Association between Hypochromic Microcytic Anaemia and Febrile Seizure in patients admitted at Pelonomi Tertiary Hospital in Bloemfontein

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Declaration

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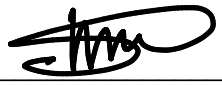
Declaration of Authorship

I, Dr. Thozamile Madikizela, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Paediatrics at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.



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All researchers declare that they have no conflict of interest and know no other situation of real, potential, or apparent conflict of interest. They undertake to inform the University there be any change in these circumstances.

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Abstract

BACKGROUND: Febrile seizure, which affects 2-5% of neurologically healthy children, is the most common type of seizure in children. The seizures occur in the context of a global iron deficiency that stands out as the most common nutritional deficiency especially prevalent in developing countries. Studies carried out to determine the association between febrile seizures and iron deficiency anaemia have yielded contradictory results.

OBJECTIVES: To evaluate the association, if any, between febrile seizures and hypochromic microcytic anaemia (iron deficiency anaemia). Hypochromic microcytic anaemia is a surrogate for iron deficiency anaemia.

METHODS: This was a retrospective cross-sectional study. A study population consisting of 244 patients, admitted over the period of twelve months with infective febrile illnesses, was included in the study. The age group was between six months and five years. In addition, 55 of these patients had febrile illnesses complicating with febrile seizures while 189 had febrile illnesses without febrile seizures. All of the patients' full blood counts (FBC) were used to determine if each had hypochromic microcytic anaemia. The following FBC parameters were used: haemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW). Chi-square and Fischer's exact tests were conducted for statistical interpretation. Finally, a comparison of full blood count parameters between the children with febrile seizures and those without was made.

RESULTS: The mean age of all patients included in the study was 17 months. The study had 153 male patients who constituted 62.7% of the study population. The majority of patients had lower respiratory tract infection. Full blood count parameters of patients with febrile seizures and those without febrile seizures were similar. There was no statistical significance between the two groups (p=0.826).

CONCLUSION: The results of the study, based on hypochromic microcytic anaemia used as surrogate for iron deficiency anaemia, showed no association between iron deficiency anaemia and febrile seizures.

This was due to the lack of difference between patient with febrile seizures and those without febrile seizures, with no statistical difference.

Keywords

Febrile illness, anaemia, seizures, epilepsy, iron deficiency, febrile seizures, paediatrics, children, fever

Acronyms and Abbreviations

CT	Computerized Tomography
FS DOH	Free Stated Department of Health
Hb	Haemoglobin
HSREC	Health Sciences Research Ethics Committee
MCH	Mean corpuscular haemoglobin
MCHC	Hypochromic microcytic anaemia
MCV	Mean corpuscular volume
MRI	Magnetic Resonance Imaging
RDW	Red cell distribution width

Definitions

Anaemia	**:**	A condition in which the number of red cells or haemoglobin concentration is lower than normal for age and gender. [1, 4]
Iron	**:**	A trace element that is essential for life, which assists in many physiological processes such as DNA synthesis, forms part of haemoglobin that is essential for oxygen transport and energy production, and works as the cofactor in neuron's metabolism amongst other functions. [2,3,5]
Iron deficiency anaemia	**:**	Anaemia that is caused by a decrease in the production of haemoglobin due to insufficient iron in the body that can be due to inadequate intake or excessive loss. [4]
Hypochromic microcytic anaemia	**:**	Anaemia in which the circulating red blood cells are smaller than the normal size and have decrease red colour. [2,4]
Fever	**:**	An elevated body temperature of more than 37.5 degrees Celsius. [3]
Seizure	**:**	A condition due to an abnormally excessive discharge of neuronal activity in the brain which results in signs and symptoms that include loss of

		both consciousness and generalised body movement.[2]
Febrile seizure	**:**	Seizure occurring in a child with fever aged 6 months to 60 months who does not have an intracranial infection nor pathology, metabolic disturbance, and no history of afebrile seizure. [1]
Infection	**:**	An invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present in our body. [3]
Acute febrile illness	**:**	An illness characterised by a rapid onset of fever. The most common illnesses presenting with fever are acute infections regardless of the location in the body. [1]
Opinion room	**:**	A room in paediatric ward of the Pelonomi Tertiary Hospital that functions as a referral unit for acute paediatric cases. Referred patients are examined here first and the decision made on whether the patient needs to be admitted to the paediatric ward or not.
Meditech computer system	**:**	The computer-based system used at the Pelonomi Tertiary Hospital to register patients and produce discharge summaries and inpatient department reports.

Full blood count : A test that counts all the cells, namely red blood cells, white blood cells and platelets, which make up the blood. (3)

1. CHAPTER 1: LITERATURE REVIEW

1. Literature review

1.1 Febrile seizure

Febrile seizures occur in children with fever of greater than 38 degrees Celsius and without evidence of intracranial pathology [1]. The following are the most important conditions to exclude before a diagnosis of febrile seizure is made: Central nervous system infection, head trauma, epilepsy, electrolytes imbalance, hypoglycaemia, drug use or drug withdrawal [1]. Febrile seizures are the most common type of childhood seizures and affect 2-5% of neurologically healthy children between 6 months and 5 years [1, 13]. Age of peak incidence is 18 months [1, 10, 11]. Their high incidence and the fact that they tend to recur pose a great challenge in paediatric care. [1] The seizures affect children of lower socio-economic status more and this could be because of less access to healthcare services. [1]

Febrile seizures are classified into simple and complex febrile seizures depending on physical characteristics, duration and their recurrence patterns [1]. Simple febrile seizures are generalised tonic-clonic seizures that last less than 15 minutes (usually less than 5 minutes) [1]. They are followed by brief postictal drowsiness and an almost instant recovery [1, 13]. Simple febrile seizures do not recur within 24 hours and have no postictal neurological abnormalities [1]. Nonetheless, complex febrile seizures usually last more than 15 minutes [1, 2]. They can be focal, prolonged, and or recur within 24 hours [1]. They can also be associated with postictal neurological abnormalities [1, 2, 13]. Furthermore, febrile status epilepticus is a severe form of complex febrile seizure that is characterised by prolonged or recurrent seizures without full regain of consciousness [1]. It usually continues for more than 30 minutes [1]. Loss of consciousness happens to both simple and complex febrile seizures [1].

The exact causes of febrile seizures are unknown [13]. Some studies suggest that the causes are multifactorial with genetic and environmental factors playing major roles [1, 13]. There is strong evidence, from genetics perspective that family history of febrile seizures plays a role in the seizures' occurrence [1]. The developing brain is thought to be vulnerable to the effects of fever and cytokines; hence, febrile seizures occur mostly in children less than the age of three years [1, 13]. In this age group, the brain is still at peak of maturation process and brain neurons are easily excitable [1]. The deficiency of the following micronutrients has also been suggested as predisposing factors for febrile seizures: zinc, vitamin B 12, folic acid, selenium, calcium, magnesium and iron [1, 13]. The common underlying factor is the vulnerability of the developing brain to the effects of fever and cytokines especially interleukin 1 and 6 [1, 13].

Respiratory tract infection, especially upper respiratory tract infection, is the most common cause of febrile illness and fever [1]. About 80% of this infection is viral infection [1]. The rest is usually due to a bacterial infection affecting the respiratory system, gastrointestinal system and other systems [1]. Some studies have suggested that certain vaccines are associated with febrile seizure by causing fever [1]. Examples of such vaccines include the pneumococcal and influenza vaccine [1, 13]. Nevertheless, it has been noted that the risk of febrile seizures is minimal [1]. Some perinatal conditions that considered as risk factors for febrile seizures include intrauterine growth retardation (IUGR), prematurely born child, neonate treated with corticosteroids and prolonged stay in nursery [1, 13]).

Febrile seizures mostly occur during the first day of fever [1]. Simple febrile seizures account for 80-85% of all febrile seizures [1, 3]. A typical simple febrile seizure presents itself as a generalised tonic-clonic seizure and through a rolling back of the eyes with loss of consciousness [1]. Similar to other tonic-clonic seizures regardless of the cause, a child having a seizure may experience difficulty in breathing, cyanosis, pallor and foaming in the mouth [1]. Febrile status epilepticus can also happen but it is not common [1, 13]. This is when a seizure continues more than 30 minutes or is intermittent without regaining consciousness [1]. Febrile status epilepticus has increased risk of neurological sequelae such as hippocampal abnormalities [1, 3].

