin test (TST) measures the type IV delayed hypersensitivity response to a purified protein derivative (7). The WHO recognises "definite case of tuberculosis" when MTB complex (*M. tuberculosis,* M*. bovis, M. africanum, M. bovis* BCG strain*, M. pinnipedii, M. microti, M. caprae* and *M. canettii*) is identified from a patient sample (26).

The diagnosis of TBM remains challenging as signs and symptoms are non-specific and may overlap with that of other bacterial meningitis (15). Culture of CSF for MTB is the gold standard and studies have reported variable sensitivities (10% to >80%), which may be improved by increasing cerebrospinal fluid (CSF) volume analyzed, thorough microscopic examination and multiple samples being examined (11,12,27). Smear sensitivity on CSF for MTB is less than 10%, (11,12,28) and this is influenced by the volume of CSF collected (ideally 10- 20mls of CSF is required). In the absence of bacteriological confirmation of TBM, a typical CSF biochemical and cell profile includes

lymphocyte predominance, high protein, low glucose and low chloride (11,12,23). In addition to CSF findings, a history of TB contacts, a positive TST, suggestive CXR for pulmonary TB and brain CT scans are adjunctive tools used for clinically diagnosing TBM (17).

HIV associated TBM has both diagnostic and therapeutic challenges (27), coupled with a variable in-hospital case fatality rate in adult patients (63% vs. 18%; OR 7.4:95% CI 3.0-18.5, p=0.000) (29) and when compared to another study showed no difference (27, 30). Exudates in HIV-infected compared to HIV-uninfected adults are minimal, thinner and serous in nature. They contain fewer lymphocytes and epithelial cells (10,31), making the diagnosis more challenging (23). Computed Tomography (CT) scan of the brain in HIV-infected TBM cases shows features that are less specific, with hydrocephalus being uncommon, and ventricular dilatation being secondary to cerebral atrophy from HIV-infection, (10,23) resulting in an atypical radiological presentation of TBM in HIV-infected individuals, which could lead to delayed or missed diagnosis and delayed treatment initiation.

The Bacille Calmette-Guérin (BCG) vaccination provides protection against severe forms of TB such as TBM (64%) (32). It is a live attenuated form of *Mycobacterium bovis*. An estimated 90-94% BCG vaccine coverage was reported in 2009 and 2010 in South Africa (33). There is no significant difference in BCG- vaccinated and -unvaccinated children with respect to clinical presentation, outcome and CT scan findings in children with TBM (34,35). In the South African setting all children including

those born to HIV-infected mothers, receive the BCG vaccination (16). There is limited data on the effectiveness of BCG vaccination in preventing pulmonary TB (8,35,36).

Adenosine deaminase (ADA) is an enzyme required for T lymphocyte proliferation and differentiation. It converts adenosine into ionosine. ADA is released by the activated T cells in response to the MTB bacilli (37,38). The ADA test fails to differentiate between TBM and bacterial meningitis when used alone (39), however, once bacterial meningitis has been ruled out, an ADA value above 9.5 µ/L improves the specificity for diagnosing TBM in adults (40) . Furthermore, among children, ADA between 1-4 µ/L (sensitivity >90% and specificity <80%) helped to exclude TBM, while a value > 8µ/L (sensitivity <59% and specificity >96%) aided in establishing the diagnosis of TBM (21). High CSF ADA values are non-specific and may also be raised in conditions such as lymphoma (32) . Therefore, even though values have been established, ADA does not always help in the diagnosis of TBM as levels may be increased in other conditions as well.

1.2 Literature review

The incidence of TBM in both HIV-infected and -uninfected children remains largely unknown in high HIV/TB burden areas. The possible reasons for this include the inability to confirm TBM on culture, the non-specific biochemical findings and the under reporting in resource constrained settings.

In the pre-HAART era, a study conducted in the Western Cape by Berman *et al.* reported an incidence of TBM from 1985 to 1987 as 10 per 100 000 in children <15

years of age. The incidence of notified TBM stratified by age was reported as 31.5, 17.1, 4.8, 0.7 per 100 000 in children aged <1 year, 1-4 years, 5-9 and 10-14 years, respectively, (13) which was higher than that reported in other countries (0 per 100 000 in France between 1980-1984; 2.4 per 100 000 in Columbia in 1984 in the 0-4 year age group)(13).This could have been an underestimate as not all cases may have been notified. Following this, Hesseling *et al.* reported an estimated population based incidence of culture-confirmed TB in HIV-infected and -uninfected children in the Western Cape, between 2004 and 2006, of which 11% (27 of 245) had TBM. Hesseling et al. estimated the incidence of TBM to being 120 per 100 000 (95% CI: 28-258) in HIV-infected children and 7.9 per 100 000 (95% CI: 5-11) in HIV-uninfected children (16). These estimates likely underestimated the incidence of TBM due to only reporting on culture confirmed cases and some possibly having been missed due to lack of referrals. Furthermore, those children with an unknown HIV status were classified as being HIV-uninfected, which may have further resulted in underestimating the incidence of TBM in HIV-infected children or an overestimation in HIV-uninfected children. Although Asian countries have a high burden of TB, the incidence of TBM in Hong Kong was only reported as 0.369 per 100 000 in the study period 1993 to 2000 (41). TBM is uncommon in high-income countries with an incidence of 0.155 per 100 000 cases across all age groups reported over a two year period in France (42).

Three paediatric studies have compared the clinical presentation, biochemical and radiological features of TBM between HIV-infected and -uninfected children (22,23,28) (Table1). In these studies the mean age ranged from 34-38 months, among HIV-

uninfected children compared to 23-39 months in HIV-infected children (22, 23, 28). Although non-specific to TBM, clinical differences observed between HIV-infected and -uninfected children with TBM included greater frequency of lymphadenopathy, clubbing, hepato-splenomegaly and otorrhoea; all of which could have been attributable to the underlying HIV-infection (23). Also, HIV-infected children with TBM had lower haemoglobin (<8 gm/dl) (28), while significant reactivity of the tuberculin skin test (TST) was more common among HIV-uninfected children (22). These studies, however, had small numbers of HIV-infected children and there were very few (35.5% versus 20.8% (23); 10% versus 6.66% (22)) microbiologically confirmed cultures. In the study by Van der Wreet et al, including 56 HIV-infected and 34 HIV-uninfected children, CSF findings did not differ between the groups and the frequency of culture confirmed MTB was 35.5% in HIV-infected compared to 20.8% in HIV-uninfected children (OR=2.1;95% CI 0.78-5.66). HIV infected children were more likely to have abnormal CXR with hilar lymphadenopathy (60.1%) compared to HIV-uninfected children (37.3%; OR=2.59; 95% CI 1.05-6.37). Brain CT scan findings such as obstructive hydrocephalus was significantly lower in HIV infected (72%) compared to HIV-uninfected children (97.9%; OR=0.06, 95% CI 0.01-0.49), similarly basal enhancement was significantly less common in HIV -infected children (37.5% vs. 71.4 %;) OR=0.24; 95% CI 0.08-0.70) (23).

Table1: Paediatric Studies comparing HIV-infected and HIV-uninfected children with Tuberculosis Meningitis (TBM)

Author	Yr	No. Of cases	Mean Age (mo)	Method of Diagnosis	CSF findings				Radiological Findings				Mortality		
						HIV +	HIV-	p-value		HIV+	HIV-	p-value	HIV+	HIV-	p-value
Topley et al. (22)	1998	30 HIV - 10 HIV+	HIV+ 23.4 HIV- 38.7	Culture or Combination of clinical, CSF findings and TB found at another site [MTB culture on CSF: HIV-: 2/30(6.7%) HIV+: 1/10(10.0%)]	Mean Poly mm^3	18	50	NS	Hydrocephalus	80%	63%	NS	30%	0%	0.010
					Mean Lymphocytes mm^3	219	13	NS	Basal Enhancement.	40%	53%	NS			
					Mean Protein g/dl	4.5	1.8	NS	Tuberculoma	20%	27%	NS			
					Mean Glucose mmol/l	2.0	2.0	NS	Basal ganglia infarcts	40%	30%	NS			
									Cerebral atrophy	40%	17%	NS			
Karande et al. (28)	2005	115 HIV - 8 HIV+	HIV+ 34.5 HIV- 67	Based on a clinical case definition	Cells >100 mm^3	50%	43%	1.000	Hydrocephalus	50%	46%	0.168	12%	23%	0.779
					Protein >1g/dl	50%	60%	0.815	Basal Inflammation	62%	77%	0.597			
									Tuberculoma	25%	22%	0.232			
									Cerebral infarcts	37%	44%	0.993			
								OR (95% CI)				OR (95% CI)			OR (95% CI)
Van der Weer et al. (23)	2006	34 HIV- 56 HIV+	HIV+ 39 HIV- 38	Culture positive or Combination of radiological and clinical findings [MTB culture on CSF and gastric aspirates: HIV+: 35.5% HIV-: 2.0%	Mean Poly mm^3	46	51	0.99 (0.99-1.00)	Hydrocephalus	72%	97%	0.06 (0.01-0.49)	23%	0%	35.6 (1.98-640.2)
					Mean Lymp mm^3	212	178	1.00 (0.00-1.00)	Infarcts	50%	40%	1.47 (0.54-4.04)			
					Mean Protein g/dl	2.4	1.9	1.08 (0.88-1.34)	Basal Exudate	37%	71%	0.24 (0.08-0.70)			
					Mean Glucose mmol/l	2.7	2.0	1.26 (0.87-1.83)							

*__Key:__ Yr: year, Mo: Months, HIV +: HIV infected, HIV-: HIV uninfected, Poly: Polymorphocytes, Lymph: Lymphocytes, OR: Odds ratio.

Of the three studies among HIV-infected children with TBM, one study used only clinical criteria for diagnosing TBM (28), (Table 1) while the other two used a combination of clinical and microbiological criteria (22, 23). Overall, these three paediatric studies showed no difference in CSF biochemistry and CSF cell count findings between HIV-infected and -uninfected children (22, 23, 28) (Table 1). A study conducted among 214 HIV-uninfected children demonstrated that most (80%) children display “typical” biochemistry (lymphocyte predominance, high protein, low glucose) findings on CSF (25) .

