Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: F

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A New Dysmorphic Syndrome

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Highlights

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VOLUME 20 ISSUE 1 VERSION 1.0

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Global Journal of Medical Research: F
Diseases
Cancer, Ophthalmology & Pediatric

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Volume 20 Issue 1 (Ver. 1.0)

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Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
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Offset Typesetting

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Packaging & Continental Dispatching

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Contents of the Issue

Global Journal of Medical Research: F
Diseases
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Reverse Slanting of Split Eyebrows and Palpebral Fissures: A New Dysmorphic Syndrome

By Aamir Jalal Al Mosawi

Abstract- A dysmorphic syndrome is suspected in the presence of more than three minor anomalies which are variations of normal morphological features that are considered of little or no known medical, surgical, or cosmetic significance; more than one major anomaly which is an abnormality that has major medical, surgical or cosmetic significance; and one major anomaly with two or more minor anomalies are also suggestive of congenital syndrome.

Many congenital syndromes are associated with different combinations of hypertelorism (with or without flat mid-face), epicanthic folds, convergent squint, low set ears, upward and downward slanting of the palpebral fissures, and eyebrows abnormalities occurring in association with hypotonia and developmental delay.

The aim of this paper is to describe the occurrence of a new congenital syndrome with the novel association of unique eyebrows abnormalities (splitting with a relatively thick upward slanting medial parts and thin non-slanting lateral parts) with downward slanting palpebral fissures, bilateral convergent squint, hypertelorism with flat mid-face, epicanthic folds, large ears, developmental delay, and infantile hypotonia mostly attributed to congenital myopathy.

GJMR-F Classification: *NLMC Code: QS 675*

REVERSESLANTINGOFSPLITEYEBROWSANDPALPEBRALFISSURESANEWDYSMORPHICSYNDROME

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I. Introduction

A dysmorphic syndrome is suspected in the presence of more than three minor anomalies which are variations of normal morphological features that are considered of little or no known medical, surgical, or cosmetic significance; more than one major anomaly which is an abnormality that has major medical, surgical or cosmetic significance; and one major anomaly with two or more minor anomalies are also suggestive of congenital syndrome. Many congenital syndromes are associated with different combinations of hypertelorism (with or without flat mid-face), epicanthic folds, convergent squint, low set ears, upward and downward slanting of the palpebral fissures, and eyebrows abnormalities occurring in association with hypotonia and developmental delay [1,2,3,4].

The aim of this paper is to describe the occurrence of a new congenital syndrome with the novel association of unique eyebrows abnormalities (splitting with a relatively thick upward slanting medial parts and thin non-slanting lateral parts) with downward slanting palpebral fissures, bilateral convergent squint, hypertelorism with flat mid-face, epicanthic folds, large ears, developmental delay, and infantile hypotonia mostly attributed to congenital myopathy.

II. Case Report

A thirteen-month old boy who was the first born child to non-consanguineous parents was first seen at the pediatric neuropsychiatry clinic of the Children Teaching Hospital of Baghdad Medical City because of motor developmental delay. The child had hypotonia during infancy, and was not crawling and was unable to sit without support for long time. He has just started babbling. The boy has distinctive facial features (Figure-1) including:

1. Highly specific unique eyebrows abnormalities consisting of splitting with a relatively thick upward slanting medial parts and thin non-slanting lateral parts.
2. Downward slanting palpebral fissures.
3. Epicanthic folds.
4. Hypertelorism.
5. Depressed nasal bridge.
6. Large ears.
7. Convergent squints of both eyes.

Brain MRI was performed at the age of one month showed normal findings.

Screening for several inborn errors of metabolisms has already revealed no abnormality.

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Figure 1: The boy unique eyebrows abnormalities consisting of splitting with a relatively thick upward slanting medial parts and thin non-slanting lateral parts in association with downward slanting palpebral fissures, bilateral convergent squint, hypertelorism with flat mid-face, epicanthic folds, and large ears.

EMG and nerve conduction studies were performed at the age of seven months (Table-1).

Nerve conduction study (Table-1) was performed by surface and needle electrode on:

Right and left median nerve.
Right ulnar nerve.
Right and left sural nerve.
Right and left common peroneal nerves.

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Repetitive nerve stimulation with supra-maximal stimulation of the right ulnar, right facial and right axillary at low rates (3Hz) was performed. The right and right axillary decrement test with 10 pulses (5 trials) showed 1% decrement of motor response.

Needle electromyography (EMG) study was performed on:

Right FDI.
Right deltoid.
Right biceps.
Right and vastus medialis.
Right anterior.

Needle electromyography (EMG) stud showed:

No spontaneous activity.

No myotonic discharges.

The average duration of 20 motor units:

Right deltoid= 5.1 msec (n=8.3 msec).
Right biceps = 4.8 msec (n=8.1 msec).
Right vastus medialis = 4.1 msec (n=8.3 msec).
Right tibialis anterior = 5.3 msec (n= 10.2 msec).
Left tibialis anterior = 5.2 msec (n= 12.5 msec).

30-40% polyphasia of short duration low amplitude was observed.

EMG and nerve conduction studies suggested chronic diffuse non dystrophic myopathic of moderate severity mostly resulting from congenital myopathy.

The proximal lower limb muscles were more severely involved.

Table 1: Summarizes the clinical features of the new syndrome

	Sensory			Motor			
Nerve	Latency msec/cm	Amplitude μV	SNCV m/sec	Muscle	DML Msec /cm	MNCV msec /cm	F-wave Latency
Right median	2.1	26.6	56.2	ABP	3.1	50.2	16.5
Right ulnar	1.9	27.3	56.6	ADM	2.9	51.2	17.2
Right common peroneal				Tibialis Ant. EDB	3.3 4.2	40.2	35.3
Left common peroneal				Tibialis Ant. EDB	3 4.1	40.3	36.3
Left sural	2	15.3	44.6				
Table 4.1: The finding of EMG and nerve conduction studies which were performed at the age of seven months							

Table 2: The clinical features of the new syndrome

Sporadic occurrence
Non consanguineous parents
Splitting of eyebrows with a relatively thick upward slanting medial parts and thin non-slanting lateral parts
Downward slanting palpebral fissures
Epicanthic folds
Hypertelorism
Depressed nasal bridge
Large ears
Convergent squints of both eyes.
Infantile hypotonia attributed to congenital myopathy

Acknowledgement

The author would to express his gratitude for the parents of the patients who accepted publishing his photos.