Diagnosis is mainly clinical from history and physical examination [1]. A detailed history should be taken to ascertain the presence of a fever and to find the source of the fever [1, 13]. In most cases, the source of fever will be obvious from the history that include the child having a diarrhoea or coughing or a runny nose. If the source of the fever cannot be identified, depending on the age of the patient and the clinical status, it is recommended to do a full septic work up [13]. Further inquiry must include family history of febrile seizures and a determination of whether the immunisations are up to date [1, 13]. All central nervous pathologies, such as any CNS trauma, history of epilepsy and neurodevelopmental issues, must be excluded from the history [1, 3]. Clinical examination should be carried out to confirm what would have been found from the history and to exclude meningitis through the absence of meningism especially if a child is more than 12-18 months [1, 3]. The important differentials to be excluded through history and clinical examination are as follows: rigors, febrile delirium, febrile syncope, breath holding spells, reflex anoxic seizures and evolving epileptic syndromes [13].

No investigations are usually necessary [1, 3, 13]. If there is a suspicion of other causes of a seizure, the necessary investigations are done and individualised [1]. The initial tests include full blood counts (FBC), urea, electrolytes and creatinine (UEC), calcium, magnesium and phosphate (CMP) and glucose [1, 13]. A lumber puncture (LP) is also done to exclude meningitis, especially in children less than 12 months of age, or if meningitis cannot be excluded clinically [1]. LP is individualised in those more than 12 months of age [1]. In the case of status epilepticus and or if there is a suspicion that the patient might be having meningitis, LP is mandatory to do [1]. Amplitude integrated electroencephalography (aEEG) can be considered in complex febrile seizures or seizures not associated with fever [1-2]. Brain imaging can also be individualised as per indication but this is usually not necessary [13].

Febrile seizures usually occur at home or somewhere else out of the health care facilities and rarely happens in hospital settings [1]. The diagnosis is made with inference from the history as the seizures usually would have resolved by the time of presentation to health care facility [1]. Simple

febrile seizures do not warrant admission, especially if the child is clinically well [1, 13]. Admission only occurs when the child presents with complex febrile seizures [1, 2, 13]. If the seizures occur in a health care facility, they are managed like any other seizure [1, 13]. The ABCDE (away, breathing, circulation, disability and exposure) approach is used [1, 13]. If seizures last more than five minutes, benzodiazepines that include lorazepam intravenous (IVI), diazepam IVI or per rectum, is used [1, 13]. Finally, a child that presents with status epilepticus are treated as an emergency with the algorithm for treating status epilepticus used, and if necessary, an admission to ICU is done [1, 3, 13].

Simple febrile seizures are benign and do not have neurological sequelae. However, febrile seizures can be very frightening to parents and some clinicians [1]. Parent might even think that their child is going to die or there will be severe brain damage [1]. This necessitates proper parent counselling about the nature and natural history of febrile seizures [1]. The risk of epilepsy is 0.5% higher in children with simple febrile seizures compared to those without febrile seizures [1]. Children with complex febrile seizures have a 6% higher risk of epilepsy. Recurrent or prolonged febrile seizures are associated with an increased risk of neurological abnormalities such as disruption of white matter maturation or damage in hippocampus leading to epileptogenesis [1-2].

Febrile seizures have a good prognosis [1]. They are self-limiting and children usually outgrow them by the age of 6 years [1, 13]. One third of children with febrile seizures have a risk of recurrence [1]. About 75 % of that recurrence occurs within one year and 90% within 2 years [1]. The following factors increase the risk of recurrence of febrile seizures: a child having the first episode of a febrile seizure while less than 15 months old, shorter interval between fever onset and seizure, family history of febrile seizures, a child who goes to day-care or crèche, complex febrile seizures and frequent febrile illness [1]. A higher number of risk factors increases the chances of seizure recurrence [1, 13]. The growth and neurodevelopment of children with febrile seizures are not affected especially if it is simple febrile seizures [1, 2, 3].

Febrile seizure prevention using anti-epileptic drugs is not recommended [1, 2, 13]. Studies demonstrate that the adverse effects of anti-epileptic drugs outweigh their benefits [1, 13,]. Antipyretic drugs, such as paracetamol and ibuprofen, are effective to lower the fever and discomfort. However, they have not been proven to prevent febrile seizures [1, 13]. Other measures that are used to lower body temperature, such as tepid sponging, removing clothes and direct fanning, have not been proven to lower the risk of febrile seizures [1]. Effective vaccination to reduce morbidity of febrile illnesses is universally encouraged [1]. Finally, proper counselling and reassuring of the parents about the nature and natural history of febrile seizures is very important prior to discharge [1, 3].

1.2 Iron deficiency anaemia

Anaemia is a decrease in the number of total red blood cells or haemoglobin (Hb) below normal for sex and age [2, 4]. The World Health Organization (WHO) defines anaemia in terms of ages as haemoglobin less than [4, 12, 14]:

- 11g/dl in children aged 6-59 months
- 11.5g/dl in children aged 5-11 years
- 12g/dl in children aged between 12-14 years

Anaemia can be classified as mild, moderate and severe based on how low the haemoglobin is in the blood [4, 14]. For example, in the 6-59 months age, Hb between 10-10.9g/dl is mild while that between 7 and 9.9 is moderate and less than 7g/dl is severe. Further classification is based on red cell characteristics and underlying mechanism [4, 14]:

a) Underlying mechanisms
    - Decrease bone marrow output issues such as
        - Bone marrow aplasia/infiltrate
        - Ineffective hematopoiesis
        - Substrate deficiency
        - Erythropoietin (EPO) insufficiency

- peripheral loss/destruction issues such as
    - Bleeding
    - Sequestration
    - Hemolysis

b) Red cell characteristics (red cell size, chromia and morphology)

- Hypochromic microcytic anemia
- Macrocytic normochromic anemia
- Normochromic normocytic anemia
- Morphology e.g. leuco-erythroblastic

(4, 12, 14)

Anaemia itself is not a disease but a sign or manifestation of an underlying condition [(2)]. It is common in developing countries to witness more than one underlying condition present in one patient [(2)]. The following are the common underlying conditions: nutritional deficiencies as in the case of iron deficiency anaemia, chronic infections as in the case of HIV and TB, and intestinal infestation leading to chronic blood loss [(2, 3)]. Finally, it is of paramount importance to establish the cause of anaemia before treating it [(2)].

Iron deficiency anaemia is anaemia due to lack of iron [(2, 3, 4)]. This leads to a decrease in red cell production and results in hypochromic microcytic anaemia [(2, 3)]. It is the most common cause of anaemia occurring in 15% of the world's population [(2, 3)]. Up to 50% of children in developing countries have been shown to be affected [(2)]. The children at highest risk fall in the six months to three years and adolescent age group [(2)].

Iron deficiency anaemia can be due to inadequate iron intake (nutritional or iron malabsorption) or excessive iron loss due to chronic bleeding [(4)]. Predisposing factors begin in the prenatal period and extent until adolescent age [(2)]. All the predisposing factors ultimately lead to the depletion of iron stores and iron deficiency, which eventually leads to iron deficiency anaemia. The perinatal examples include low birth weight, conditions that lead to perinatal bleeding like such as twin-to-twin transfusion, and haemorrhagic disease of a newborn [(2)]. Infections such as viral, bacterial and parasitic also contribute by impairing iron uptake and utilisation [(2)].

Diagnosis is made with good history taking that narrows down the differentials [2, 3]. Depending on the age of the child, the history aiming to pick up any predisposing factors can start from the prenatal and perinatal period [3]. Dietary and bleeding history are also very crucial components, which help in the diagnosis of iron deficiency anaemia [2, 3]. Furthermore, history should exclude any comorbidities and chronic illnesses such as TB and HIV.

The symptoms are usually vague at the beginning of iron deficiency anaemia and include lethargy, pica and irritability [2, 3, 4]. The development of iron deficiency anaemia is a gradual process such that the body usually adapts and the child may not feel sick most of the time [2, 4]. The diagnosis is usually an incidental finding when the child presents at the hospital for another illness such as pneumonia [2]. Physical examination is another important component [2, 3]. It assists in deciding on further laboratory examination [2]. Pallor is the cardinal sign for anaemia while Koilonychia is a common sign of iron deficiency anaemia [2,4].

Initial investigations include a full blood count (FBC) [2, 3, 4]. A peripheral blood smear, reticulocyte count and FBC help in making the diagnosis [2, 3]. The important parameters of the FBC for anaemia diagnosis are haemoglobin (Hb) concentration, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH) and red cell distribution width (RDW). The FBC, peripheral smear and reticulocyte count results assist in determining the tests to do next [3, 4, 14].