Chest X-ray features varied between the three paediatric studies, with no difference in CXR features observed between HIV-infected and -uninfected children with TBM by Karande et al, (28), whereas more abnormalities on CXR’s of HIV-infected children compared to -uninfected children with TBM were reported in the other two studies (22,23). This could, however, been due to non-TB opportunistic and chronic lung conditions such as LIP (lymphocytic interstitial pneumonia) and bronchiectasis in HIV-infected children (23).

CT scan findings in TBM include hydrocephalus, basal meningeal enhancement, infarction and tuberculoma (27) .There is conflicting data as to whether the frequency of meningeal enhancement is less common in HIV-infected children not on HAART compared to HIV-uninfected children (22,23).Furthermore, obstructive hydrocephalus was observed to be less common in HIV-infected compared to -uninfected TBM cases by van der Wreet et al. (23). Thus, the paucity of CT scan changes among HIV-infected

children with TBM may lead to delayed diagnosis, which could contribute to increased mortality (23).

TBM was associated with a higher mortality in HIV-infected compared to -uninfected children, in the studies by Topley et al, (30% vs. 0%; p=0.01) (22) and by van der Wreet et al. (23% vs. 0%; OR 35.60; 95% CI 1.98-640.29) (23). Furthermore, Topley *et al.* reported that 52% of HIV-uninfected children were normal at 6 to 12 months compared to 0% of HIV-infected children (p=0.01) (22), and similarly van der Weert *et al.* reported complete recovery in 60% (33/55) of HIV-uninfected children compared to 29% (10/3%) of HIV-infected children (OR 0.28; 95% CI 0.11-0.69) (22). The study by Karande *et al.* reported that only 19% (22/115) of HIV-uninfected children and 37% (3/8) HIV-infected children made a complete recovery (28).

Among adults with TBM, similar to paediatric patients, most studies did not identify clinical, CSF laboratory cell count or biochemistry differences between HIV-infected and -uninfected adults (43-45). Clinical features of TBM between HIV-infected and -uninfected adults were similar (31,43,46) except that HIV-infected adults had more cognitive impairment (31). There were 2 adult studies that reported a significantly lower CSF protein and cell count in HIV-infected adults with TBM compared to HIV-uninfected adults (29,31) (Table 2). One of these studies showed that HIV-infected adults had decreased pleocytosis and protein compared to -uninfected adults (29), while another study that looked only at HIV-infected adults showed a neutrophil dominance (47). Adult studies also show that ventricular dilatation and infarction are more common in

HIV-infected patients (43), whereas meningeal enhancement and obstructive hydrocephalus are more common among HIV-uninfected adults (31) (Table 2).

Adults who received Highly Active Antiretroviral Treatment (HAART) prior to, or who are started on HAART during treatment for TBM had lower mortality rates (2,48). The majority of adult studies also demonstrated a higher mortality (up to 63%) among HIV-infected adults compared to 17.5%% in HIV-uninfected adults (29, 31). Two studies in adults with TBM, however, demonstrated a trend that surviving cases had fewer neurological sequelae among HIV-infected compared to HIV-uninfected adults (43,49) (Table 2).

Table2: Adult studies comparing HIV-infected and HIV-uninfected adults with Tuberculosis Meningitis (TBM)

Author	Year	No. Of cases	Age Yrs	Method of Diagnosis	CSF findings				Radiological Findings				Mortality		
						HIV +	HIV-	p-value		HIV+	HIV-	p-value	HIV+	HIV-	p-value
Scutte,CM (43)	2001	20 HIV+ 17 HIV-		Definite: Culture positive Probable: TB at another site and clinical, and laboratory features of TB, and improvement on TB treatment	Mean Poly mm^3	69	87	0.62	Abnormal CTB	79%	75%	0.780	40%	41%	0.900
					Mean Lymph mm^3	164	96	0.07	Hydroceph	50%	35%	0.370			
					Mean Protein g/dl	2.88	3.47	0.51	Infarcts	40%	6%	0.010			
					Mean Glucose mmol/l	1.90	1.70	0.66							
					ADA (µ/L)	12.60	13.50	0.67							
Cecchini,D (29)	2008	101 HIV+ 40 HIV -	Median 33	MTB isolated on CSF culture	Cell count Cells/ml	47	167	0.02							
					Pleocytosis (%>4 cells /ml)	80%	85.2%	<0.001							
					Mean Protein g/l	0.77	1.15	0.01							
					Mean Glucose mg/dl	28	23	0.170							
Katrak,SM (31)	2000	22 HIV+ 31 HIV-	Median: HIV + 33 HIV – 33	Definite: Culture confirmed Highly probable: laboratory, improvement on TB treatment, TB form another site Probable: above criteria not met	WCC ($\times 10^{-3}$/ul)	0.14	0.2	0.03	Basal exudate	33%	81%	0.002	36%	9%	0.03
					Mean Protein g/l	1.24	1.75	0.01	Hydroceph	5.5%	63%	<0.001			
					Mean Glucose mmol/l	0.46	0.50	NS	Infarcts	38%	40%	NS			
					AFB + (%)	1	3	NS							

*__Key:__ Yr: year, Mo: Months, HIV +: HIV infected, HIV-: HIV uninfected, CTB: CT brain, Hydroceph: Hydrocephalus

This study, conducted in partial fulfilment of a Masters of Medicine in Paediatrics (MMed) degree, aims to determine the impact of childhood HIV-infection and changes in management thereof on the epidemiology of TBM in Soweto, South Africa. A retrospective review of clinical and laboratory records of children admitted with TBM to Chris Hani Baragwanath Hospital (CHBH) was undertaken.

1.3 Hypothesis and objectives

The aim of this study was to describe the epidemiology, including incidence trends of TBM, associated with improved access to HAART for HIV-infected individuals, in a setting with a high prevalence of paediatric HIV-infection.

Primary objective:

- To determine temporal changes in the incidence of TBM in HIV-infected and HIV-uninfected children between 2006 to 2011.

Secondary objective:

- To compare the clinical and laboratory characteristics of TBM between HIV-infected and HIV-uninfected children

2.0 STUDY DESIGN AND METHODS

2.1 Study design

A retrospective, descriptive study of the incidence and clinical findings of culture-confirmed and all-categories of TBM in HIV-infected and HIV-uninfected children was undertaken for the period January 2006 to December 2011 at Chris Hani Baragwanath Academic Hospital (CHBAH).

2.2 TBM definition

Cases in the study were classified as confirmed, probable or possible TBM based on criteria (see Table 3). Patients who did not fit the criteria of confirmed, probable or possible, due to incomplete records, below were labelled as “undefined”. These patients were included in all the analysis. In addition, the undefined group were extrapolated into the confirmed, probable and possible group based on the percentage of these groups to the overall TBM.

Table 3: Adapted diagnostic criteria used by van Well and van der Weert (23,50) **to diagnose Tuberculosis Meningitis (TBM)**

Confirmed TBM	Probable TBM	Possible TBM
Mycobacterium tuberculosis identified on CSF culture	Signs and symptoms of meningitis and Suggestive CSF finding : lymphocytosis, elevated protein, low glucose(all of these) ***and*** 2 or more of the following -Poor weight gain -Household contact with sputum smear-positive tuberculosis -CT scan compatible with TBM -Chest radiograph compatible with TB -Positive tuberculin skin test* -Other clinical specimens positive for AFB	Patients who were treated empirically without fulfilling the criteria for confirmed or probable TBM

*The TST is performed by injecting 0.1ml of PPD on the left anterior forearm and is read 48 to 72 hours later (i.e. Mantoux method). A positive skin reaction is measured by the transverse diameter of the induration and this is indicative of MTB infection. A positive response is indicated by a diameter of ≥10mm in an HIV-uninfected child and a diameter of ≥5mm in an HIV-infected or severely malnourished child (6). However, any reaction in HIV-infected children is usually considered as significant in this setting. A positive TST does not differentiate infection from disease (6).

2.3 Study population

Chris Hani Baragwanath Academic Hospital (CHBAH) is situated in Soweto, south of Johannesburg and is the only public hospital serving this area. It serves a population of approximately 1.4 million mainly black Africans, including 398 555 children under 15 years of age (51). The area falls under Region D of the Johannesburg metropolis. Medical treatment, including anti-retrovirals and anti-TB drugs is provided for free to all children attending public health care facilities. It is estimated that 90% of all

hospitalisations involving individuals from Soweto would occur at CHBAH, the only public-hospital in the area.

HIV prevalence and management in study population

The prevalence of HIV in Gauteng was estimated to be 18-19% in adults and 4-5% in children during the study period (52). It is estimated that about 65% of the children who were eligible for HAART were on treatment in 2006 in this province compared to 96% in 2011 (52) (Figure 1)

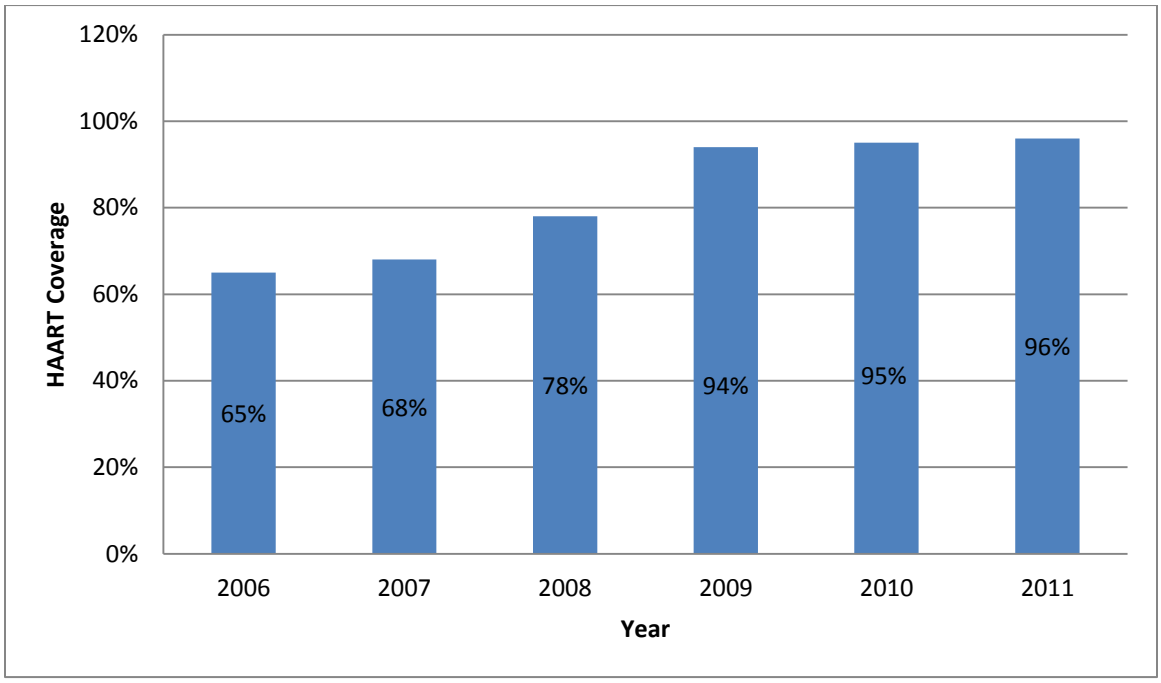

Figure 1: Highly Active Antiretroviral Treatment (HAART) coverage in children age 0-14 years, across study period 2006-2011 in Gauteng, in whom HAART was required (52)

During the study period, the criteria for initiating children on HAART included either recurrent admissions/ prolonged hospital-admissions or WHO stage 3 or 4 disease or if

clinically asymptomatic a CD4 count of <20% in children less than 18 months or <15% in those >18 months age. The first line HAART regimen in children less than 3 years of age included lamivudine, stavudine and lopinavir/ritonavir and lopinavir/ritonavir was substituted for efavirenz in older children (53).