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Global Journal of Medical Research: F
Diseases
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Successful Management of Spondylodescitis in a three Years Old Girl: A Case Report

By Khadija Saleh, AL Zahraa Hamed, Ali AL Sharqi & Hilal Al Hashami
Sultan Qaboos University

Abstract- Spondylodescitisis considered one of the rare diseases that cause back pain. The disease pathology is not yet been clearly known, however, in most patients the disease thought to be spreading hematogenously from a previously existing site of infection. We report two years and 11months old child, previously healthy girl, presented to the emergency department with twoweeks' history of weakness of the lower extremities and lumbar back pain with slightly arched back. She had a complete recovery with early intervention and complete course of antibiotics.

Keywords: *spondylodiscitis, MRI, Staph. aureus, antibiotics, case report, Oman.*

GJMR-F Classification: *NLMC Code: WE 346*

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Successful Management of Spondylodescitis in a three Years Old Girl: A Case Report

Khadija Saleh [α], AL Zahraa Hamed [σ], Ali AL Sharqi [ρ] & Hilal Al Hashami [ω]

Abstract- **Spondylodescitisis considered one of the rare diseases that cause back pain. The disease pathology is not yet been clearly known, however, in most patients the disease thought to be spreading hematogenously from a previously existing site of infection. We report two years and 11months old child, previously healthy girl, presented to the emergency department with twoweeks' history of weakness of the lower extremities and lumbar back pain with slightly arched back. She had a complete recovery with early intervention and complete course of antibiotics.**

Keywords: spondylodiscitis, MRI, Staph. aureus, antibiotics, case report, Oman.

I. Introduction

Spondylodiscitis (SD), is infectious process of the spine involving vertebral bodies and intervertebral discs. It remains a rare condition with an estimated incidence of around one to two cases in 30000.[2] this case report describes spondylodescitis in tow years old girl who presented with acute back pain, irritability and inability to walk. SD although it's rare disease, it should be kept as one of the deferential diagnosis in children present with non-traumatic back pain. *Staphylococcus aureus* is the causative agent of SD accounting for 80% of the cases. [2,7,14,15,16,17,18] Treatment of SD is usually a combination of both pharmacological and non-pharmacological.

II. Case Presentation

A two years and 11 months old toddler, previously healthy girl, presented to the emergency department in a tertiary center in Muscat, Oman in 2019, with a two weeks' history of weakness of the lower extremities with back pain and slightly arched back. There was no history of trauma, unexplained weight loss, or any other systemic manifestation. There was no history of fever, joint pain or skeletal deformity, skin rash, seizure, or photophobia. She was not known to have any chronic diseases. She was up to date with her vaccinations. Her parents reported no exposure to individuals with similar symptoms. In addition, none of her family members and neighbors had recently suffered from chronic cough or unexplained weight loss. There was no history of previous admission.

On admission, she was irritable, her vital signs were within normal ranges. Her physical examination revealed normal gait with slight hyperextension of lower back. In addition, she was bearing weight with support due to pain and there was slight pain in lower back while flexion and extension of the back. Otherwise, no muscle wasting, full range of movement of all joints actively and passively, with normal tone, power and reflexes. A blood investigations revealed complete blood count: Hemoglobin of 9.2 g/dl, increase in platelet count of 736 * 10^9 /L, normal white cell counts and slight increased level of acute phase reactants C-reactive protein(CRP)of 11.1 mg/L and erythrocyte sedimentation rate of 42mm/hour. Pelvic X-RAY was done and was reported as normal and Ultrasound hip showed no fluid in hip joint. She was started on non-steroidal anti-inflammatory medications and on vancomycin and ceftriaxone but the next day vancomycin was switched to flucloxacillin and child showed clinical improvement. Lumbar puncture was done as she was inactive and it showed normal microscopy, count, protein and glucose, with negative CSF and blood culture. On day five of admission, MRI done which showed bone marrow changes of L4 and L5 vertebrae associated with endplates irregularities and mild destruction, with loss of intervertebral space and indentation of thecal sac suggestive of spodilodescitis due to pyogenic or granulomatous infection. Child was tested for Q fever, tuberculosis and brucellosis in which all test were negative. Child was treated with ceftriaxone and flucloxacillin for two weeks as she was responding to the treatment and finally discharged on oral cefdinir for four weeks after consultation with infectious disease doctor. On subsequent follow up as outpatient she showed marked improvement and she started to regain her full movement of lower limbs.

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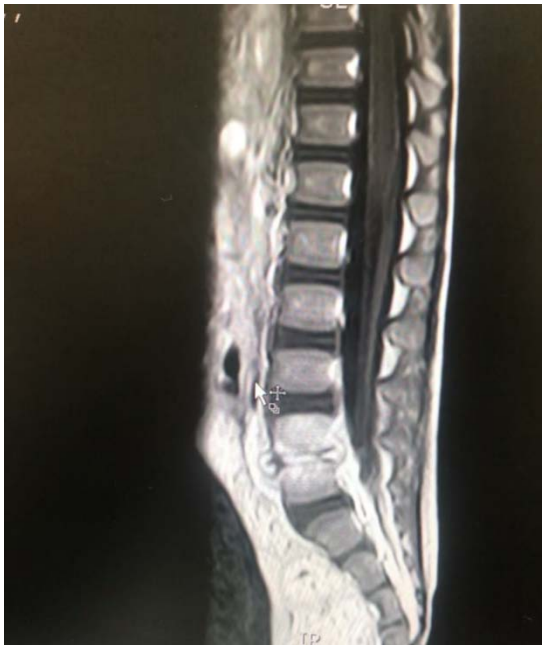

Figure 1: MRI of the patient reported in the case show, Bone marrow changes of L4 and L5 vertebrae associated with endplates irregularities and mild destruction, loss of intervertebral space and indentation of the thecal sac suggestine of spondylodescitis due to pyogenic or granulomatous infection.