Iron deficiency usually precedes iron deficiency anaemia and it is characterised by low ferritin levels [14]. Failure to correct iron deficiency leads to iron deficiency anaemia [14]. The typical findings of iron deficiency anaemia for age 6-59 months are as follows [2]:

Microcytosis (MCV of less than 70fl)

Hypochromia (MCH of less than 25pg)

Reduced serum iron of less than 40ug/dl

Increased iron binding capacity of more than 450ug/dl

Decreased transferrin saturation of less than 16% and

Decreased serum ferritin of less than 10ng/ml [2].

Cases in which the history and clinical examination are suggestive of iron deficiency anaemia demand that iron studies should not be carried out [2]. Instead, a trial of treatment with iron is recommended provided a follow up is guaranteed [2]. Therefore, only in the absence of a response should iron studies be carried out [2].

The most important differential diagnoses to exclude are anaemia of chronic disease, thalassemia and sideroblastic anaemia [2, 3, 4, 14]. If the exclusion of these cannot be made from history, further laboratory studies can be done to exclude them [4]. Iron studies can also be done to confirm iron deficiency anaemia [2, 14]. Typical findings of iron studies are low serum iron, high transferrin, high total iron binding capacity and low ferritin [3, 4].

Treatment for iron deficiency anaemia starts with clearly identifying the cause [14]. The most common cause in children is dietary issues (lack of iron intake) [2]. The next step is to examine the severity of anaemia (mild, moderate and severe) [2, 14]. In mild to moderate cases, dietary modifications to incorporate iron-rich foods and oral iron supplementation are cornerstones of treatment [3, 14]. The elemental iron dose of 4-6mg/kg/day has a good response within four weeks [2, 4]. In rare situations where oral supplements are ineffective, such as in iron malabsorption, intolerance or adherence issues, intravenous iron can be used [2]. If the patient has severe symptomatic anaemia and/or is bleeding, a blood transfusion of packed red cells is done [2]. After haemoglobin (Hb) has normalized, treatment with iron supplements continues for 4-6 months in order to replenish iron stores [3, 4].

1.3 Association between febrile seizures and iron deficiency anaemia

Iron deficiency anaemia and febrile seizures are both common conditions in childhood [5]. The peak ages for febrile seizures is 14 to 18 months [5, 6]. This age group coincides with that of iron deficiency anaemia, which is from 6 months to 24 months [5, 6]. Iron plays an important physiological role in neurological function [5]. It plays a role in neurotransmitters metabolism such as GABA and serotonin [5, 6]. Iron is involved in enzyme metabolism like monoaminoxide and aldehidoxidase [5]. It also plays a role in myelin formation and brain energy metabolism mostly as a cofactor [5, 6]. Neurological symptoms of iron deficiency include weak memory, motor developmental delay, behavioural disturbances, poor attention span and learning deficit [7].

Various existing studies evaluated the association between febrile seizures and iron deficiency anaemia [6]. The results have not been consistent [6, 7]. Most of the studies show that iron deficiency anaemia is a modifiable risk factor for febrile seizures [5, 6, 7, 8, 9]. However, some studies show no association between both conditions [10, 11, 16]. No studies evaluating this association between these conditions have been published in South Africa. The above conflicting results were used as motivation to conduct the study. The fact that no similar studies have been conducted in South Africa, and in the Free State Province in particular, also contributed as a motivation for the study.

Case-control studies that were conducted elsewhere suggest that iron deficiency anaemia is a modifiable risk factor for febrile seizures [5, 8, 9]. Sreenivasa B *et al* conducted a prospective case control study in India in 2014. The total number of patients was 200 (100 cases and 100 control) in the age group 6 months to 6 years with the study conducted from July 2010 to October 2014. The results showed that iron deficiency anaemia was a modifiable risk factor for febrile seizures [5]. Tariq Saeed et al. also conducted a case control prospective study e in Pakistan in 2012 over a period of six months with total of 100 patients (50 cases and 50 control) [8]. The results show that iron deficiency anaemia is a risk factor for febrile seizures [8]. GHOSAL S *at al* also did a prospective case control study in Bangladesh in 2017 over a period of one year. The age group of children under study was 5 months to 6 years and these constituted a patient study population of 120 (60 cases and 60 control). The results show that iron deficiency anaemia was associated

with febrile seizures [9]. It is also shown that low serum ferritin levels <7ng/ml was associated with 49 times of having febrile seizures.

A prospective cohort study carried out by Srinivasa *et al.* shows the difference in iron deficiency anaemia between the children with febrile seizures and those without was statistically significant($p<0.05$) [6]. The study was done over a period of one year with 208 children aged 6 months to 5 years. The study population was divided into 108 cases and 100 controlled [6]. The conclusion made is that iron deficiency anaemia is a modifiable risk factor for febrile seizures [6]. The recommendation made highlighted the need to screen for and treat iron deficiency anaemia early to prevent febrile seizures [6]. Khan SA *et al* did a comparative study in Pakistan over a period of one year, which consisted of two groups as case and control with 80 patients in each group. The results show that febrile seizures were twice as common in children with iron deficiency anaemia compared to those children without iron deficiency anaemia (p value=0.003) [7].

Some studies show contrary results as they found no association between iron deficiency anaemia and febrile seizures [10, 11, 16]. Hasim G *at al*. report in a study carried out in Turkey that there was no statistical significant relationship between iron deficiency anaemia and febrile seizures [10]. The results of the study conducted by Ghasem M *at al*. in Iran also show no association between iron deficiency anaemia and febrile seizures [11].

The systematic and meta-analysis study done by Byung Ok Kwak *at al.* suggests that iron deficiency anaemia is associated with increased risk of febrile seizures (OR, 0.198; 95% CI, 1.26-3.13; p=0.003) [15]. This systematic review and meta-analysis consisted of 17 studies enrolling 2 416 children with febrile seizures and 2 387 children as control. The recommendation made calls for the need to conduct well-designed interventional studies to assess if iron supplement can prevent febrile seizures in the 3 months-6 years age group.

The above-mentioned meta-analysis makes it clear that there is a strong association between iron deficiency anaemia and febrile seizures and recommends the carrying out of an intervention study on the management of iron deficiency anaemia to prevent febrile seizures. This raises a question about those other studies, which show contrary results. Could it be that they were not conducted well or the number of sampling was not sufficient? Another important fact I observed is that those studies that showed no association between febrile seizures and iron deficiency anaemia are quite few compared to the other studies that showed the association between these two conditions.

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2. CHAPTER 2: ARTICLE MANUSCRIPT

Abstract

BACKGROUND: Febrile seizure is the most common seizure in children. It affects 2-5% of neurologically healthy children. Iron deficiency is the most common nutritional deficiency worldwide especially in developing countries. Studies carried out to determine the association between febrile seizures and iron deficiency anaemia have produced contradictory results.

OBJECTIVES: To evaluate the association, if any, between febrile seizures and hypochromic microcytic anaemia (iron deficiency anaemia). Hypochromic microcytic anaemia has been used as surrogate for iron deficiency anaemia.

METHODS: This was a retrospective cross-sectional study. Here, 244 patients admitted over the period of twelve months with infective febrile illnesses were included in the study. The age group was between six months and five years with 55 of these patients suffering from febrile illnesses complicating with febrile seizures while 189 had febrile illnesses without febrile seizures. Full blood counts (FBC) of all the patients were used to determine if each patient had hypochromic microcytic anaemia. The following FBC parameters were used: haemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW). Chi-square and Fischer's exact tests were conducted for statistical interpretation. A comparison of full blood count parameters was made between the children with febrile seizures and those without.

RESULTS: The mean age of all patients included in the study was 17 months with 153 of the 244 patients (62.7%) being males . The majority of patients had lower respiratory tract infection. Full blood count parameters of patients with febrile seizures and those without febrile seizures were similar. There was no statistical significance between the two groups (p=0.826).

CONCLUSION: The results of the study, based on hypochromic microcytic anaemia used as surrogate for iron deficiency anaemia, showed no association between iron deficiency anaemia and febrile seizures. This was due to a lack of difference between patient with febrile seizures and those without febrile seizures, with no statistical difference.

Introduction

Febrile seizures are the most common types of childhood seizures [(1)]. They occur in 2-5% of neurologically healthy children between the ages of 6 months to 5 years [(1, 13)]. The exact causes of

febrile seizures are unknown [1]. Genetic and environmental factors have a strong association with febrile seizures [1, 13]. The peak incidence age is 18 months, which overlaps with that of iron deficiency anaemia, which is from 6 months to 24 months [5, 6, 7]. Iron deficiency is the most common micronutrient deficiency worldwide and can be easily treated and prevented [2, 4]. Iron deficiency anaemia and febrile seizures are both common conditions especially in developing countries.