In 2010 the ART-management guidelines were amended in which Abacavir replaced Stavudine as one of the first line anti-retroviral treatments. The eligibility criteria for initiating HAART was changed so enable all children <1 year of age to be initiated immediately on HAART upon confirmation of being HIV-infected, as well as children between 1-5 years with a WHO stage 3 / 4 or CD4 count< 25% (or CD4 count <750 cells/ mm^3) and children older than 5 years with a WHO stage 3 /4 or a CD4 count of <350 cells/mm^3 (54).

The prevention of mother to child transmission programme (PMTCT) started in South Africa in 2001 as a pilot programme in a few selected areas with national implementation in 2004 (55). This initial programme included single doses of nevirapine to the mother during labour and to the newborn following delivery (55). This was changed in 2004 whereby mothers with CD4+ counts <200 cells/mm^3 or WHO stage 4 disease irrespective of CD4 count received HAART during pregnancy and those with CD4+ counts >200 cells/mm^3 received nevirapine during labour. Additionally, newborns of HIV-infected mothers received nevirapine post-delivery (56). In April 2008 the PMTCT programme was further modified with mothers with CD4+ counts <200 cells/mm^3 being recommended to receive HAART and those with CD4+ >200 cells/mm^3

recommended to receive zidovudine (AZT) daily during the pregnancy from 28 weeks. Furthermore, it was recommended that the mother also receives a single dose of nevirapine during delivery and AZT at the onset of labour and on a 3 hourly basis during labour. In addition, recommendations for the HIV-exposed newborn included a single dose of nevirapine at delivery and zidovudine twice daily for 7days or 28 days in mothers who received either greater or less than four weeks of antiretroviral treatment respectively (55). In 2010, all HIV-infected females with a CD4 < 350 cell/ mm^3 or a WHO stage 3 or 4 disease received HAART during pregnancy. Those with a CD4 count of > 350 cell/ mm^3 received AZT from 14 weeks with a single dose of NVP and AZT 3 hourly during labour with a single dose of tenofavir and FTC after delivery (57) .In addition, AZT is discontinued in the newborns, and replaced this with nevirapine from birth to six weeks of life if the mom was on HAART or chose to use formula feed for her child and continued as long as the mother continued to breastfeed and was not on HAART (54). All babies born in Soweto receive the BCG vaccination at birth irrespective of maternal HIV-infection status.

Laboratory Methods

Laboratory methods used by the National Health Laboratory Service (NHLS) for culturing MTB, include CSF being processed using the N-acetyl-L-cysteine-NaOH method. From 2006 onwards the BACTEC™ MGIT 960™ TB System (Mycobacterium Growth Indicator Tube) (Becton Dickinson, Sparks, Maryland) was used.

The CSF cell counts were done using the Fuchs-Rosenthal counting chamber. Protein, glucose and chloride are measured as part of the CSF chemistry profile which use the

Roche Modular® and Cobas® 8000 (Roche Diagnostics, North America, USA) analysers. This is an automated system that utilises reagent specific kits. Typical CSF features of TBM in both adults and children include a leucocytosis of 10-1000 × 10^6 cells/l, predominantly lymphocytes. An elevated protein of >0.5g/l and a decreased CSF glucose to blood ratio of <0.5 is suggestive of TBM (27).

CSF ADA was measured on the Cobas Mira® analyser (Roche Diagnostics, North America, USA) using the kit from (Diazyme® Laboratoriess, Poway, USA). It is based on the deamination of adenosine to ionosine. Ionosine is then converted to hypoxanine by PNP (purine nucleoside phosphorylase) . The amount of adenosine that generates one µmol of ionosine equates to one unit of ADA (58).

CT scan and Chest X-ray findings were based on information documented in the patients medical records, with CXRs generally having been interpreted by the attending clinicians and brain CT scan by radiologists.

The management of TBM at CHBAH included treatment with 4 drugs for 6 months [rifampacin (15-20mg/kg); Isoniazid (15-20mg/kg), Pyrazinamide (30-40mg/kg) and Ethionamide (15-20mg/kg)]. In addition prednisone is given at 2mg/kg for 4 weeks and then tapered over 2 weeks (6). INH prophylaxis has not been part of the national TB guidelines until 2013.

2.4 Study method

A record review of all children admitted to general paediatric wards at CHBAH, with a discharge diagnosis of TBM, between January 2006 and December 2011 was analysed. Cases were identified from an electronic database of paediatric hospitalisations at CHBAH. These were cross referenced with TB notification books and records from the TB centre database. The TB centre database maintains a record of all children notified to have been initiated on TB treatment at CHBAH. Additionally, TBM cases managed in the neurosurgery wards were also identified from admission registries in these wards. Available subject medical-records were reviewed and clinical information extracted. Laboratory data was retrieved from the NHLS database on all patients, including those whose medical records were unavailable. Children with a positive bacterial culture on CSF other than MTB were excluded from the study. Children referred from other hospitals and not resident in Soweto, as well as those who started TBM treatment outside of the study period were excluded (Figure 2).

Approval to conduct the study at CHBAH was obtained from the Medical Advisory Committee at the hospital and the Human Research Ethics Committee (HREC) at the University of Witwatersrand (HREC number: M120542).

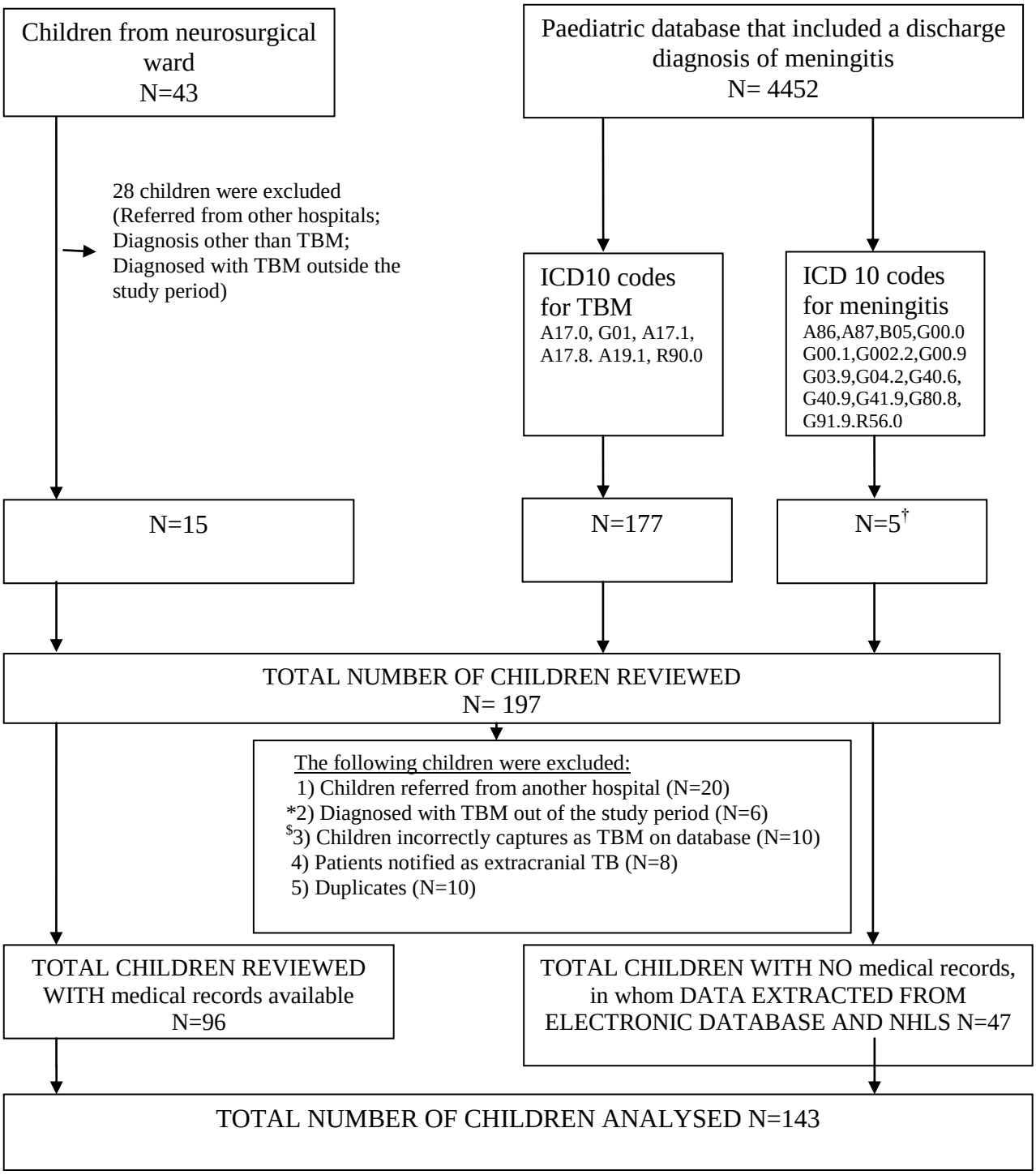

Figure 2: Schematic representation of study methods used to collect data in children with Tuberculosis meningitis (TBM)

*TBM reflected in the discharge summary however patient started on TBM treatment prior to study period
$TBM diagnosis incorrectly reflected on the discharge summary and database
†These 5 cases were captured as bacterial meningitis on the database, but were TBM

2.5 Statistical Analysis

Estimated incidence of TBM were calculated and stratified by HIV-infection status to yield stratum specific incidence. Due to a low percentage (8 of 143, 5.6%) of culture-confirmed cases, all categories of TBM were combined for the incidence estimates.