III. Discussion

We report almost three years old girl with two weeks' history of lower back pain and lower limb weakness in whom MRI spine showed destructive changes of L4 and L5 with high ESR of 42 mm/hour and all other tests were not significant. When lumbar pain is accompanied by significant irritability, (as was evident in our case), this should lead pediatrician to include infectious discitis among the differential diagnosis. Kang et al[1] reported that approximately 60% of discitis and SD cases were diagnosed in children <3 years old showed that irritability was the most common among all other symptoms at the time of disease presentation. Discitis and SD are infectious processes of the spine involving vertebral bodies and intervertebral discs. It remains a rare condition with an estimated incidence of around one to two cases in 30,000 [2]. In our case, there was no delay in establishing the correct diagnosis where it has been established in the fifth day of admission. Delays of diagnosis for four to six months have been reported [2,3,4]. These delays are attributed to the often non-specific clinical presentation of children with discitis or SD and their inability to describe the site of discomfort [2,5,6]. This delay can lead to an increase risk of permanent abnormalities [7]. Its pathophysiology has not yet been clearly established, but in most patient, pathogens reach the spine hematogenously, starting from a previously existing site of infection [2,7]. A prodrome with a distant focus of infection has been identified in most cases. Mylona et al[8], described these to include the genitourinary tract 17%, skin and soft tissue 11%, intravascular devices 5%, gastrointestinal tract 5%, respiratory tract 2% and the oral cavity 2%. A wide range of pathogens can cause this disease and many studies showed it is primarily monomicrobial bacterial infection. Many attempts to identify the causative pathogen of Discitis and SD of children through blood and/or vertebral aspiration cultures have failed to identify the organisms; causing related problems in selecting the most appropriate antibiotic therapy [9,10,11,12,13]. When positive, pyogenic bacteria are usually detected, with *Staphylococcus aureus* being the cause of discitis and SD in approximately 80% of the cases that occur in first months of life and in most of those that develop in older children [2,7,14,15,16,17,18]. The most specific imaging method to diagnose discitis is MRI[19]. Intravenous antibiotic treatment, analgesia and physical rehabilitation treatment showed complete recovery in most cases. Treatment include pharmacological like antibiotics and non-pharmacological such as physiotherapy and bed rest[20]. Mortality has dropped from 25-26% [21,22] to less than 5% [23] with antibiotics treatment.

IV. Conclusion

SD is a rare disease which represents an important disease in children and should be kept as deferential diagnosis in patients presented with back pain. High suspicion of the disease result in early treatment which reduce the risk of bone lesions requiring surgical interventions or the development of a permanent alteration of spine mobility. Clinical presentation varies according to age, however as in our case back pain, irritability, and walking difficulties are common signs and symptoms of the disease. MRI is a best modality to confirm the diagnoses of the disease. Antibiotics are the drugs of choice, taking into account covering *S. aureus* and Gram-negative organisms. Discontinuation of antibiotic depends on resolution of symptoms and the normalization of ESR or CRP.

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Global Journal of Medical Research: F
Diseases
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Anaphylaxis and Epinephrine Auto-Injector use: A Survey of Pediatric Trainees

By Shaza Mohammed Elhassan MD, MS, Dr. Med Carolin Beck, Ahmed Alhammadi MD & Mehdi Adeli MD

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Method: This study was a two-phase cross-sectional surveybased study of 94 pediatrics trainees in phase one and 84 in phase two at the only tertiary hospital in Doha, Qatar. The primary outcome was trainees' knowledge related to anaphylaxis management and Epinephrine auto-injector (EpiPen®) use.

Results: 44 (46%) trainees claimed they had not received training about how to treat anaphylaxis. There was a discrepancy between claimed knowledge of how to treat anaphylaxis 86 (90%) and actual knowledge as none of the trainees' level answered all the questions correctly. Moreover 41 (49%) were unaware that EpiPen® should be administered IM in the lateral part of the thigh and 24 (28%) did not know it should be used in case of anaphylaxis.

GJMR-F Classification: *NLMC Code: QV 126*

ANAPHYLAXISANDEPINEPHRINEAUTOINJECTORUSEASURVEYOFPEDIATRICTRAINEES

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Results: 44 (46%) trainees claimed they had not received training about how to treat anaphylaxis. There was a discrepancy between claimed knowledge of how to treat anaphylaxis 86 (90%) and actual knowledge as none of the trainees' level answered all the questions correctly. Moreover 41 (49%) were unaware that EpiPen® should be administered IM in the lateral part of the thigh and 24 (28%) did not know it should be used in case of anaphylaxis.

Conclusion: Deficient knowledge about Epinephrine injection site, concentration and mode of administration among pediatric trainees were the most concerning outcome. Overconfidence in anaphylaxis management in senior trainees was worrisome. Continuing medical education, coupled with training opportunities to apply knowledge and practice skills, is needed to improve trainees' knowledge.

I. Introduction

Anaphylaxis is a life-threatening event, which requires urgent and prompt medical attention. Its exact incidence in pediatric is unknown, because few epidemiologic studies to date have examined the incidence of anaphylaxis in the general pediatric population.1 Available UK estimates suggest that approximately 1 in 1333 of the population of England has experienced anaphylaxis at some point in their lives.2 Lifetime prevalence based on international studies is estimated at 0.05-2%.3 This translates to a major impact on quality of life and healthcare costs. 4 Increase in diagnosis of anaphylaxis and hospitalizations were reported from multiple countries. 5-8 Pediatric trainees are at the frontline managing children at risk for anaphylaxis in the hospital and at community level. In many instances, they are the first medical responders. Their fundamental knowledge is crucial in all sorts of emergencies including anaphylaxis. Clinical diagnosis of anaphylaxis is based on consideration of the patient's presenting symptoms and signs and on ruling out other sudden-onset multisystem diseases.1 9 10 Epinephrine is the first-line and lifesaving medication of choice in anaphylaxis. Its use is recommended in guidelines issued by the World Allergy Organization.1 9 Epinephrine should be injected by the intramuscular route in the mid-anterolateral thigh as soon as anaphylaxis is diagnosed or strongly suspected, in a dose of 0.01 mg/kg of a 1:1,000 (1 mg/mL) solution, to a maximum dose of 0.3 mg in children and the patient should be placed on the back with the lower extremities elevated. Intravenous epinephrine is potentially hazardous and should be avoided except in an intensive care setting.1