Studies have been done outside of South Africa to determine the association between iron deficiency anaemia and febrile seizures [5, 6, 7, 8, 9, 10, 11,16]. The results are often contradictory. Iron deficiency anaemia has been shown to be a risk factor for febrile seizures such that recommendations to screen for and treat iron deficiency anaemia to prevent febrile seizures have been made in these international studies [5, 6, 7, 8, 9]. Other studies show contrary results that show no association between iron deficiency anaemia and febrile seizures [10, 11, 16]. Pisacane *at al* report in their study that iron deficiency increased the risk of febrile seizures while Kobrinsky *at al* found contrary results [16]. Hasim G *at al* reported in a study he did in Turkey that there was no statistical significant relationship between iron deficiency anaemia and febrile seizures [10]. The results of the study conducted by Ghasem M *at al* in Iran also show no association between iron deficiency anaemia and febrile seizures [11].

There are no studies that determine the above conditions' association in the South African context. The aim of the study was to determine the association between hypochromic microcytic anaemia and febrile seizures in South African children, particularly in Bloemfontein. The results of the study could be used to create awareness on the association between the two conditions if there is any association. The results could also provide motivation to screen for iron deficiency anaemia in children with febrile seizures and initiate early treatment to minimise the risk of a febrile seizure in young children.

Hypochromic microcytic (HCMC) anaemia was used in the study as a surrogate for iron deficiency anaemia. This arose from the reality that data was collected retrospectively and iron studies are not routinely done in all patients admitted with febrile seizures. The most common cause of HCMC anaemia in the study setting is iron deficiency anaemia.

This research study was a retrospective, analytical cross-sectional study. The objective of the study, done at Pelonomi Tertiary Hospital from January 2019 to December 2019, was to determine the association between hypochromic microcytic anaemia and febrile seizures.

Methods

This study is based on a retrospective medical record review. The study design was an analytical, cross-sectional study conducted at Pelonomi Tertiary Hospital in Bloemfontein, Free State, South Africa. The study consisted of all children between the ages of 6 months and 5 years, admitted into the paediatric ward with acute infective febrile illnesses with or without febrile illness from 1 January 2019 to 31 December 2019. These patients also had a positive history of fever and or documented fever and had full blood count taken on admission or during the cause of stay in the hospital as per inclusion criteria. Children with history of the conditions such as neurodevelopmental delay, thalassemia, on iron therapy, metabolic seizure e.g. hypoglycaemia, hyponatremia etc., central nervous system (CNS) pathology e.g. epilepsy, cerebral palsy (CP), asphyxia history etc. and CNS infection e.g. meningitis and encephalitis were excluded in the study. The patients included in the study using the above-mentioned information amounted to 244. Most of the patients included in the study had the following conditions on admission as acute infective febrile illnesses: respiratory tract infection, urinary tract infection, gastrointestinal infection.

The researcher used the admission register book in the ward to identify the file numbers of patients admitted with acute infective febrile illnesses during the study period. The files were retrieved from the records department while electronic summaries on the Meditech system were also referred to. The researcher was responsible for screening each patient according to the inclusion and exclusion criteria, as well as the data collection process.

The data collection form was designed with the REDCap® (Research Electronic Data Capture) software. Study data was collected and managed using the REDCap® database hosted at the

University of the Free State. The REDCap® is a secure web-based software platform designed to support data capture for research studies. A pilot study was performed on 10 patients to assess the feasibility of the study prior collecting the rest of the data. The researcher entered the data in the database. The data was exported in Excel format for statistical analysis. The data was analysed by the Department of Biostatistics at the University of Free State. Data was summarised by means of frequencies and percentages for categorical data and means and standard deviation or medians and percentiles for numerical data. Subgroups were compared using 95% confidence intervals for difference in means or medians. Appropriate statistical testing (t-test, Mann-Whitney or Chi-squared test) was performed at 5% significant level.

Approval to conduct the research study was obtained from the Free State Department of Health while an ethics approval was obtained from the Health Science Research Ethics Committee (HSREC) of the University of Free State. The study was a retrospective medical record review, thus patient consent was not required. Each patient record was assigned with a numerical value in order to remove any patient identifying data.

Results

The study population consisted of 244 patients as explained in the methodology. Demographic characteristics, including gender and age are presented for the study population. The majority of patients included in the study were male (62.7%). The age of included patients ranged from six months to five years (median 17.0 months).

Table 1 summarises the type of acute infective febrile illnesses recorded for each patient upon admission. Most patients, 184 out of 244 (75.4%), presented with lower respiratory tract infection. There were also upper respiratory tract infections which were 26 out of 244 (10.6%) patients) and gastrointestinal tract infections which were 16 out of 244 (6.5%) patients).

Table 1: Febrile illness

	Frequency	Percentage (%)
Upper respiratory tract infection	24	10.0
Lower respiratory tract infection	182	74.5
Urinary tract infection	10	4.0
Gastrointestinal tract infection	16	6.6
Other (n=12)	12	4.9
Conjunctivitis	1	0.4
Kawasaki disease	1	0.4
Measles	1	0.4
Neck abscess	1	0.4
Pansinusitis	1	0.4
Septic arthritis	1	0.4
Submandibular abscess with fever	1	0.4
Tonsillitis	1	0.4
Source not identified	1	0.4
Source not recorded	1	0.4
Source of fever not recorded	2	0.9

The full blood count results of patients are presented in Table 2.

Table 2: Full blood count results of all patients (both with and without febrile seizures (FS))

First column represents children without FS and the second column represents those with FS

	Hb g/dL	Hb g/dL	MCV fL	MCV fL	MCH pg/cell	MCH pg/cl	RDW %	RDW %
n	189	55	189	55	189	55	189	55
Mean (SD)	11.1	11.3	78.6	78.3	24.7	24.5	14.8	14.6

Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, RDW: red cell distribution width

A summary of the nutritional status of the study population is presented in Table 3.

Table 3: Nutritional status: study population (those with febrile seizures and those without)

	without FS	With FS
MAM	20	8
SAM	9	1
Normal	156	45
Overweight	4	1

MAM: moderate acute malnutrition, SAM: severe acute malnutrition

The results indicated that most patients (82.3%) had normal nutritional status. Patients affected by moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) comprised 15.5% of the study population (11.5%) and (4.0%), respectively. The nutritional status did not seem to affect febrile seizures frequency, as is the case with other studies (refer table 4).

In total, 55 patients (22.5%) presented with febrile seizures, compared to 189 patients (77.4%) who did not present with febrile seizures. The duration of seizures was recorded to last less than 15 minutes in 30 patients (63.8%) compared to a duration of more than 15 minutes in 17 patients (36.2%).

A total of 29 patients (11.8%) had hypochromic microcytic anaemia on full blood count compared to 215 (88.1%) who did not have a hypochromic microcytic anaemia on full blood count (refer table 5). Out of 55 patients who presented with febrile seizures, only 4 were discharged on anti-convulsants.

The result of the aim of the study, namely, the association between hypochromic microcytic anaemia and a febrile seizure was evaluated using Chi-square and Fischer's exact tests (refer to Table 5). The p-value>0.05(0.826) indicate that there is no statistically significant association. The relative risk of those with anaemia compared to those without anaemia to have febrile seizures is 1.1 (95% CI 0.5; 2.2).The results from the statistical analysis are presented in Table 4 below.

Table 4: Statistical analysis results

	No	**Yes**
Febrile seizures	189	55
Hypochromic microcytic anaemia	215	29
Chi-Square	p=0.826	
Fisher's Exact Test. Two-sided Pr <= p	p=0.815	

Table 6: Discharged on antiepileptics (n = 244)

	Frequency	**Percentage (%)**
No	240	98.36
Yes	4	1.64

Discussion

The aim of this study was to determine the association between hypochromic microcytic anaemia and febrile seizures in the study population. Hypochromic microcytic anaemia was used as surrogate for iron deficiency anaemia. This was a retrospective study, hence iron studies results, which would confirm iron deficiency and iron deficiency anaemia were not available, as is not done routinely.

The sample consisted of 244 patients. The results showed no association between febrile seizures and hypochromic microcytic anaemia (iron deficiency anaemia), with a $p > 0.5$ ($p=0.826$).