The numerator for incidence calculation were the number of TBM episodes hospitalised during each year and the denominator was based on mid-year population estimates from the Gauteng Department of Health and Social development for region D (Soweto) in Johannesburg as per the Statistics South Africa report (51) (Table 4). HIV prevalence in the study population between the years 2006 to 2011 was estimated from the projections of the Gauteng- Sub-district Population Mid-year Estimates 2005-2012 (52) (Table 4).

To compare the differences between HIV-infected and -uninfected children, odds ratios were used for categorical variables. Continuous variables such as CSF and blood results were analysed using Mann Whitney U tests as the data was not normally distributed. A 95% confidence intervals for parameters of interest were reported and p-values<0.05 were considered statistically significant. STATISTICA version 12 SP2 (statsoft.com) and STATA version 10.0 (College Station, Texas, USA) was used for data analysis and to calculate the confidence intervals, risk ratios and incidences of TBM.

Table 4: Population denominators and HIV prevalence by age-group in Soweto (Region D, Johannesburg), South Africa[1,2]

Year	Age	Total population[1]	HIV prevalence[2]
2006	0 - 1 years	22382	5.7%
	1 – 4 years	96797	3.9%
	5 -14 years	225563	1.2%
2007	0- 1 years	21326	5.2%
	1- 4 years	95807	4.1%
	5 -14 years	234353	1.5%
2008	0 - 1 years	21077	4.6%
	1 – 4 years	95198	4.1%
	5 -14 years	241801	1.8%
2009	0 - 1 years	21627	4.0%
	1 – 4 years	95152	4.2%
	5 -14 years	247918	2.0%
2010	0 - 1 years	22660	3.9%
	1 – 4 years	95773	3.9%
	5 -14 years	252732	2.3%
2011	0 - 1 years	23777	3.9%
	1 – 4 years	96887	3.7%
	5 -14 years	256627	2.5%

[1]Population estimates for region D (Soweto) in Johannesburg as per Statistics South Africa (51)

[2]HIV prevalence in the study population was estimated from the projections of the AIDS and Demographic models developed by the Actuarial Society of South Africa (52)

3.0 RESULTS

3.1 Demographic information of all children hospitalised with all categories of TBM between 2006 and 2011

A total of 143 TBM cases were identified for the period 2006 to 2011, including 74 (51%) males and 69 (49%) females (Table 5). The overall median age was 3.7 years (range 0.07-14.18) and the majority of children were underweight for age with a median z-score of -2.25 (range -5.68 to 1.93). Forty-seven (32.9%) of the children were HIV-infected, whilst HIV-infection status was unknown among 9 (6.3%) children. CD4+ cell counts were obtained in 43 of the 47 (91%) HIV-infected children, of which 30 (69.8%) were categorised as severely immunocompromised based on the WHO immunological classification of HIV (Table 5).

Culture of MTB on CSF was requested in 50 of 143 (35.0%) TBM cases, of which MTB was cultured in 8 cases (16.0% of 50; or 5.6% of overall TBM cases). A further 46 (32.1%) children were classified as probable TBM, 48 (33.6%) as possible TBM and 41(28.7%) were undefined (Table 5).

Demographic data was further analysed by combining the confirmed and probable TBM cases. Similar median ages and weight for age z-scores were noted compared to all TBM cases (Table 6). Children with possible TBM, however, tended to be older (median 5.95) and less malnourished (median weight for age z-score of -1.2) compared to HIV-uninfected children (Table 7).

Table 5: Demographic information of children hospitalised with all categories of Tuberculosis meningitis (TBM) between 2006 and 2011

Year	2006	2007	2008	2009	2010	2011	Overall
Total no. of cases	**24**	**22**	**26**	**36**	**23**	**12**	**143**
Confirmed	1(4.2%)	0(0%)	2(7.7%)	4(11.1%)	1(4.4%)	0(0%)	8(5.6%)
Probable	12(50%)	9(40.9%)	6(23.1%)	11(30.6%)	6(26.1%)	2(16.6%)	46(32.2%)
Possible	5(20.8%)	8(36.4%)	11(42.3%)	12(33.3%)	7(30.4%)	5(41.7%)	48(33.6%)
Undefined	6(25%)	5(22.7%)	7(26.9%)	9(25%)	9(39.1%)	5(41.7%)	41(28.6%)
HIV-infected	7(29.2%)	9(40.9%)	10(38.5%)	10(27.8%)	7(30.4%)	4(33.3%)	47(32.9%)
HIV-uninfected	17(70.8%)	12(54.5%)	13(50.0%)	22(61.1%)	15(65.2%)	8(66.7%)	87(60.8%)
HIV-unknown	0	1(4.6%)	3(11.5%)	4(11.1%)	1(4.4%)	0	9(6.3%)
Age (%)							
Median(years)	3.17	2.7	3.89	5.47	5.16	4.3	3.70
Range(years)	0.13-10.79	0.57-14.18	0.29-12.9	0.08-11.63	0.53-12.48	0.4-13.04	0.07-14.18
<1 yr	4(16.6%)	2(9.1%)	5(19.2%)	6(16.7%)	1(4.3%)	3(25.0%)	21(14.7%)
1-<5yrs	10(41.7%)	12(54.5%)	10(38.5%)	10(27.8%)	10(43.5%)	3(25.0%)	55(38.4%)
5 - <15yrs	10(41.7%)	8(36.4%)	11(42.3%)	20(55.6%)	12(52.2%)	6(50.0%)	67(46.9%)
Male Gender (%)	12(50%)	16(72.7%)	13(50.0%)	19(52.7%)	6(26%)	8(66.6%)	74(51.7%)
Weight for Age (<10yrs)	n=20	n=15	n=13	n=27	n=16	n=9	n=100
Median Z-scores	-2.41	-2.56	-1.70	-2.34	-1.77	-2.26	-2.25
Range	-5.68 to -0.08	-5.01 to 0.91	-3.75 to 1.93	-3.51 to 0.13	-4.75 to 0.68	-3.73 to-0.61	-5.68 to 1.93
HIV-infected with CD4	n=5	n=8	n=9	n=10	n=7	n=4	n=43
*Severe immunocompromised (%)	2/5(40.0%)	6/8(75.0%)	8/9(88.8%)	8/10(80.0%)	4/7(57.1%)	2/4(50.0%)	30(69.8%)

***WHO Immunological classification for established HIV infection(CD4% or absolute number per mm3)** (59)

	Not significant	**Mild**	**Advanced**	**Severe***
<11 months	>35%	30-35%	25-29%	<25%
12-35 months	>30%	25-30%	20-24%	<20%
36-59 months	>25%	20-25%	15-19%	<15%
>5yrs	>500	350-499	200-349	<200/<15%

Table 6: Demographic information of children hospitalised with confirmed and probable Tuberculosis meningitis (TBM) between 2006 and 2011

Year	2006	2007	2008	2009	2010	2011	Overall
Total no. of cases							
Confirmed and Probable	13	9	8	15	7	2	54
HIV-infected	5(38.5%)	4(44.4%)	4(50.0%)	4(26.7%)	4(57.1%)	0	21(38.9%)
HIV-uninfected	8(61.5%)	5(55.6%)	4(50.0%)	8(53.3%)	3(42.9%)	2(100%)	30(55.6%)
HIV-unknown	0	0	0	3(20.0%)	0	0	3(5.5%)
Age (%)							
Median(years)	2.39	2.15	3.89	3.74	8.01	5.87	3.81
Range(years)	0.27-8.58	0.86-11.69	0.7-12.91	0.17-10.72	1.13-12.03	5.74-6.16	0.17-12.91
<1 yr	3(23.1%)	2(22.2%)	1(12.5%)	4(26.7%)	0	0	10(18.5%)
1-<5yrs	7(53.8%)	3(33.3%)	4(50.0%)	4(26.7%)	2(28.6%)	0	20(37.1%)
5 - <15yrs	3(23.1%)	4(44.5%)	3(37.5%)	7(46.6%)	5(71.4%)	2(100%)	24(44.4%)
Male Gender(%)	8(61.5%)	6(66.6%)	5(62.5%)	9(60%)	1(14.2%)	0	29(53.7%)
Weight for Age (<10yrs)							
Median Z-scores	-2.68	-3.00	-2.38	-2.95	-3.18	-1.91	-2.81
Range	-5.68 to -0.87	-3.87 to -0.14	-3.75 to -1.64	-4.87 to -0.88	-4.75 to -0.66	-2.26 to -1.56	-5.68 to -0.14
HIV-infected with CD4	n =4	n =4	n = 3	n = 4	n = 4	n =2	21
*Severe immunocompromised(%)	2(50.0%)	3(75.0%)	2(66.0%)	2(50.0%)	2(50.0%)	0(0%)	11(52.3%)

WHO immunological classification for established HIV infection(CD4% or absolute number per mm3) (59)

	Not significant	Mild	Advanced	Severe*
<11 months	>35%	30-35%	25-29%	<25%
12-35 months	>30%	25-30%	20-24%	<20%
36-59 months	>25%	20-25%	15-19%	<15%
>5yrs	>500	350-499	200-349	<200/<15%

Table 7: Demographic information of children hospitalised with possible Tuberculosis meningitis (TBM) between 2006 and 2011