These guidelines advise that epinephrine via the intramuscular route should be given by first medical responders. 11 Early administration of epinephrine effectively reduces morbidity and mortality in human anaphylaxis, whereas delayed administration of epinephrine is associated with increased mortality because epinephrine becomes progressively less effective in reversing anaphylaxis with the passage of time.12 13 cardiovascular side effects and overdoses were significantly more likely with intravenous epinephrine compared to intramuscular administration. 14 Plumb and colleagues found that junior doctors today seem to be no better at correctly identifying the clinical need for, and correct dose and route for administration of, adrenaline than their predecessors a decade earlier.15 Deaths have been reported from the inappropriate use of epinephrine in the context of allergic reaction.16 17 The latest NICE guideline 2016 recommended sufficient and appropriate training of healthcare professionals in management of patients with anaphylaxis.18 Immunologists Pete Storey and Penny Fitzharris stated that the knowledge gap regarding anaphylaxis was not unique to the United Kingdom.

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1. "We need to rethink how we train doctors and nurses in the care of all aspects of the management of this life-threatening condition," they wrote,
2. "We know that some patients die because they are not given adrenaline soon enough, or at all, or are given it by the wrong route.
3. "This is a longstanding and international problem. Doctors, especially those in emergency departments need to be skilled and confident in the care of these patients."19

The primary objective of our study was to evaluate the level of knowledge regarding anaphylaxis and its management in our pediatric training program. The secondary objective was to compare knowledge between the most junior and most senior residents for any observed knowledge gap. Understanding key knowledge gaps and their underlying reasons are vital to optimizing the training at medical school and/or during the training program, thus ensuring that a fatal outcome to a reversible condition is avoided. This furthermore will give the chance to implement training interventions at the right time points of pediatric training.

II. Methods

a) Study Design

This study was a two-phase cross-sectional study where verbal consent was taken from the trainees after explaining the objectives of the study. Questionnaires with pre-determined multiple-choice questions and one open ended question were handed out to the trainees. Phases one and two were 1 month apart. The reason for the two-phase study was to reinforce the accuracy of the responses. The study was approved by the IRB and Hamad Medical Corporation Hospital Committee.

b) Setting

The study was conducted at Hamad Medical Center (HMC), the only tertiary hospital in the state of Qatar. In phase one, the participants were approached after the morning report and asked to fill a questionnaire. They were divided into six groups according to their training level. Each questionnaire took about 3 minutes to complete. Phase two questionnaire was started 1 month after completed Phase one. The surveys were collected immediately after they were completed. 12 trainees were reached via WhatsApp® only. Their responses were received electronically. Each round of surveys took around 7 days to complete

c) Participants

Our six trainee groups included interns, who rotate in all specialties one year prior to residency program, and pediatric residents divided into post-graduate year 1 (PGY1), post-graduate year 2 (PGY2), post-graduate year 3 (PGY3), post-graduate year 4 (PGY4), and pediatric fellows from all pediatric subspecialties. The study was done between February and March 2015.

d) Selection criteria

We selected all trainees in the pediatric department including interns, residents and fellows. We only excluded those who were not willing to participate. Sample Size

The questionnaires were distributed to 96 trainees. For sample size refer to Figure 1.

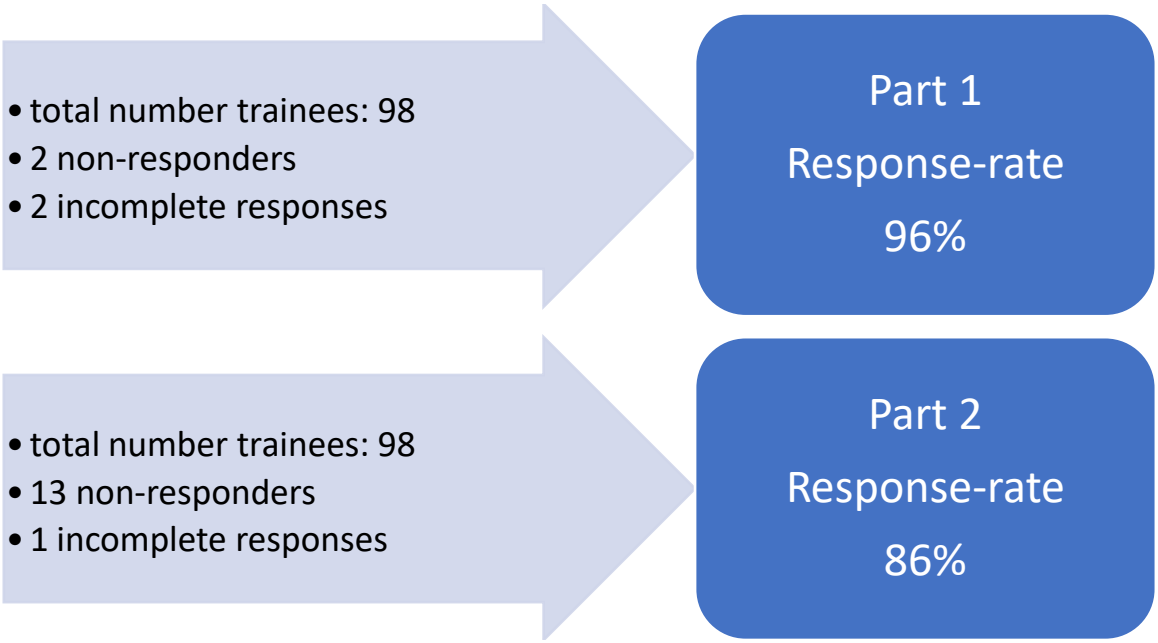

Figure 1: Sample size

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e) Study tools

Participants were informed verbally about the questionnaire and paper surveys were distributed to the trainees for both phases one and two. Survey administered questions were in English language. The interview questions were created based on previous studies and the clinical expertise of the investigator group.