Most of patients with febrile seizures had respiratory tract infection, followed by acute gastroenteritis and urinary tract infection. This is in keeping with literature in general regarding causes of febrile illnesses that trigger febrile seizures [1, 13]. Most of the patients with febrile seizures were males, which is also in keeping with the literature [1, 13]. Some patients with febrile seizures were examined in the opinion room and not admitted. No full blood count was done on these patients hence, they could not be included in the study as it was a retrospective study. This contributed to having fewer patients with febrile seizures in the study. This could have affected the results due to fewer patients during the study period. The inclusion of these patients, if they had full blood count results, would have led to an increase in number of patients included in the study. Larger sample sizes yield more reliable the results.

Numerous studies have been done to determine the association between febrile seizures and iron deficiency anaemia [5]. The results have been inconsistent [5]. This was a major motivation behind this study, which sought to evaluate the association between the two above-mentioned conditions in our study population. The first study by Pasacane *et al* in Italy in 1990s showed that iron deficiency anaemia was a risk factor for febrile seizures [6]. Shazia AK *et al* carried out a study in Pakistan with the results showing that iron deficiency anaemia was twice as common in children with febrile seizures compared to those without febrile seizures [7]. Another study conducted in India by Bharat G *et al* suggested that iron deficiency anaemia is a modifiable risk factor for febrile seizure.

The study by Eiham B *et al* in Iran, which was a prospective case control study, showed contrary results [16]. The results showed that iron deficiency anaemia was less frequent in children who had febrile seizures than those without. It also stated that iron deficiency anaemia was not protective against febrile seizures [16]. Ghasem *et al* also did a study, which showed no association and therefore did not show iron deficiency anaemia as a risk factor for febrile seizures [17]. The results of the later are similar to the findings of our study, which showed no association between iron deficiency anaemia and febrile seizures.

The conclusion from the two meta-analysis studies indicated that both conditions have an association whereby iron deficiency anaemia was shown as a modifiable risk factor for febrile seizures [15, 18]. Byung O *et al* did the meta-analysis in 2017 that consisted of 17 studies, which enrolled 2 416 children with febrile seizures and 2 387 children without febrile seizures, as control group. The results from the combined data suggested that iron deficiency anaemia was associated with increased risk of febrile seizures (OR of 1.98; 95% CI,1.26-3.1;P=0.003). Parviz K *et al* also did a meta-analysis in 2018, which consisted of 38 studies [18]. Their results showed that there was a strong association between iron deficiency anaemia and febrile seizures whereby iron deficiency anaemia increased the risk of febrile seizures with OR of 2.36(95% CI: 1.72-3.24; p<0.001). They also recommended screening for and treating iron deficiency anaemia in all children with febrile seizures [18]. Both high quality meta-analysis studies suggest that there is a strong association between febrile seizures and iron deficiency anaemia, which is what our study did not show. This could be due to several factors that include our study being a retrospective and therefore very limited with the study sample small that this could have affected results negatively.

Conclusion

This study's results showed no association between febrile seizures and iron deficiency anaemia in children between age of 6 months and 5 years that were admitted at Pelonomi academic hospital over a period of twelve months. Further studies, preferably prospective case control studies with a larger sample in the same population, should be conducted to evaluate the association between iron deficiency anaemia and febrile seizures. The studies should include iron studies, which will confirm the diagnosis of iron deficiency anaemia.

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3. APPENDICES

Appendix A: Letter of Approval from the Research Ethics Committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

17-Jul-2020

Dear **Dr Thozamile Madikizela**

Ethics Clearance: **Association Between Hypochromic Microcytic Anaemia and Febrile Seizure in Patients Admitted at Pelonomi Tertiary Hospital in Bloemfontein from January 2019 to December 2019**
Principal Investigator: **Dr Thozamile Madikizela**
Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2020/0686/2807**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00011992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa

Appendix B: Permission from the FS DOH

health
Department of Health
FREE STATE PROVINCE

01 June 2020

Dr. T Madikizela
Dept. of Paediatrics and Child Health
UFS

Dear Dr T Madikizela

Subject: Association between hypochromic Microcytic Anaemia and Febrile Seizure in Patients Admitted at Pelonimi Tertiary Hospital in Bloemfontein from January 2019 to December 2019.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of **Pelonomi Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeelats@fshealth.gov.za / makenamr@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- **Please discuss your study with Institution Manager on commencement for logistical arrangements see 2nd page for contact details.**
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- **As part of feedback you will be required to present your study findings/results at the Free State Provincial health research day**

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 2/6/2020

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za
www.fs.gov.za

Appendix C: Permission from the Evaluation Committee

UNIVERSITY OF THE FREE STATE
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YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

MASTER OF MEDICINE

This is to certify that the Departmental Research Meeting approved of the following MMed research protocol:

DATE OF MEETING	24 January 2020

DEPARTMENT	Paediatrics and Child Health
STUDENT NUMBER	2017444023
INITIALS AND SURNAME OF CANDIDATE	Dr. T. Madikizela
NAME OF DEGREE	MMed Paed
SUPERVISOR	Dr. Patricia Jardim Eleftheriou
CO-SUPERVISOR	Not Applicable

TITLE OF THE RESEARCH PROJECT
Association between hypochromic microcytic anaemia and febrile seizures in patients admitted at Pelonomi hospital from January 2019 to December 2019

Annatjie Bouwer

Digitally signed by Annatjie Bouwer
Date: 2020.04.29 10:42:56 +02'00'

RESEARCH CHAMPION — **DATE** 29 April 2020

SUPERVISOR(S) — **DATE** 30/04/2020

HEAD OF THE DEPARTMENT — **DATE** 29/04/2020

Appendix D: Biostatistics Approval Letter

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

24 April 2020

For attention: Health Sciences Research Ethics Committee
Faculty of Health Sciences

Title of project:

Association between hypochromic microcytic anaemia and febrile seizures in patients admitted at Pelonomi Hospital from January 2019 to December 2019.

Researchers:

T. Madikizela

I have given input regarding the above mentioned project's protocol on the following aspects of the protocol, namely the study design, sample, measurement, measuring instrument and statistical analysis.

Yours faithfully

Mpendulo Mamba

Departement Biostatistiek
Department of Biostatistics
T: +27(0)51 401 3114/5/6/7, F: +27(0)51 401 3641
205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
P.O. Box/Posbus 339 (G31), Bloemfontein 9300, South Africa/Suid-Afrika, www.ufs.ac.za

Appendix E: Permission from HOD

The Chair: Health Sciences Research Ethics Committee
Dr SM Le Grange
For Attention: Mrs M Marais
Block D, Room 104,
Francois Retief Building
Po Box 339 (G40)
Nelson Mandela Drive
Faculty of Health Sciences
University of the Free State
Bloemfontein
9300

28 April 2020

Dear Dr SM Le Grange

Dr T Madikizela (Student number: **2017444023**)

Protocol title: Association between hypochromic microcytic anaemia and febrile seizures in patients admitted at Pelonomi hospital from January 2019 to December 2019

I, Dr Nomakhuwa Elizabeth Tabane, hereby grant Thozamile Madikizela permission to conduct the above mentioned research project. The research will be completed in accordance with myself as Head of Department of Paediatrics and Child Health and Dr Patricia Jardim Eleftheriou as supervisor of this study.

Yours faithfully

29/04/2020

Dr N.E.Tabane
HOD: Paediatrics and Child Health Department
Universitas Academic Hospital and UFS: Faculty of Health Sciences
Bloemfontein
9300

Date

Department of Paediatrics and Child Health / Departement Pediatrie en Kindergesondheid
205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
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Appendix F: Data collection form

Confidential

ective study to determine the association between hypochromic microcytic anemia and febrile seizures done at Pelonomi hospital over a period of one year

Data Collection Form

Record ID	_____
Patient Date of Birth	_____
Age at presentation	_____
Gender/Sex	○ Male ○ Female
Febrile Illness	☐ Upper respiratory tract infection ☐ Lower respiratory tract infection ☐ Urinary tract infection ☐ Gastrointestinal tract infection ☐ Other
If other febrile illness, please specify	_____
Febrile seizures?	○ Yes ○ No
Duration of febrile seizures (indicate in minutes)	_____
FBC-HB	_____
FBC-MCV	_____
FBC-MCH	_____
FBC-RDW	_____
Hypochromic microcytic anaemia present?	○ Yes ○ No
C-reactive protein	_____
Was lumbar puncture done?	○ Yes ○ No
Was brain imaging(MRI/CT) done?	○ Yes ○ No

Confidential

Patient discharge on anti-epileptic?	○ Yes ○ No
Nutritional status:	○ Normal ○ Overweight ○ MAM ○ SAM

Appendix G: Author guidelines

Author Guidelines

Author Guidelines

Please view the Author Tutorial for guidance on how to submit on Editorial Manager.