Year	2006	2007	2008	2009	2010	2011	Overall
Total no. of cases							
Possible	5	8	11	12	7	5	48
HIV-infected	1(20.0%)	3(37.5%)	4(36.4%)	4(33.3%)	3(42.9%)	2(40.0%)	17(35.4%)
HIV-uninfected	4(80.0%)	5(62.5%)	5(45.5%)	8(66.7%)	4(57.1%)	3(60.0%)	29(60.4%)
HIV-unknown	0	0	2(18.1%)	0	0	0	2(4.2%)
Age (%)							
Median(years)	5.95	3.2	7.52	6.95	5.16	5.96	5.95
Range(years)	1.22-10.94	1.2-13.20	0.46-11.42	0.38-11.63	0.53-12.48	1.28-9.61	0.46-13.20
<1 yr	0	0	2(18.2%)	2(16.7%)	1(14.3%)	0	5(10.4%)
1-<5yrs	1(20.0%)	6(75.0%)	3(27.3%)	1(8.3%)	2(28.5%)	2(40.0%)	15(31.3%)
5 - <15yrs	4(80.0%)	2(25.0%)	6(54.5%)	9(75.0%)	4(57.2%)	3(60.0%)	28(58.3%)
Male Gender(%)	1(20.0%)	6(75.0%)	6(54.5%)	7(58.3%)	0	4(80.0%)	24(50.0%)
Weight for Age (<10yrs)							
Median Z-scores	-1.19	-1.2	0.16	-1.84	-1.82	-1.44	-1.2
Range	-4.33 to -0.08	-2.56 to 0.91	-2.68 to 1.93	-4.77 to 0	-3.81 to 0.59	-3.55 to -0.61	-4.77 to 1.93
HIV-infected with CD4	n =0	n =2	n = 4	n = 4	n =3	n =2	16
*Severe immunocompromised(%)	0(0.0%)	1(50.0%)	4(100.0%)	4(100.0%)	3(100.0%)	1(50.0%)	13(81.3%)

WHO immunological classification for established HIV infection(CD4% or absolute number per mm3) (59)

	Not significant	Mild	Advanced	Severe*
<11 months	>35%	30-35%	25-29%	<25%
12-35 months	>30%	25-30%	20-24%	<20%
36-59 months	>25%	20-25%	15-19%	<15%
>5yrs	>500	350-499	200-349	<200/<15%

3.2 The Incidence of TBM in HIV-infected and HIV-uninfected children.

The overall incidence (per 100 000) of TBM in 2006 was 6.96 (95%CI 4.46-10.36) and peaked in 2009 at 9.87 per 100 000 (95% CI: 6.91-13.60) (Figure 3).This was followed by decrease in 2010 and 2011. In HIV-infected children across all age groups, the incidence of TBM per 100 000 also trended to increase steadily from 89.35 (95% CI: 35.93-184.02) in 2006 to 101.10 (95% CI: 48.49-185.80) in 2009 and subsequently declined in 2010 to 66.63 (95% CI: 26.80-137.70) and to 36.26 (95% CI: 9.88-92.81) in 2011 (Figure 3). With regard to HIV-uninfected children, the incidence of TBM ranged from 5.05 (95% CI: 2.94 - 8.08) in 2006 to 2.19 (95% CI: 0.94-4.30) per 100 000 in 2011 (Figure 3).

The incidence for period 2006-2009 was compared to that in 2010-2011. The overall incidence (per 100 000) of TBM was 7.61 (95% CI: 6.24-9.19) in 2006-2009 and 4.68 (95% CI: 3.26-6.50) in 2010-2011, with an overall reduction of 38.55% (95% CI: 10.04-58.03; p=0.011) (Table 8). In HIV-infected children across all age groups the incidence of TBM in 2006-2009 was 101.43 (95% CI: 71.05-140.40) compared to 51.07 (95% CI: 25.50-91.37) in 2010-2011, i.e. rate reduction of 49.6% (95% CI: 1.1-74.4; p=0.042) (Table 8). There was no significant reduction in TBM incidence in HIV-uninfected children comparing the period 2006-2009 (4.63) with 2010-2011 (31.60; p=0.116) (Table 8).

When analysing the composite of confirmed and probable TB cases between 2006-2009 and comparing this to 2010-2011, a significant rate reduction of 62.08% (95% CI:

22.43-81.46; p=0.005) was observed overall, as well as in HIV-uninfected children (61.9%; 95% CI: 5.7-85.4; p=0.04), whereas no reduction was observed in HIV-infected children. The incidence of “possible” TBM did not change over the study periods (p=0.164) (Table 8).

Furthermore, we conducted a sensitivity analysis to proportionately include the number of cases that were “undefined” into one of the above two mentioned groups based on the prevalence of confirmed/probable and possible TBM among those for whom it was known. The results were similar to that of the crude analysis, except that the reduction in the HIV-infected with confirmed/probable was now significant (95% CI: 20.85-75.27; p-value=0.004) (Table 8).

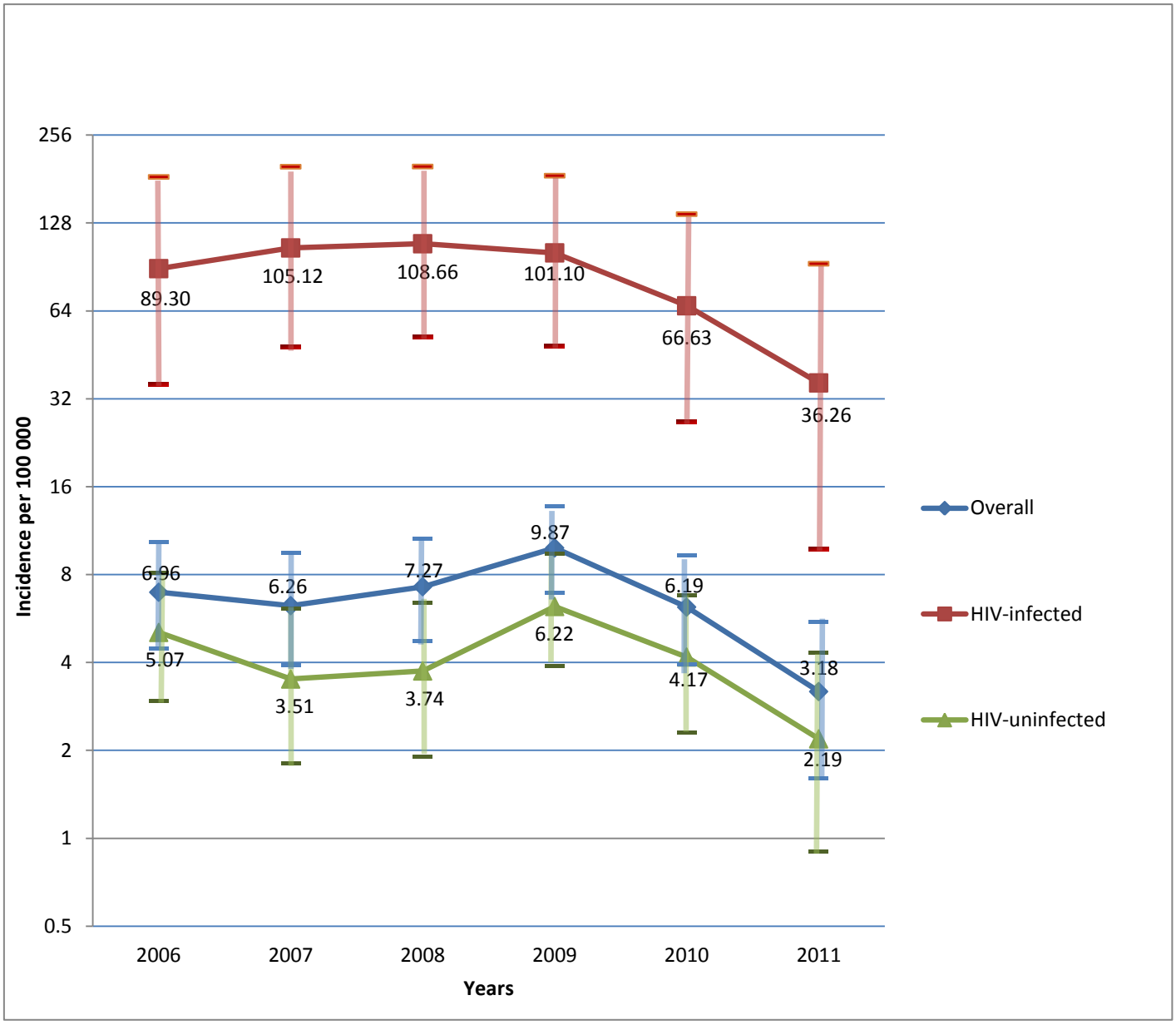

Figure 3: Yearly incidence of Tuberculosis meningitis (TBM) from 2006-2011 stratified by HIV status

Table 8: Comparing the incidence of Tuberculosis meningitis (TBM) between 2006-2009 to 2010-2011

	2006-2009	2010-2011	Comparing 2006-2009 and 2010- 2011 IRR[a]	p-value[^]	%Reduction[#]
	Incidence of TBM [95%CI] (N of TB cases)	Incidence of TBM [95%CI] (N of TB cases)			
Overall TBM	7.61[6.24-9.19]	4.68[3.26-6.50]	0.61[0.41-0.89]	0.011	38.6[10.0-58.0]
	108	35			
HIV-infected	101.43[71.05-140.40]	51.07[25.50-91.37]	0.50[0.25-0.98]	0.042	49.6[1.1-74.4]
	36	11			
HIV-uninfected	4.63[3.56-5.19]	3.16[2.01-4.75]	0.68[0.42-1.10]	0.116	31.6[-10.1-57.5]
	64	23			
Confirmed TB and Probable	3.17[2.31-4.24]	1.20[0.55-2.28]	0.37[0.18-0.77]	0.005	62.1[22.4-81.5]
	45	9			
HIV-infected	47.9[27.91-76.68]	18.57[5.06-47.55]	0.38[0.13-1.15]	0.076	61.2[-15.3-86.9]
	17	4			
HIV-uninfected	1.81[1.17-2.67]	0.68[0.22-1.61]	0.38[0.14-0.99]	0.040	61.9[5.7-85.4]
	25	5			
Possible TB	2.54[1.78-3.51]	1.60[0.82-2.80]	0.63[0.32-1.21]	0.164	36.8[-21.4-67.1]
	36	12			
HIV-infected	33.81[17.47-59.05]	23.22[7.54-54.17]	0.68[0.24-1.94]	0.477	31.3[-94.9-75.8]
	12	5			
HIV-uninfected	1.59[0.99-2.41]	0.96[0.87-1.98]	0.60[0.25-1.41]	0.242	39.4[-41.8-74.1]
	22	7			
Confirmed and Probable TB extrapolated to include undefined	14.23[3.23-5.44]	1.87[1.02-3.14]	0.44[0.24-0.79]	0.004	55.8[20.9-75.3]
	60	14			
HIV-infected	59.17[36.63-90.43]	18.57[5.06-47.55]	0.31[0.10-0.91]	0.024	68.6[8.5-89.2]
	21	4			
HIV-uninfected	24.58[17.02-34.34]	1.24[0.56-2.35]	0.50[0.24-1.05]	0.060	49.6[0.5-75.8]
	34	9			
Possible TB extrapolated to include undefined	3.38[2.49-4.48]	2.81[1.74-4.29]	0.82[0.49-1.38]	0.474	17.1[-38.5-50.3]
	48	21			
HIV-infected	42.26[23.66-69.70]	32.5[13.07-66.96]	0.76[0.31-1.88]	0.565	23.1[-88.5-68.6]
	15	7			
HIV-uninfected	2.17[1.46-3.10]	1.93[1.05-3.23]	0.88[0.47-1.67]	0.714	11.2[-67.5-52.9]
	30	14			