A total number of 12 questions was given to the trainees (Table1). In each phase one and two, there were two demographic questions plus four knowledge related questions.

Table 1: Questionnaire Part 1

What's your Gender	Male	Female						
What's your Level of training?	Intern	PGY-1	PGY-2	PGY-3	PGY-4	Fellow		
Question 1 Do you know how to treat Anaphylactic shock due to Food Allergy?	1.1 Yes, and I got training about it.	1.2 Yes, but I did not get training about it	1.3 Maybe, I forgot how to treat despite my training	1.4 No, and I did not get any training.				
Question 2 What is the lifesaving drug in this case?	2.1 Antihistamine	2.2 Methylprednisolone	2.3 Terbutaline	2.4 Norepinephrine	2.5 Epinephrine	2.6 IV fluids	2.7 oxygen	
Question 3 Which route would you use to administer the treatment?	3.1 Oral	3.2 Nebulizer or inhaler	3.3 IV	3.4 SC	3.5 IM	3.6 Rectal	3.7 Via continuous mask inhalation	3.8 In the heart
Question 4 What dose would you give?	4.1 0.001mg/kg from 1:1,1000 solution	4.2 0.01mg/kg from 1:1,1000 solution	4.3 1mg/kg	4.4 2mg in 2ml nebulizer solution	4.5 1 liter / minute	4.6 I don't know		

Table 2: Questionnaire Part 2

What's your Gender	Male	Female				
What's your Level of training	Intern	PGY-1	PGY-2	PGY-3	PGY-4	Fellow
Question 5 Have you heard of Epinephrine Autoinjector / Epipen?	5.1 Yes	5.2 No	5.3 I can't remember			
Question 6 Do you know when to use it (which case)? -> Advised to stop here if answer "no"	6.1 Yes	6.2 No				
Question 7 Please write down which case it is used for	7.1 No answer	7.2 Correct answer (anaphylaxis)	7.3 Other answer (wrong)			
Question 8 Where would you give it?	8.1 lateral part upper arm SC	8.2 lateral part thigh IM	8.3 frontal part upper arm IM	8.4 frontal part thigh SC	8.5 lateral part thigh IM or SC	8.6 no answer

f) Variables

Three variable themes were included in the questionnaire:

1. Demographic data i.e. gender and training level,
2. Anaphylaxis-related questions i.e., lifesaving medications, route of administration and dosage,
3. Epinephrine auto-injector (EpiPen®) knowledge-related questions. Outcomes

The outcomes of importance were:

1. Knowledge related to anaphylaxis management and EpiPen® use among pediatric trainees;

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2. Identification of possible gaps in trainees' knowledge among different levels of training related to anaphylaxis management, with the aim to target teaching accordingly.

g) Data sources/measurement

This study aimed to assess pediatric trainees' knowledge in acute management of anaphylaxis as primary objective. Secondary objective was to assess possible knowledge gaps between the different trainees' levels, to evaluate whether the educational deficiencies are found at medical school or postgraduate training, so targeted training can be implemented accordingly. Statistical Analysis Descriptive statistics were used to summarize the demographics and level of training of the participants. We assessed knowledge related responses amongst trainees using frequencies along with percentages (univariate analysis). To compare knowledge between the most junior and most senior trainees, we used the fisher exact test (multivariate analysis).

A two-sided P value <0.05 was considered statistically significant. Surveys with missed data were not included in the analysis. All statistical analyses were performed using statistical package SPSS, version 19.0 (IBM Corporation, Armonk, NY).

III. Results

A total of 98 trainees were approached for both phases one and two, from whom we analysed 94 (96% response rate) for phase one and 84 surveys (86% response rate) in phase two (Figure 1). Most participants were females and pediatric fellows in both parts as seen in table 3.

Table 3: Demographics for phases 1 and 2*

Variable	Part 1 N=94	Part 2 N=84
Gender		
a. Male	40 (41.5%)	39 (46%)
b. Female	56 (58.5%)	45 (54%)
Training level		
a. Interns	7 (7%)	4 (5%)
b. Pgy1	20 (21%)	15 (18%)
c. Pgy2	19 (20%)	17 (20%)
d. Pgy3	11 (12%)	10 (12%)
e. Pgy4	9 (9%)	7 (8%)
f. Pediatric fellows	30 (31%)	31 (37%)

Knowledge related responses

Table 4: Knowledge related responses

Knowledge related responses	Trainees N (%)
Q1. Do you know how to treat Anaphylaxis? Did you receive any training about it?	
a. Yes and I got training about it.	46 (48)
b. Yes, but I did not get training about it.	40 (42)
c. May be, I forget how to treat despite my training.	4 (4)
d. No, and I did not get any training.	4 (4)
Q2. What is the lifesaving drug in this case?	
a. Antihistamine	3 (3)
b. Norepinephrine	2 (2)
c. Epinephrine	89 (92)
Q3. Which route would you use to administer the treatment?	
a. I.V	6 (6)
b. S.C	12 (13)
c. I.M	76 (80)
Q4. What dose would you give?	
A. 0.001mg/kg from 1:1000 solution	4 (4)
B. 0.01mg/kg from 1:1000 solution	77 (80)
C. 1mg/kg	4 (4)
D. 2mg in 2ml nebulizer solution	1 (1)
F. Not sure	8 (8)
Q5. Have you heard about the EpiPen®?	
A. Yes	71 (85)
B. No	11 (13)
C. Not sure	2 (2)

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Q6. Do you know when to use it (which case)? A. Yes B. No	 60 (72) 24 (28)
Q7. Please write down which case it is used for Not sure Anaphylaxis Other	 23 (27) 60 (72) 1 (1)
Q8. Where would you give it? a. Lateral part upper arm SC b. Lateral part thigh IM c. Frontal part upper arm IM d. Frontal part thigh SC e. Lateral part thigh IM or SC f. Not sure	 3 (4) 43 (51) 4 (5) 5 (6) 12 (14) 17 (20)

Table 4 shows knowledge related responses for all participants. Of notice 44 (46%) of the trainees responded they received no training about how to treat anaphylaxis. While 86 (89%) claimed they know how to treat anaphylaxis, 41 (49%) trainees were unaware that epinephrine should be administered in the lateral part of the thigh by intramuscular route and 24 (28%) trainees did not know that the EpiPen® is used in case of anaphylaxis.