To submit a manuscript, please proceed to the *SAJCH* Editorial Manager website: Editorial Manager

To access and submit an article already in production, please see the guidelines here.

Author Guidelines

Please take the time to familiarise yourself with the policies and processes below. If you still have any questions, please do not hesitate to ask our editorial staff (tel.: +27 (0)21 532 1281, email: submissions@hmpg.co.za).

Article Processing Charges

All articles published in the South African Journal of Child Health are open access and freely available online upon publication. This is made possible by applying a business model to offset the costs of peer review management, copyediting, design and production, by charging an article-processing charge (APC) of R3 180 (ex Vat) for all articles published. The charge applies only to Research articles submitted after 1 Jan 2019. The APC is standard and does not vary based on length, colour, figures, or other elements.

When submitting a Research article to the SAJCH, the submitting author must agree to pay the APC should the article be accepted for publication. The APC is payable when your manuscript is editorially accepted and before production commences for publication. The submitting author will be notified that payment is due and given details on the available methods of payment. Prompt payment is advised; the article will not enter into production until payment is received.

Queries can be directed to claudian@hmpg.co.za.

Please refer to the section on 'Sponsored Supplements' regarding the publication of supplements, where a charge is applicable. Queries can be directed to dianes@hmpg.co.za or claudian@hmpg.co.za

Authorship

Named authors must consent to publication. Authorship should be based on: (*i*) substantial contribution to conceptualisation, design, analysis and interpretation of data; (*ii*) drafting or critical revision of important scientific content; or (*iii*) approval of the version to be published. These conditions must all be met for an individual to be included as an author (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Protection of rights to privacy

Patient

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- The manuscript must be in Microsoft Word or RTF document format. Text must be 1.5 line spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes). Pages and lines should be numbered consecutively.
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
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- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

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Research

Guideline word limit: 3 000 words (excluding abstract and bibliography)

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

- May include up to 6 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.

- **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
- **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
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Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide evidence of consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
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- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
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- Ensure each table has a concise title and column headings, and include units where necessary.
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Rather:

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- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
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Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx/doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references
- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

  South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

  South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

  South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

  South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

  Rex v Jopp and Another 1949 (4) SA 11 (N)
  Rex v Jopp and Another: Name of the parties concerned
  1949: Date of decision (or when the case was heard)
  (4): Volume number
  SA: SA Law Reports
  11: Page or section number
  (N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.
  NOTE: no . after the v

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Appendix H: Turnitin report

Association Between Hypochromic Microcytic Anaemia And Febrile Seizures

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1	Pinnaka Subbarao, Puttagunta Sree Apoorva, Padmasani Venkat Ramanan. "TO EVALUATE THE RELATIONSHIP BETWEEN IRON DEFICIENCY AND FEBRILE SEIZURES", Journal of Evolution of Medical and Dental Sciences, 2019 Publication	1%
2	Alexander KC Leung, Kam Lun Hon, Theresa NH Leung. "Febrile seizures: an overview", Drugs in Context, 2018 Publication	1%
3	Shailaja Potdar, Sunil Junagade, Jayesh Panot, Vandana Kumavat, Mohit Rojekar V, Aniruddha Malgaonkar, Madhuri Bhusare. "CASE-CONTROL STUDY OF IRON DEFICIENCY ANAEMIA IN FEBRILE SEIZURES", Journal of Evolution of Medical and Dental Sciences, 2017 Publication	1%
4	Han Na Jang, Hoi Soo Yoon, Eun Hye Lee. "Prospective case control study of iron deficiency and the risk of febrile seizures in children in South Korea", BMC Pediatrics, 2019 Publication	1%

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Appendix I: Copy of the research protocol approved by the HSREC

REASEARCH PROTOCOL

Association between Hypochromic Microcytic Anaemia and Febrile Seizure in patients admitted at Pelonomi Tertiary Hospital in Bloemfontein from January 2019 to December 2019

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Faculty of Health Sciences

University of the Free State

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Selected definitions

Anaemia: a condition in which the number of red cells or haemoglobin concentration within them is lower than normal for age and gender [1, 4].

Iron: a trace element that is essential for life which is involved in many physiological processes such as DNA synthesis, forms part of haemoglobin which is essential for oxygen transport, energy production and also works as the cofactor in neuron's metabolism amongst other functions. [2,3,5]

Iron deficiency anaemia: anaemia which is due to decreased haemoglobin production due to insufficient iron in the body which can be due to inadequate intake or excessive loss. [4]

Hypochromic microcytic anaemia: anaemia in which the circulating red blood cells are smaller than the normal size and have decrease red colour. [2,4]

Fever: an elevated body temperature of more than 37.5 degrees Celsius [3]

Seizure: a condition due to an abnormally excessive discharge of neuronal activity in the brain which results in signs and symptoms like loss of consciousness, generalized body movement.[2]

Febrile seizure: seizure occurring in a febrile child between the age of 6 months to 60 months who does not have an intracranial infection nor pathology, metabolic disturbance and no history of afebrile seizure.[1]

Infection: an invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present in our body.[3]

Acute febrile illness: an illness characterized by rapid onset of fever. The most common illnesses presenting with fever are acute infections regardless or the location in the body. [1]

Opinion room: The room in paediatric ward of Pelonomi Tertiary Hospital which functions as a referral unit for acute paediatric cases. Referred patients are seen here first and the decision is made if the patient needs to be admitted to the paediatric ward.

Meditech computer system: the computer based system used at Pelonomi Tertiary Hospital for registering patient discharge summaries and inpatient department reports.

Full blood count: A test that counts all the cells that make up the blood, namely red blood cells, white blood cells and platelets.[3]

Introduction

Febrile seizure is the most common type of childhood seizure which occurs in 2-5% of neurologically healthy children between the ages of 6 months to 5 years. The exact cause of a febrile seizure is unknown, but genetic and environmental factors have been shown to have strong association with its occurrence. The peak incidence age is 18 months, which overlaps with that of iron deficiency anaemia which is from 6 months to 24 months. Iron deficiency is the most common micronutrient deficiency worldwide and can be easily treated and prevented. Iron deficiency has been postulated to be a risk factor for febrile seizures. Several studies have been done to look at the association between febrile seizures and iron deficiency however, none could be found that had been done in the South African population and none in Bloemfontein in particular. This is a great motivation for this study to be conducted.

1. Literature review

1.1. Febrile seizure

Febrile seizure is a seizure which occurs in neurologically healthy children and associated with fever of more than 38 degrees Celsius, without evidence of intracranial pathology. It is the most common type of childhood seizure affecting 2-5 % of neurologically healthy children between 6 months to 5 years. Age of peak incidence is 18 months. [1, 10, 11]

It is classified as simple or complex febrile seizure. Simple febrile seizure is generalized and lasting less than 15 minutes (usually less than 5 minutes), not recurring within 24 hours and no postictal neurological abnormalities. Complex febrile seizure is focal, prolonged or recurrent within 24 hours or associated with postictal neurological abnormalities. [1-2]

Febrile seizure has multifactorial causes. The common underlying factor is the vulnerability of the developing brain to the effects of fever. Other contributing factors are genetic predisposition (e.g. family history) and environmental factors. Viral infection is the most common cause of febrile illness (about 80 %) mostly affecting the respiratory system. The rest is usually due to bacterial infection affecting the respiratory system and gastrointestinal system. Other predisposing factors for febrile seizure include zinc deficiency as well as deficiencies in vitamin B 12, folic acid, selenium, calcium or magnesium.[1]

Mostly, febrile seizure happens during the first day of the fever. Simple febrile seizure accounts for 80-85% of all febrile seizures. Typical presentation involves loss of consciousness. Foaming at the mouth, difficulty in breathing and pallor or cyanosis may also occur. There is generalized tonic-clonic movement of the limbs and rolling back of the eyes. A severe form, which is called febrile status epilepticus, can also happen but is not common. This seizure continues more than 30 minutes without regaining consciousness. [1, 3]

Diagnosis is mainly clinical (history and clinical examination). No investigations are necessary unless there is a suspicion of other causes of a seizure and in that case the investigations are individualized. If blood tests are done, they usually include, full blood counts (FBC), urea,

electrolytes and creatinine (UEC), calcium, magnesium and phosphate (CMP) and glucose. Lumber puncture (LP) is also individualized especially in patients above 12 months of age. There is a strong recommendation to do LP in all patients under 12 months to exclude meningitis. Amplitude integrated electroencephalography (aEEG) is not routinely recommended for simple febrile seizure. It can be considered in complex febrile seizures or seizures not associated with fever. [1-2]