*IRR=Incidence Risk Ratio (Population denominators were added for 2006 to 2009 and for 2010 to 2011
^ χ^2 test or Fischer test
[#]%reduction between Early and Established HAART era

3.3 "Confirmed and probable" TBM stratified by age

The incidence (per 100 000) of confirmed and probable TBM stratified by age was highest in children <1 year age from 2006 (13.40; 95% CI: 2.76-39.17) to 2009 (18.5; 95%CI: 5.04-47.35), with no cases reported in 2010 and 2011 in this age group (Figure 4).

3.4 "Possible" TBM stratified by age

There were no cases of possible TBM in the <1 year age group in 2006 and 2007, however in 2009 the incidence (per 100 000) was 9.25 (95% CI: 1.12-33.4) and 4.41 (95% CI: 0.11-25) in 2010. In the 1-5 year age group, the highest incidence was noted in 2007 with 6.26 per 100 000 (95% CI: 2.30-13.63) and a decline was noted in 2009 of 1.05 per 100 000 (95% CI: 0.02-5.86) (Figure 5).

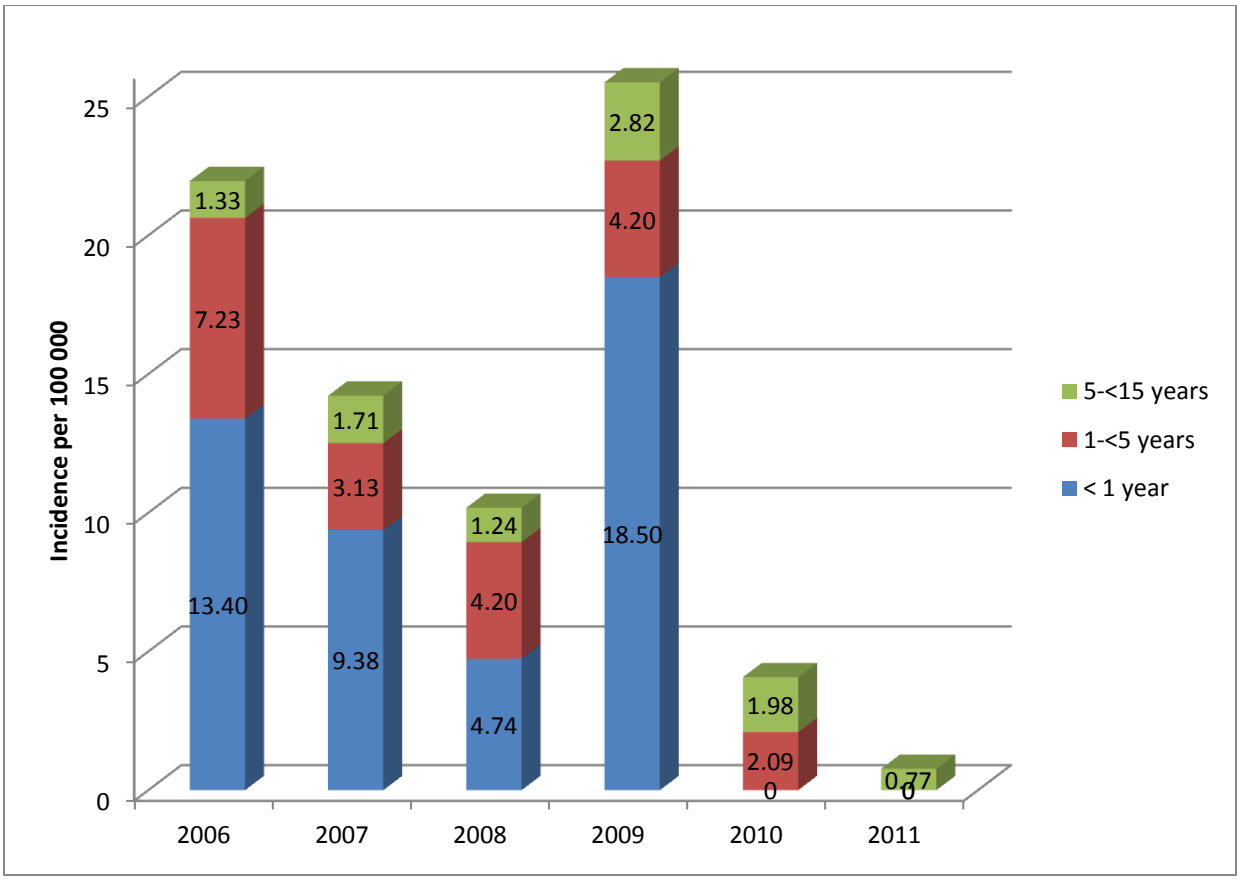

Figure 4: Incidence of "confirmed and probable" Tuberculosis meningitis (TBM) from 2006-2011 stratified by age

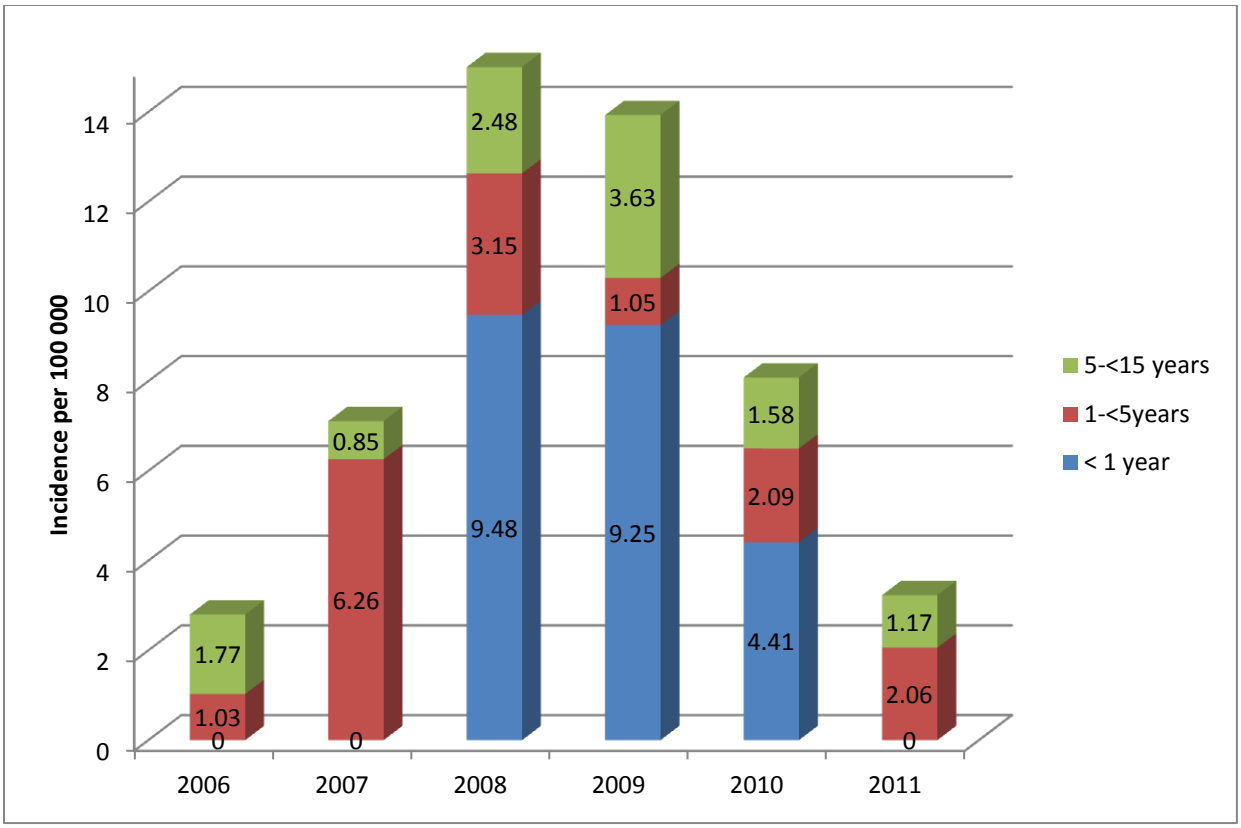

Figure 5: Incidence of possible Tuberculosis meningitis (TBM) from 2006-2011 stratified by age

3.5 Clinical symptoms and signs in children with TBM

Constitutional symptoms of TB, such as coughing, weight loss and night sweats were similar between HIV-infected and -uninfected children. The odds of having a previous history of TB was significantly higher among HIV-infected children compared to -uninfected children (OR 4.63; 95%CI: 1.40-15.22; p= 0.011)(Table 9). TB exposure from family or household contact was present in 38.2% of HIV-infected and 22.4% of -uninfected children (OR 2.95; 95%CI: 0.75-5.30; p= 0.163) (Table 9).

Symptoms suggestive of meningitis such as headache, fever, neck pain, vomiting, photophobia and seizures were similar in both groups; with a high proportion of children presenting with fever (50.0% HIV-infected and 60.3% HIV-uninfected) and neck pain (58.8% HIV-infected and 55.2% HIV-uninfected) (Table 9 and 10). HIV-infected children presented less commonly with irritability (23.5%) compared with HIV-uninfected children (43.1%; OR 0.22; 95%CI: 0.07-0.71; p=0.010). Paralysis, seizures, hemiplegia, long tract signs (long tract signs: spasticity, hyper-reflexia and clonus associated with upper motor neuron pathology) and cranial nerves showed no significant difference between HIV-infected and -uninfected children.