In table 5 we compared the knowledge related responses between the most junior and most senior trainees in the residency program, to explore whether the training programs were well equipped with the necessary tools to provide trainees with the necessary knowledge and skills to treat anaphylaxis Comparing the most junior and most senior trainees, there was no statistical difference in knowledge related responses except that all 9 (100%) senior residents claimed to know how to treat anaphylaxis compared to only 14 (74%) of junior residents (p-value 0.01).

Table 5: Comparing knowledge related responses of PGY1 to PGY4

Correct responses to knowledge questions Questionnaire Part 1	PGY1 N=19 (%)	PGY4 N=9 (%)	P value (fischer exact test)
Q1. Do you know how to treat anaphylactic shock due to food allergy? Yes, and I got training about it.	4 (21)	7 (78)	0.01
Yes, but I didn't get training about it.	10 (53)	2 (22)	0.27
Maybe/No.	5 (26)	0	0.24
Q2. What is the lifesaving drug in this case? Epinephrine	18 (95)	9 (100)	0.9
Q3. Which route would you use to administer the treatment? I.M	19 (100)	8 (89)	0.6
Q4. What dose would you give? 0.01mg/kg from 1:1000 solution	15 (79)	9 (100)	0.3
Correct response to knowledge questions Questionnaire Part 2	PGY1 N=15(%)	PGY4 N=7(%)	P value
Q5. Have you heard about EpiPen®? Yes	11 (73)	7 (100)	0.3
Q6. Do you know when to use it? Yes	10 (67)	7 (100)	0.2
Q7. Please write down which case it is used for? Anaphylaxis	10 (67)	7 (100)	0.2
Q8. Where would you give it? Lateral part of the thigh	7 (47)	5 (71)	0.5

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As summarized in figure 2, pediatric fellows (12 fellows or 30%) and PGY1 (10 residents or 25%) were more likely to report that they did not receive training compared to other categories.

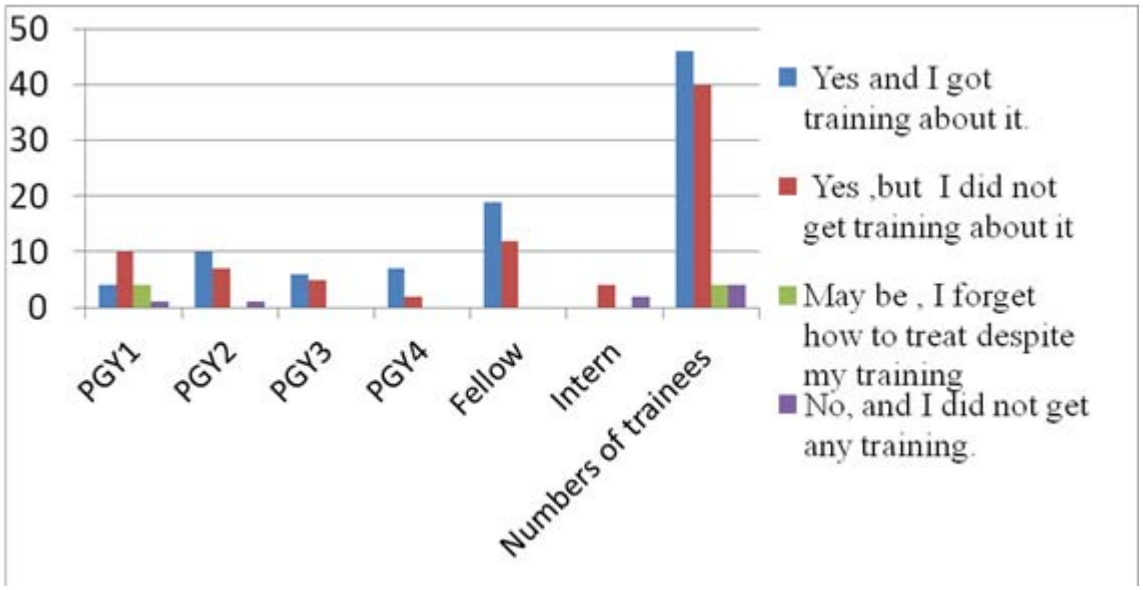

Figure 2: Knowledge of anaphylaxis treatment and training received among all trainees (% of total number by trainee level)

IV. Discussion

There are notable findings from our study. Despite the vital importance of knowing the emergency treatment of anaphylaxis, of significance is the observation that none of the trainees' categories answered all the questions correctly. Surprisingly significant number of the total trainees 44 (46%) claimed they did not receive any training about how to treat anaphylaxis. Almost half of the trainees 41 (49%) were not aware that the EpiPen® should be administered in the lateral part of the thigh by intramuscular route. Moreover, 24 (28%) of trainees did not know that EpiPen® is used in case of anaphylaxis. Our study showed that 13 (15%) have never heard about epinephrine auto-injectors from which the most junior trainees represent about half.

These worrisome results indicate that both medical schools and training programs need to consider restructuring their existing educational agenda to better address low prevalence high consequence conditions like anaphylaxis and other emergencies. There is an urgent need for improving training in the recent international consensus.20 There was an obvious discrepancy between claimed and actual knowledge in our study. While 86 (89%) of the trainees claimed they knew how to manage anaphylaxis, when they were asked more detailed questions, half of them were unaware that epinephrine should be administered in the lateral part of the thigh by intramuscular route and one third did not know that the EpiPen® is used in case of anaphylaxis.