Febrile seizure is benign and does not have any neurological sequela. Febrile seizure increases the incidence risk of epilepsy by 0.5% compared to the general population. Complex febrile seizure has more incidence risk of epilepsy up to 6 %. Recurrent or prolonged febrile seizures is associated with increased risk of neurological abnormalities e.g. disruption of white matter maturation, damage in hippocampus leading to epileptogenesis. [1-2]

Most children with a simple febrile seizure do not need to be admitted to the hospital especially if they are clinically neurologically well. Admission is done when they present with a complex febrile seizure. If the child presents with status epilepticus, she/he is treated as an emergency and the algorithm of treating status epilepticus is used. [1, 3]

Febrile seizure has a very good prognosis, it is self-limiting and children outgrows it by the age of 6 years. One third of children with a febrile seizure has a risk of recurrence and about 75 % of that recurrence occurs within one year and 90% within 2 years. The following factors increase the risk of recurrence of febrile seizure: the first episode in a child less than 15 months old, shorter interval between fever onset and seizure (e.g. less than 1 hour), family history, day-care attendee, complex febrile seizures, and frequent febrile illness. The more the number of risk factors the higher the chances of recurrence. Most of children with febrile seizure have normal growth and development. [1, 2, 3]

Febrile seizure prevention using anti-epileptic drugs such as sodium valproate and phenobarbitone is not recommended because adverse effects of these drugs outweigh the benefits. Antipyretic drugs such as paracetamol and ibuprofen are effective to lower the fever and discomfort however have not been proven to prevent a seizure. Effective vaccination to reduce morbidity of febrile illnesses must be universally encouraged as a way to reduce febrile

illnesses. Proper counselling and reassuring of the parents prior to discharge is very important and must include all the above mentioned factors. [1, 3]

1.2. Iron deficiency anemia

Anemia, according to World Health Organization (WHO), is defined as hemoglobin less than:

- 11g/dl in children aged 6-59 months
- 11.5g/dl in children aged 5-11 years
- 12g/dl in children aged between 12-14 years

Anemia can be classified based on red cell characteristics and or underlying mechanism.

c) Red cell characteristics (red cell size, chromia and morphology)
    - Hypochromic microcytic anemia
    - Macrocytic normochromic anemia
    - Normochromic normocytic anemia
    - Morphology e.g. leuco-erythroblastic and micro/macroangiopathic

d) Underlying mechanisms
    - Decrease bone marrow output
        - Bone marrow aplasia/infiltrate
        - Ineffective hematopoiesis
        - Substrate deficiency
        - Erythropoietin (EPO) insufficiency
    - Peripheral loss/destruction
        - Bleeding
        - Sequestration
        - Hemolysis [4,12]

Iron deficiency anemia is an anemia due to lack of iron as a substrate, which leads to a decrease in red cell production resulting in hypochromic and/or microcytic anemia. It is the most common

cause of anemia occurring in 15 % of the world's population. It can be due to inadequate iron intake (e.g. nutritional deficiency or iron malabsorption) or excessive iron loss mostly due to bleeding.[4]

Proper diagnosis is made with good history taking, with the main focus on dietary and bleeding history. A good history also helps to exclude other causes of anemia and narrow the differential diagnosis even before laboratory results are available. Physical examination is another important component. It also assists in deciding on further laboratory examination. Pallor is the cardinal sign for anemia. Koilonychias are also commonly seen in iron deficiency anemia. [2, 4]

Full blood count, differential count, reticulocyte count and microscopic smear are the starting point of the investigations. These results will determine which tests to do next.

In iron deficiency anemia, the typical findings will be as follows:

- microcytic and/or hypochromic red cells
- low ferritin
- raised transferrin

Most important differential diagnoses to be excluded are anemia of chronic disease, thalassemia and sideroblastic anemia. [3, 4]

Mainstay treatment for iron deficiency anemia is oral iron supplements (oral ferrous sulphate). The dose of 4-6mg/kg/day usually has a good response within four weeks. In rare situations whereby oral supplements are ineffective e.g. in iron malabsorption or intolerance, intravenous iron is used. If the patient has severe symptomatic anemia and/or is bleeding, a blood transfusion of packed red cells is done. After hemoglobin (Hb) has normalized, treatment continues for 4-6 months which is aimed at replenishing iron stores. [3, 4]

1.3 Association between febrile seizures and iron deficiency anemia

There has been exploration of the association between iron deficiency anemia and febrile seizures. Iron plays an important physiological role in neurological function for example in neurotransmitter metabolism like GABA and serotonin and enzyme metabolism like

monoaminoxide and aldehidoxidase. It also plays a role in myelin formation and brain energy metabolism mostly as a cofactor. Several studies have been done elsewhere in the world except in South Africa and in Bloemfontein in particular which suggest that iron deficiency anemia is one of the risk factors for febrile seizures. [5, 6,7,8,9, 11]

A study that was done in 2015 in Basaveshwara Medical College Hospital Chitradura in India by Sreenivasa at al. showed an association between iron deficiency anemia and febrile seizure. It was a case control prospective study done from July 2010 to October 2014. There were 100 cases (children within the age group of the study presenting with febrile seizures) and 100 controls (children within the age group of the study presenting with febrile illness but without seizures). Both cases and controls were matched in terms of age (which was between 6 months and 6 years) and sex. In both cases and controls, febrile illnesses were similar, being upper and lower respiratory tract infection and gastroenteritis. It was observed that serum ferritin was low in cases (29.5 ± 21.3) compared to controls (53.3± 37.6) and red blood cell distribution width(RDW) was high in cases (16.8±1.3) and low in controls (12.7±1.1). [5]

Srinivasa et al conducted a cohort study in India looking at iron deficiency anemia in children with febrile seizures. The study was done by the department of pediatrics, KIMS Bangalore from July 2013 to June 2014. Children were in the age group of between 6 months and 5 years. The study consisted of 208 children who met inclusion criteria and of which 108 were cases (children with simple febrile seizures) and 100 were controls (children with febrile illness without seizures). 37 out of 108 cases had iron deficiency anemia (39.96%) whereas only 22 out of 100 controls had iron deficiency anemia (22%). The difference in relation to iron deficiency anemia among the two groups was significant with p value of <0.05. [6]

A study that was done in Pakistan by Khan SA et al also showed an association between iron deficiency anemia and a febrile seizure. It was a comparative study done in Kuwait teaching hospital Peshawar and Hayatabad Medical complex Peshawar from January 2015 to December 2015. Total of 160 children were included of which 80 were cases (children with febrile seizures) and 80 were controls (children with febrile illness without seizures). The age group was between six months and five years. The results were as follows; hemoglobin level was 10.48±1.16g/dl in

the cases group while in the controls group it was 12±2.31g/dl with a p-value of 0.004. The MCV level was 71±6.63 in cases group and 77±6.00 in the controls group with p -value of 0.002. The serum ferritin was 10±1.82 in the cases group and 15±2.12 in the controls group with the p-value of 0.002. The results showed the frequency of iron deficiency anemia to be 38% in cases group while the rate in the controls group was 20%. They concluded that febrile convulsions were twice as common in children with iron deficiency anemia compared to those children without iron deficiency anemia. [7]

Tariq Saeed et al did a case control prospective study at Holy Family Hospital, Rawalpindi from 1st of October 2011 to 31st march 2012 in the pediatric ward. The study had a total of 100 children, 50 as cases (children with febrile seizures) and 50 as controls (children with fever but no seizures). The age group was between 6 months to 5 years. Serum ferritin levels were compared. In the cases group, serum ferritin levels were 6.9 ± 0.9 microgram per liter(ug/l). In the controls group ferritin levels were 7.9 ± 1.72ug/l. It was shown that in the cases group 32 out of 50 patients had low ferritin levels while in the control group only 21 out of 50 patients had low ferritin levels. That showed a positive association between iron deficiency anemia and febrile seizures. [8]

A case control study was conducted at pediatric department of SSMC & Mitford Hospital over a period of one year. There were 60 cases (children with febrile seizures) and 60 controls (children with fever without seizures). Both cases and controls were matched according to age group which was between 5 months and 6 years. Causes of febrile illness in the two groups were similar. The results showed that a significant number of cases had low ferritin levels, low MCV and low MCH which were all statistical significant as compared to control group. The p-value of < 0.001 in all 3 parameters (MCV, MCH and ferritin), therefore showing that iron deficiency anemia increased the risk of febrile seizures. [9]

2. Defining the research

2.1. Research motivation

Iron deficiency anemia and febrile seizure are both common conditions. Studies done outside of South Africa have shown an association between these two conditions. [5, 6, 7, 8, 9, 10, 11] During literature review search, no studies were found to have been done and or published about the above conditions' association in the South African context. This study seeks to determine this association in South African patients. This will bring awareness to the health care providers of the association between the two conditions so that iron deficiency anemia can be screened for and treated early to minimize the risk of a febrile seizure in young children. Also to always investigate and treat iron deficiency anemia in all patients who present with febrile seizures to prevent possible recurrence. The hypochromic microcytic (HCMC) anaemia will be used in the study as a surrogate for iron deficiency anaemia because this is a retrospective study and iron studies are not routinely done in all patients admitted with febrile seizures. In our setting the most common cause of HCMC anaemia is iron deficiency anaemia.