Table 9: Clinical symptoms suggestive of tuberculosis in children with Tuberculosis meningitis (TBM) n=92

Symptoms	HIV-infected n=34	HIV-uninfected n=58	Odds Ratio	p-value
Cough (Any duration)				
Yes	20(58.9%)	26(44.8%)	2.11(0.58-7.64)	0.252
No	4(11.7%)	11(19.0%)		
Not recorded*	10(29.4%)	21(36.2%)		
Night sweats				
Yes	3(8.8%)	2(3.4%)	2.50(0.25-24.72)	0.432
No	3(8.8%)	5(8.6%)		
Not recorded	28(82.4%)	51(88.0%)		
Weight loss				
Yes	10(29.4%)	11(19.0%)	0.30(0.01-8.31)	0.480
No	1(3.0%)	0		
Not recorded	23(67.6%)	47(81.0%)		
Loss of Appetite				
Yes	15(44.1%)	20(34.5%)	6.80(0.34-136.04)	0.209
No	0	4(6.9%)		
Not recorded	19(55.9%)	34(58.6%)		
Previous history of TB				
Yes	11(32.4%)	5(8.6%)	4.63(1.40-15.22)	0.011
No	19(55.9%)	40(69.0%)		
Not recorded	4(11.7%)	13(22.4%)		
TB exposure˟				
Yes	13(38.2%)	13(22.4%)	2.00(0.75-5.30)	0.163
No	16(47.1%)	32(55.2%)		
Not recorded	5(14.7%)	13(22.4%)		
Vomiting				
Yes	13(38.2%)	19(32.8%)	1.07(0.40-2.84)	0.883
No	14(41.2%)	22(37.9%)		
Not recorded	7(20.6%)	17(29.3%)		
Headache				
Yes	10(29.4%)	14(24.1%)	0.71(0.08-5.95)	0.755
No	2(5.9%)	2(3.5%)		
Not recorded	22(64.7%)	42(72.4%)		
Neck pain				
Yes	20(58.8%)	32(55.2%)	0.97(0.35-2.66)	0.956
No	9(26.5%)	14(24.1%)		
Not recorded	5(14.7%)	12(20.7%)		

*Not recorded: No record of symptom in file
˟TB exposure: Indicates household contacts

Table 10: Clinical signs suggestive of tuberculosis in children with Tuberculosis meningitis (TBM) n=92

Signs	HIV-infected n=34	HIV-uninfected n=58	Odds Ratio	p-value
Fever				
Yes	17(50.0%)	35(60.3%)	0.7(0.27-1.84)	0.478
No	11(32.4%)	16(27.6%)		
Not recorded*	6(17.6%)	7(12.1%)		
Confusion				
Yes	5(14.7%)	6(10.3%)	0.83(0.20-3.42)	0.800
No	13(38.2%)	13(22.4%)		
Not recorded	16(47.1%)	39(67.3%)		
Irritability				
Yes	8(23.5%)	25(43.1%)	0.22(0.07-0.71)	0.010
No	14(41.2%)	10(17.2%)		
Not recorded	12(35.3%)	23(39.7%)		
Drowsiness				
Yes	9(26.5%)	19(32.8%)	0.49(0.18-1.37)	0.177
No	19(55.9%)	20(34.4%)		
Not recorded	6(17.6%)	19(32.8%)		
Photophobia				
Yes	5(14.7%)	4(6.9%)	1.56(0.31-7.13)	0.610
No	10(29.4%)	12(20.7%)		
Not recorded	19(55.9%)	42(72.4%)		
Paralysis				
Yes	4(11.8%)	16(27.6%)	0.32(0.09-1.12)	0.070
No	20(58.8%)	26(44.8%)		
Not recorded	10(29.4%)	16(27.6%)		
Seizures				
Yes	14(41.2%)	32(55.2%)	0.6(0.26-1.65)	0.370
No	14(41.2%)	21(36.2%)		
Not recorded	6(17.6%)	5(8.6%)		
Hemiplegia				
Yes	2(5.9%)	12(20.7%)	0.22(0.04-1.09)	0.060
No	32(94.1%)	44(75.9%)		
Not recorded	0	2(3.4%)		
Long tract signs†				
Yes	4(11.8%)	13(22.4%)	0.44(0.13-1.5)	0.190
No	29(85.3%)	42(72.4%)		
Not recorded	1(2.9%)	3(5.2%)		
Cranial Nerve Palsy				
Yes	1(2.9%)	10(17.2%)	0.14(0.017-1.177)	0.070
No	32(94.2%)	46(79.3%)		
Not recorded	1(2.9%)	2(3.5%)		

*Not recorded: No record of sign in file

†Long tract signs: spasticity, hyper-reflexia and clonus associated with upper motor neuron pathology

3.6 Clinical, laboratory and brain CT scan investigations in children with TBM

A high proportion of children's TST results were not recorded (n=50/100; 50%). HIV-infected children were less likely to have a reactive TST (11.1%) compared to HIV-uninfected children (32.8%; p=0.002)(Table 11).There was also a lesser proportion of HIV-infected children (27.6%) who had positive sputum or gastric washing (including smear and/or culture) results for *Mycobacterium tuberculosis* compared to HIV-uninfected TBM cases (12.8%; p=0.025). Brain CT scan findings such as tuberculoma, basal enhancement and infarcts were generally uncommon; but occurred more frequently in HIV-uninfected children. Notably, 5/19 (26.3%) of HIV-infected children had radiologic evidence of hydrocephalus compared with 27/55 (49.1%) of HIV-uninfected children, however this was not statistically significant (p= 0.096) (Table 11).

Table 11: Radiological and clinical investigations performed in children admitted with Tuberculosis Meningitis (TBM)

Investigation	HIV-infected	HIV-uninfected	Odds ratio	p-value
Mantoux test†	**n=36**	**n=64**		
Reactive	4(11.1%)	21(32.8%)	0.09(0.02 -0.43)	0.002
Non-reactive	10(27.8%)	15(23.4%)		
Not recorded*	22(61.1%)	28(43.8%)		
Chest X-ray	**n=34**	**n=58**		
Suggestive of PTB	9(26.5%)	13(22.4%)	1.03(0.14 -7.52)	0.970
Not suggestive of PTB	2(5.9%)	3(5.2%)		
Not recorded	23(67.6%)	42(72.4%)		
Sputum‡	**n=47**	**n=87**		
Positive	6(12.8%)	24(27.6%)	0.29(0.10 -0.86)	0.025
Negative	21(44.7%)	25(28.7%)		
Not recorded	20(42.5%)	38(43.7%)		
CT brain findings	**n= 19**	**n = 55**		
Hydrocephalus				
Yes	5(26.3%)	27(49.1%)	0.37(0.11-1.16)	0.096
Tuberculoma				
Yes	4(21.0%)	14(25.4%)	0.97(0.27-3.39)	0.963
Basal enhancement				
Yes	3(15.7%)	19(34.5%)	0.35(0.09-1.37)	0.133
Evidence of Infarct				
Yes	1(5.2%)	9(16.3%)	0.28(0.03-2.40)	0.248

*Not recorded: No record of symptom in file

†Mantoux test- Purified Protein Derivative (PPD) tuberculin injected intra-dermally to asses for TB infection in children

‡Sputum-*Mycobacterium tuberculosis* cultured on sputum samples taken in children suspected to have TBM

3.7 Cerebrospinal Fluid Count in children with TBM

Although the CSF biochemistry showed no difference between HIV-infected and -uninfected children, the median protein level was elevated (>0.5g/dl) in both groups (Table 12). CSF glucose levels were similar in both groups. A higher lymphocyte count was noted among HIV-uninfected children compared to HIV-infected children, but this was not statistically significant. ADA was requested on 93 of the 143 (65.0%) of children, of whom 19 (20.4%) had levels above 8.0µ/L (Table 12).

Table 12:Cerebrospinal fluid (CSF) biochemistry results comparing HIV-infected to -uninfected children

CSF Biochemistry results	**HIV-infected**	**HIV-uninfected**	**p-value**
CSF protein (g/dl)	**n = 46**	**n = 82**	
Median	0.935	1.03	0.990
Range	0.02-7.5	0.1-6.9	
Chloride (mmol/l)	**n=46**	**n = 81**	
Median	117	117	0.354
Range	61-130	87-134	
Glucose (mmol/l)	**n = 46**	**n = 82**	
Median	2.4	2.6	0.704
Range	0-5.8	0.3-6.1	
Polymorphs (cu/mm)	**46**	**82**	
Median	2	5.5	0.622
Range	0-1000	0-10001	
Lymphocytes (cu/mm)	**n = 46**	**n = 82**	
Median	12	20.5	0.400
Range	0-416	0-1490	
ADA (U/l)	**n=47**	**n=87**	
<8.0	33	42	0.291
>/=8.0	6	12	
Not recorded	8	33	

3.8 Outcomes in children with TBM

Neurological outcomes were analysed in 134 children. Outcomes were recorded on discharge only (there was no long term follow-up) as “demised or “neurological sequelae” (i.e. neurological abnormality not previously present), which was further subdivided into “returned to baseline function” (returned to function prior to the onset of TBM) or “not back to baseline function”. These findings were based on the attending-physician recorded findings (Table 13). In hospital case fatality rates were similar between HIV-infected (6.4%) and -uninfected children (6.9%). Of those discharged, 33% (26/79) of HIV-uninfected children and 22.7% (10/ 44) of HIV-infected had neurological sequelae. Neurological assessments were unavailable in 16/44 (36.4%) and 30/79 (37.9%) of HIV-infected and HIV-uninfected children, respectively, due to missing or incomplete medical record documentation (Table13). There were no difference in outcomes in HIV-infected and -uninfected children when TBM cases were stratified as “confirmed and probable” or “possible” TBM (Table 14).