Studies suggested that doctors claim to know how to treat anaphylaxis but this is often not translated into practice.19 Unlike our findings, a large survey based study of doctors and nurses in a Singapore hospital indicated not only good recognition of anaphylaxis but also a trend to over-diagnose this condition.21 A systematic review study showed that participants reported high levels of confidence in diagnosing or managing anaphylaxis at baseline and follow-up despite their limited clinical experience.22 Physicians' overestimation of their own competence may compromise the safety and clinical outcomes of patients. It may be advantageous to help trainees at all levels to become more cognizant of this disconnect.23 The incorporation of continuous medical education to practice skills is essential to maintain knowledge and competency.24 25 Though most participants knew that epinephrine is the drug of choice for treating anaphylaxis, few interns thought wrongly that antihistamine is the drug to use for treating anaphylaxis. While most of the pediatric trainees 76 (80%) acknowledged that the best mode of administrating epinephrine during anaphylaxis is I.M, 18 (20%) assumed dangerously that IV and S.Q are the standard modes of treatment during anaphylaxis. Similar to our findings, in a questionnaire-based study done in UK with a sample size of 68 foundation doctors, 27/68 (40%) chose the correct route (IM), 17/68 (25%) wrongly chose the (IV) route and 1/68 (1%) incorrectly chose either subcutaneous or nebulized routes of administration.15 Regarding the dose and concentration of epinephrine, most of the trainees except the interns acknowledged

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the right dose and concentration of epinephrine. A study of first- and second-year UK doctors in 2008 identified that even junior doctors who had completed ALS training had poor knowledge of adrenaline use and dose.26

Our study showed that 13 (15%) have never heard about epinephrine auto-injectors from which the interns and PGY1 represent about half. This might indicate gaps in the educational programs at medical schools. We anticipate that trainees' performance will continue to decline in the absence of educational reinforcement. When we compared the knowledge-related responses of the most junior and most senior trainees, we found no statistical difference between the two categories in most of the core areas. Similar to our study, a survey-based study in adult medicine by Droste et al, which compared two district hospitals with different levels of trainees showed that there was a lack of knowledge in a significant number of senior and junior doctors regarding the dose, route, and concentration of epinephrine with no much difference among trainee levels.27 Another study by Drupad HS et al of 265 subjects in which a pretested structured questionnaire was used showed no significant difference between senior and junior doctors.28 Trainees of all grades who may be the first responders at a scene of anaphylaxis should solidify their knowledge about emergencies and should be well prepared if anaphylaxis ensued. Innovative educational interventions are essential to improve and maintain trainees' knowledge and clinical competency.

V. Conclusion

Although prompt treatment with epinephrine is critically important for survival in anaphylaxis, we continue to have gaps in the critical knowledge of the frontline trainees regarding anaphylaxis management. Knowledge about epinephrine injection site, mode of administration and the lack of overall training of anaphylaxis treatment were the most concerning findings.

Continuing medical education, coupled with training opportunities to apply knowledge and practice skills, is needed to improve trainees' knowledge.

Limitations

Our study was based on self-reports. Our institution is the only tertiary center in the area and is comprised of pediatric trainees from all over the world.

Strengths

Our training program enrols medical school graduates from multiple different countries, which makes our findings more generalizable and consists of a large number of 98 trainees within a single institution. We handed out surveys are 2 time points to ascertain our findings and included comprehensive questions on anaphylaxis knowledge and treatment/ EpiPen® use, both of which are important to successfully recognize and treat such condition. We had a high response rates using both paper and electronic version of the questionnaire.

VI. Summary-Box

What is known about the subject? 1. Pediatric trainees are at the frontline managing children with anaphylaxis inthe hospital and at community level. Their fundamental knowledge of anaphylaxis treatment is crucial. 2. Studies showed that poor knowledge of anaphylaxis management impairs patients' quality of life, and leads to increased healthcare costs and preventable deaths. 3. There is limited data about pediatric trainees' knowledge of anaphylaxis management according to their level of training.

What this study hopes to add: 1. Deficient knowledge about Epinephrine injection site, concentration and mode of administration among pediatric trainees were the most concerning outcome. 2. Overconfidence in anaphylaxis management in senior trainees was worrisome 3. Continuing medical education, coupled with training opportunities to apply knowledge and practice skills, is needed to improve trainees' knowledge.

Acknowledgements

We thank Dr. Karima Becetti for her valuable review and feedback. We also thank Dr. Prem Chandra from Hamad Medical Corporation research center for support in the statistical analysis.

Disclosure Statement

The authors declare no conflict of interests.

Authors Contribution MA (Principal investigator) conceptualized the study, CB collected the data analyzed, drafted and edited the manuscript. SME analysed data and wrote the manuscript. AA presented the data in the PAAM conference. All authors read and approved the final manuscript.

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Global Journal of Medical Research: F
Diseases
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Initial Digestive Potential of Alimentary System in Newborns

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Abstract- Fatally organized alimentary system hydrolase activities in a newborn make up the initial digestive polyenzyme potential, which provides breast milk lacto trophy if combined with hydrolases. Initial digestive potential in a newborn is characterized by the results of the activity and content of lipase, α-amylase, pepsinogens (I, II), alkaline phosphatase, α1-antitrypsin in umbilical cord blood serum, amniotic fluid, aspirate gastric content in a newborn at the end of the delivery.

Systems of different hydrolases during antenatal life are asynchronous.

According to the results of the hydrolase estimation in the blood serum of the mother and the newborn, the digestive potential of the latter turns out to be much less than that of the mother's. It is the proof of the incomplete maturity of the digestive potential in the newborn. In the case of immature gestation, the concentration of hydrolases and zymogens (except lipase) in the examined bio liquids was reduced. Hydrolases of gastric contents are most informative towards the digestive potential and less informative towards amniotic fluids and umbilical cord blood serum.

Keywords: newborn, hydrolases, amniotic fluids, gastric content, blood serum, digestive potential.

GJMR-F Classification: NLMC Code: WS 205

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Initial Digestive Potential of Alimentary System in Newborns

Penzhoyan G.A. [α], Model G.Y. [σ] & Korotko G.F. [ρ]

Abstract- Fatally organized alimentary system hydrolase activities in a newborn make up the initial digestive poly-enzyme potential, which provides breast milk lacto trophy if combined with hydrolases. Initial digestive potential in a newborn is characterized by the results of the activity and content of lipase, α-amylase, pepsinogens (I, II), alkaline phosphatase, α_1-antitrypsin in umbilical cord blood serum, amniotic fluid, aspirate gastric content in a newborn at the end of the delivery.