2.2. Aim

To determine the association between hypochromic microcytic anemia and a febrile seizure. The study will be done at Pelonomi Tertiary Hospital from January 2019 to December 2019.

3. Study methods

3.1. Study setting

The study will be conducted at Pelonomi Tertiary Hospital in Bloemfontein. Children presented with acute infective febrile illnesses to the paediatric opinion room will be included in the study. Opinion room is a room in the paediatrics ward in the hospital which functions as a referral unit for acute pediatric patients. Referred patients are seen there first. They are evaluated and a decision is made if the patient needs admission to the paediatric ward. Referrals come from the

surrounding clinics, Pelonomi casualty and district level hospitals within the drainage area of Pelonomi Tertiary Hospital.

3.2. Study design

This will be a retrospective analytical cross sectional study. It will be done over a period of twelve months from the 1st of January 2019 to 31st of December 2019.

3.3. Target Study population and sampling

Target population will be the children with the age range of 6 months up to 5 completed years that presented at Pelonomi Tertiary Hospital with acute infective febrile illness over the period of 12 months from January 2019 to December 2019. Per month, approximately 50 patients present to Pelonomi opinion room with acute infective febrile illnesses and roughly 10 of those patients have febrile seizures. In a year, about 600 patients with acute infective febrile illnesses are usually seen. The study will include all patients who meet the inclusion criteria, hence no sampling will be required.

Inclusion criteria

Patients with the following will be included:

- The age range of 6 months up until 5 completed years.
- Presented with acute infective febrile illnesses.
- Positive history of fever and or documented fever.
- Full blood count results.

Exclusion criteria

All patients with the following will be excluded:

- Neurodevelopmental delay
- Known with thalassemia

- On iron therapy
- Metabolic seizure e.g. hypoglycemia, hyponatremia etc.
- CNS pathology e.g. epilepsy, cerebral palsy (CP), asphyxia history etc.
- Central nervous system (CNS) infection e.g. meningitis and encephalitis

3.4 Measurements

The researcher will use the admission register book in opinion room to identify all the patients that were admitted with acute infective febrile illnesses applying the above mentioned inclusion and exclusion criteria. Using those names and hospital numbers from admission book the files of the patients will be retrieved from the records. Meditech summaries will also be used as the source of the information. The full blood count results will also be in the files and in Meditech summaries. The researcher will be responsible for all data collection process involved above.

3.5 Data capturing

A data collection form has been designed with the REDCap (Research Electronic Data Capture) software to collect the following variables:

- The patient's date of birth
- The age at presentation
- The gender
- The nutritional status
- The body temperature
- The immunization status
- C-reactive protein
- Lumber puncture done? Yes or no
- Brain imaging e.g. MRI CT brain done? Yes or no
- The patient discharged on anti-epileptics? Yes or not
- Acute infective febrile illness
- Febrile seizure duration

- The full blood count components such as: Haemoglobin (HB)

: Mean corpuscular volume (MCV)

: Mean corpuscular haemoglobin (MCH)

: Red cell distribution width (RDW)

: Hypochromic microcytic anaemia (MCHC):

yes or no

A data collection form will be designed with the REDCap® (Research Electronic Data Capture) software. Study data will be collected and managed using the REDCap database hosted at the University of the Free State. REDCap is a secure, web-based software platform designed to support data capture for research studies.

The researcher will enter the data in the database. The researcher and study leader will have full access to the study records at all times during the data collection period. The data will be exported in Excel format for statistical analysis.

3.6 Data analysis

The data will be analyzed by the Department of Biostatistics at the University of Free State. Data will be summarized by means of frequencies and percentages for categorical data, and means and standard deviation or medians and percentiles for numerical data. Subgroups will be compared using 95% confidence intervals for difference in means or medians. Appropriate statistical testing (t-test, Mann-Whitney or Chi-squared test) will be performed at 5% significant level.

4. Statistics

4.1. Pilot study

Once approval has been obtained from Health Science Research Ethics Committee, the

researcher will commence with the pilot study. The pilot study will aid in revealing the prior unforeseen challenges of the study and to prove or disprove its feasibility. Any problems noted during the pilot study will be accounted for and changes will be made accordingly. Five files will be used for the pilot study. These files will be included as part of the study if there are no changes.

5. Value of the study

This study will be very valuable and helpful to both patients and health care workers in Bloemfontein and South Africa as a whole. It will bring awareness to health care workers of the association between iron deficiency anemia and a febrile seizure.

6. Limitation of the study

Due to the study being retrospective, results will be limited to blood tests already done. For this reason, hypochromic microcytic anemia will be used as an indicator/surrogate for iron deficiency anemia as iron studies are seldom done routinely in the study population. Another limitation will be incomplete or missing data on the files or Meditech.

7. Budget

The budget of the study will be approximately R800 (eight hundred rand). This budget will cover all the expected costs for stationery, printing and binding of the manuscript and will be on the account of the researcher. The cost will be split as follows:

- Paper/stationery - R150.00
- Printing/binding - R500.00
- Transport to and from Pelonomi hospital (5.6kmx2 at R11.2 per km) - R150
- Total R800

8. Time frame/time line

Planning of the protocol including literature review: June-July 2019

Approaching biostatistics : January 2020

Ethics committee submission : May 2020

Department of health submission: June 2020

Pilot study and Data collection : July 2020

Data analysis : August 2020

Write up : September-October 2020

9. Ethical considerations

Prior to data collection, approval for this study will be obtained from the Health Science Research Ethics Committee (HSREC) of the University of Free State as well as the Free State Department of Health.

As the study will be retrospective and data will be obtained from patient files, patient consent is not required. The researcher will be the only person responsible for collecting data. Each patient record will be assigned with a numerical value therefore excluding any patient identifying data from each case.

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11. Appendices

Appendix A: Lay term summary

Fever is the increase in body temperature caused mostly by infection. Infection is when a germ gets into the body and makes a person sick. Seizure is when the brain function is abnormal. Seizure makes the body to shake uncontrollably, a person may fall down and roll eyes. During a seizure a person loses awareness and is unable to communicate.

Anaemia is when haemoglobin is low. Haemoglobin is the most important component in the blood. It has many functions and one of them is to carry oxygen throughout the body. There are many causes of low haemoglobin. The most important cause is a lack of a substance called iron in the body. Iron is a very important substance that helps the body to stay healthy. We get iron from eating healthy food. Febrile seizure is when person who has fever develops a seizure.

Infection is very common is children. A child can have up to ten infections per year. When a child has infection, he/she also tends to have fever. Fever increases the chances of having febrile seizure. Some studies done outside South Africa have shown that a child who has fever and also has anaemeia has higher chances of having a febrile seizure than a child without anaemia . The aim of the study is to see if the same also happens in South African people (population) especially in Bloemfontein.

We shall do the study at Pelonomi Tertiary Hospital. We shall include children aged between six months and five years who were admitted between 1st of January 2019 and 31st December 2019. We'll collect information from the medical files and fill it into the data form called REDCap. We'll then send the information to a statistician for analysis. The collected information will not include anything that will lead to identification of participants. If the results show that anemia increases the chances of having a febrile seizure, then we shall make the health care workers to be aware of that and encourage them to look for and treat anaemia in all children to prevent febrile seizures. We also hope this study will motivate future studies to expand on this information.

Appendix B: Budget

The budget of this study will be approximately R800 (eight hundred rand). This budget will cover all the expected costs for stationery, printing and binding of the manuscript and will be on the account of the researcher.

The cost will be split as follows:

Paper/stationery	R150.00
Printing/binding	R500.00
Transport to and fro PAH (5.7km x2) at R11.2 per km	R150.00
Total:	R800.00