Table 13: Outcomes of children admitted with Tuberculosis meningitis (TBM)

	HIV-infected	HIV-uninfected
	n=47	**n=87**
Demised (CFR%)*	3(6.4%)	6(6.9%)
Discharged	44(93.6%)	79(90.8%)
Not recorded	0	2(2.3%)
Neurological sequelae among survivors:		
-Back to baseline º	18/44(40.9%)	25/79(31.6%)
-Not back to baseline+	10/44(22.7%)	26/79(33.0%)
-Baseline status not recorded or medical record unavailable	16/44(36.4%)	28/79(35.4%)

*CFR: case fatality rate
ºBaseline function: Back to level of function before hospitalisation with TBM
+Not back to baseline function: Had some neurological deficit after hospitalisation with TBM

Table 14: Children who demised or were discharged stratified as "confirmed and probable" or "possible" or "Undefined"

	HIV-Infected	HIV-Uninfected	p-value
Demised	**n=3**	**n=6**	
Confirmed and probable	2	3	0.850
Possible	1	2	
Undefined	0	1	
Discharged	**n=44**	**n=79**	
Confirmed and probable	19	27	0.692
Possible	16	27	
Undefined	9	24	

4.0 Discussion

The burden of TBM in our setting was greater in HIV-infected compared to -uninfected children. Overall, the incidence of TBM increased until 2009, following which we observed reduction from 2010 onward. Although the HAART coverage improved in HIV-infected children, no substantial effect was noticed on the burden of TBM in HIV-infected children up to 2009, however, the majority of cases occurred in the severely immunocompromised children. This was supported by a study conducted in the same setting between 2005 and 2009, which demonstrated no significant change in the number of cases of overall EPTB as HAART coverage increased (60). A decline in TBM was noted after 2009, which could have been from strengthening of the HAART programme and immune reconstitution among those who had been previously initiated on HAART. Furthermore, the decline in TBM from 2010 may also be due to a decrease in TB amongst adults in this endemic HIV/TB setting, which could have resulted from HIV-infected adults accessing HAART that resulted in reduced incidence of TB and consequently reduction in transmission of MTB to susceptible children in the community. However, adult TB patients with advanced AIDS (CD4 <200) have been shown to be less infectious than those with early disease (61). Furthermore, improvements in case findings, contact tracing, strengthening of TB programs, increased awareness and improved management of HIV-infected children with respect to nutrition, earlier detection of illnesses can also account for reduced transmission rates. The burden of disease was predominantly in children under 1 year of age where a more significant decline in the incidence of TBM was noted in 2010 and 2011. This

further highlights the effect of the various interventions such as HAART and PMTCT on the most vulnerable age group.

When we classified TBM as “confirmed and probable” to determine the effect on the cases of TBM that were diagnosed using a definitive outcome measure, we observed a more substantial reduction in the cases of TBM (62.1%; 95% CI: 22.43-81.46; p-value=0.005) comparing the period 2006-2009 with 2010-2011, including in HIV-infected and -uninfected children.

Clinical findings in children with TBM

In our study we observed a significant difference in the median age comparing HIV-infected (7.71 years) to -uninfected children (2.75 years), $p<0.0001$. In contrast, other paediatric studies reported similar ages between HIV-infected and -uninfected children (22,23,28). This may be explained in our setting by a failure to initiate HAART in HIV-infected children early in the course of their illness as HIV testing guidelines for infants were not well implemented over the earlier study years. In addition, 69.8% of children had severe immunological suppression by the time TBM was diagnosed.

A previous history of TB was more common in HIV-infected children (32.4%) compared to HIV-uninfected children (8.6%). It was not known if patients in the study, with previous TB, completed TB treatment or if they were compliant. These findings are nonetheless similar to other paediatric studies (30% versus 9%) (23) . Furthermore, 38.2% of HIV-infected and 22.4% of -uninfected children reported a contact with proven

pulmonary TB within the household. This emphasises the importance of screening adults and treating household contacts in order to limit the spread of infection to children (50). Additionally, instituting INH prophylaxis in HIV-infected children has been shown to reduce the incidence of TB by 72% regardless of whether the child was on HAART or not (62).

The clinical features typical of PTB such as coughing, fever, weight loss and night sweats showed no significant difference between HIV-infected and -uninfected children in this study, which is in keeping with findings reported in previous paediatric and adult studies (10,22,23,43). However, coughing was present in approximately 50% of both HIV-infected and -uninfected children which may suggest that these children often have symptoms of a cough from pulmonary TB prior to subsequent dissemination to the brain. Symptoms such as fever and neck pain, although thought to be findings of acute bacterial meningitis were also commonly found in children with TBM. Thus a child who fails to culture an organism or to respond to conventional antibiotics after an admission for suspected bacterial meningitis should be considered as possibly having TBM . Seizures were noted to be the most common sign amongst all children treated for TBM. The pathological mechanism as to why such a high proportion present with seizures could be related to increased intracranial pressure as a complication of the meningitis (17).

Investigations in children with TBM

We observed no significant difference in CSF biochemistry and cell counts between HIV-infected and -uninfected children. The typical CSF biochemistry of TBM, as similarly reported, showed a lymphocyte predominance, high protein and low glucose (50). These children are often misdiagnosed and treated for acute bacterial meningitis based on the initial neutrophil predominance (27,47,50). This further highlights the need to consider TBM in children failing to respond to conventional antibiotics and the importance of serial lumbar punctures to diagnose TBM.

Positive bacteriological culture of MTB on CSF was identified in only 5.6% of children compared to other paediatric studies which reported rates of 30% and 37% (24,25). CSF cultures were, however, only requested in 35% of patients, partly because clinicians in this setting do not routinely request MTB culture on CSF specimens due to its perceived low sensitivity for culture. Serial lumbar punctures may prove valuable as they increase the likelihood of AFB positive smears (from 39% to 87%) and positive TB cultures on CSF (from52% to 83%) in children (17,24). Furthermore, the yield of detecting a positive AFB (acid fast bacilli stain) may be improved by centrifuging 10-20 millilitres of CSF, prior to the preparation of the smear; which is not routinely done in children in our setting.

The British Infection Society guidelines do not recommend CSF ADA in children and adults as a routine test for TBM (64). This is in contrast to another paediatric study that showed that CSF ADA was a useful test for the rapid diagnosis of TBM (65). We found

that CSF ADA was not useful for diagnosing of TBM among children in our setting, as did an adult study that demonstrated limited value in HIV-infected patients (63).

The tuberculin skin test was positive in 32.8% of HIV-uninfected children and 11.1% of HIV-infected children which was similarly reported in three paediatric studies. However, Van der Wreet and Topley *et. al.* (22,23) recorded a positive tuberculin skin test in as many as 30%-45% of HIV-infected children, almost triple to what our study demonstrated. Our study did not demonstrate this, probably as a large number of children did not have documented tuberculin skin test (TST) results.

Radiological tests can assist with the diagnosis of TBM as characteristic features may be present on a CT scan (21). However, hydrocephalus, basal enhancement, infarction and tuberculomas were less commonly noted among HIV-infected children in this study, but failed to reach statistical significance. Overall, hydrocephalus and basal enhancement were found in 26.3% of HIV-infected versus 49.1% of HIV-uninfected children and 15.7% in HIV-infected versus 34.5% HIV-uninfected of children, respectively. These findings were lower compared to other paediatric studies that demonstrated hydrocephalus in 72% and 97.7% of HIV-infected and -uninfected children respectively (23), and basal enhancement in 40% of HIV-infected and 53% of HIV-uninfected children (22).

Diagnosis of TB could be improved by the Line probe assay and Xpert^{Tm}. The line probe assay identifies DNA specific to MTB on smear positive sputum or on a cultured

MTB isolate. It also identifies isoniazid, rifampacin, fluoroquinolones and aminoglycoside susceptibility. It requires sophisticated laboratory equipment and it has not yet been approved by WHO as an initial diagnostic test. The Xpert^{Tm} is a cartridge-based automated PCR test that detects MTB and rifampacin susceptibility on gastric aspirated and sputum. It has a turnaround time of 2 hours and a high sensitivity. It is currently being validated for other clinical specimens (66).

Mortality and morbidity in children with TBM

The mortality from TBM with adequate therapy has been reported as 10-20% in developed and 30-40% in developing countries (17). We reported a mortality of about 6% in both HIV-infected and -uninfected children in this setting. A high index of suspicion and early initiation of TBM treatment by clinicians in our setting could account for a lower mortality. Morbidity, only neurological, was based on short-term outcomes only (i.e. prior to discharge) and found to be higher in HIV-uninfected children (33%) compared to HIV-infected children (22.7%), however, baseline statuses of all children were not known. When comparing this to the other paediatric studies; no differences in morbidity between HIV-infected and HIV-uninfected children was reported (22,23,28).

5.0 Limitations

Several limitations were encountered in this study. This was a retrospective study and approximately 33% of the clinical and/or laboratory data was unavailable in children due to patient records being unavailable. Furthermore, there was a lack of standard history-taking; including, HIV statuses not obtained on all children, compliance with treatment in patients previously treated with TB, opening pressures of LP's (lumbar punctures) and no long term follow up of all patients discharged with TBM. Also, in HIV-infected children, treatment regimens, CD4 and viral loads were not always obtained. Furthermore, we were unable to classify TBM as confirmed, probable or possible in 28% of our cases as data was unavailable. Computed tomography scan results were not always documented and physicians may have not always recorded negative findings. Another limitation to this study was that we used population estimates as our denominator for this study, however, this was consistent over the study period. The prevalence of HIV in the study was calculated based on models from the Actuarial Society of South Africa and these models may have not accurately accounted for improvements in the PMTCT programme.

6.0 Conclusions

The incidence of TBM has decreased across the study period 2006-2011 but particularly from 2010.The majority of children in the study were HIV-uninfected who were younger at the time of presentation. Clinical features of TBM were non-specific but noted to be similar for HIV-infected and -uninfected children. Diagnosing TB in children offers huge challenges, particularly in HIV-infected children. In addition, CSF and CTB findings of TBM are less specific in HIV-infected children. Although we have shown a decline in incidence of TBM in HIV-infected and HIV-uninfected children in our setting, we believe that our study likely underestimated the incidence of TBM in this population and a high index of suspicion needs to be maintained when treating children for meningitis in high TB/HIV settings.

7.0 Recommendations

This study provides valuable information regarding the incidence of TBM. A prospective study would be beneficial to evaluate the long-term neurological outcomes of children with TBM. Our strongest recommendation would be that clinicians send TB cultures on all CSF samples. Furthermore, clinicians should avoid requesting ADA's on children as this test lacks sensitivity and specificity. Unquestionably, more research needs to be targeted at finding an improved vaccine to replace or augment the current BCG vaccine.

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