Systems of different hydrolases during antenatal life are asynchronous.

According to the results of the hydrolase estimation in the blood serum of the mother and the newborn, the digestive potential of the latter turns out to be much less than that of the mother's. It is the proof of the incomplete maturity of the digestive potential in the newborn. In the case of immature gestation, the concentration of hydrolases and zymogens (except lipase) in the examined bio liquids was reduced. Hydrolases of gastric contents are most informative towards the digestive potential and less informative towards amniotic fluids and umbilical cord blood serum.

Identification of the enzymes in three mentioned bio liquids of the newborn is advisable for the reasonable estimation of the digestive potential and precise prognosis of lacto trophy.

Resume: Initial enzyme digestive potential is presented by fatally organized alimentary system hydrolase activities in a newborn. Both lacto trophy efficacy and breast milk hydrolases depend on it.

Quantitative indicator of the potential was measured by analyzing lipase, α- amylase, alkaline phosphatase, pepsinogens (I, II) in umbilical cord blood serum, amniotic fluid, aspirate gastric content in a newborn at the end of the delivery, in venous blood of 36 new mothers with full-term pregnancy, in 40 new mothers with incomplete gestation as well as in their newborns.

During antenatal life systems of different hydrolases were formed asynchronously. In the case of immature gestation, the concentration of hydrolases and zymogens in the examined bio liquids was reduced except lipase, which was active. Hydrolases of gastric contents are most informative towards the digestive potential and its low variability, while hydrolases in the newborn umbilical cord blood serum and in the amniotic fluids are less informative. Poly-enzyme analysis of three bio-liquids of a newborn proved to be reasonable for the conclusion concerning the morph functional maturity of the digestive system of a newborn.

Initial digestive potential makes it possible to expect lacto trophy efficacy and individualize the program for both breast and mixed feeding.

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Keywords: newborn, hydrolases, amniotic fluids, gastric content, blood serum, digestive potential.

I. Introduction

Breastfeeding proved to be the "gold standard" for a newborn thanks to its unique nutritional, immune, regulatory, electrolyte, vitamin characteristics, as well as to the microbiota of the breast milk and its numerous substitutes. Milk nutrients are ingested by a newborn, but first they need to be hydrolyzed in the digestive system, this process is performed by hydrolyzing enzymes of the digestive glands and small intestine of a newborn according to the self-digestion pattern, and by first milk and mature milk enzymes according to autolytic digestion. Autolysis of lipids and proteins (casein) of first milk and milk is induced and realized in the gastric and small intestine cavities by the hydrolases of the newborn's digestive glands that developed during his antenatal period. Their hydrolases make up the initial digestive potential of the alimentary tract of the newborn. The digestive potential has not yet been investigated in perinatology, neonatology, or pediatrics either in terms of theory or in applied medicine. The notion has recently been put forward by the authors. But it should be taken into consideration that the reducing of this morpho-functional potential may threaten the development of a newborn.

II. Materials and Methods

Among seventy-six examined new mothers 36 had full-term (37-41 weeks) and 40 premature (27-36 weeks) pregnancies. Forty-seven children were born during vaginal birth and 29 by cesarean section. The investigation began after the written consent was signed by the parents under the current Federal "Law on Health Protection of Citizens" and the decision of the Ethics Committee. In newborns, anthropometric data, Apgar score and some anthropometric parameters and obstetric history were assessed under the Order of the Ministry of Health of the Russian Federation "On approval of the Order of Medical Care in the Profile «Neonatology»". The above-mentioned parameters were significantly lower in premature newborns than in mature newborn infants (see Table 1).

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Table 1: Indicators of new mothers with mature newborns (36-numerator) and premature newborns (49-denominator)

Variables	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, statistical significance
Mother's age (years)	27,67 30,75	28,0 31,5	14,0 19,0	41,0 44,0	24,5 27,5	30,5 35,0	$p < 0,5$
Gestional age (weeks)	38,03 32,00	38,0 33,0	38,0 27,0	39,0 35,0	38,0 30,0	38,0 34,0	$\downarrow p < 0,001$
Mass (g)	3546,4 1765,2	3595,0 1715,0	2460,0 670,0	4800,0 3130,0	3170,0 1335,0	3835,0 2150,0	$\downarrow p < 0,001$
Height (cm)	53,47 41,27	54,0 42,0	46,0 33,0	59,0 48,0	52,0 37,0	55,5 46,0	$\downarrow p < 0,001$
Head circumference (cm)	34,14 28,58	34,0 30,0	31,0 17,0	37,0 35,0	33,0 26,0	35,5 31,0	$\downarrow p < 0,001$
Breast circumference (cm)	33,31 26,28	33,0 27,0	27,0 16,0	37,0 33,0	32,0 24,0	35,0 29,0	$\downarrow p < 0,001$
Apgar 1 (scores)	7,9 5,5	8,0 6,0	7,0 1,0	8,0 7,0	8,0 5,0	8,0 6,0	$\downarrow p < 0,001$
Apgar 5 (scores)	8,7 6,0	9,0 6,0	8,0 1,0	9,0 8,0	8,0 6,0	9,0 7,0	$\downarrow p < 0,001$
Latency period (hours)	3,4 2,05	2,0 0,0	0,0 0,0	21,0 41,0	0,0 0,0	5,0 0,1	$\downarrow p < 0,001$

New mothers' amniotic fluids were obtained in sterile syringes and then centrifuged (10 min., 3000 revolutions). The newborns' blood was obtained from their umbilical cords; the mothers' blood was obtained from the ulnar vein.

In newborns fasting gastric content was aspirated, then it was homogenized and centrifuged (10 min., 3000 revolutions). In amniotic fluid and gastric aspirate supernatants, umbilical cord blood serum of the newborn and mother's blood serum lipase, α-amylase, alkaline phosphatase were determined by colorimetric methods with standard reagent kits for in vitro diagnostics (Roche) on a modular platform for biochemical and immunochemical analysis Cobas-8000 (module C 702). α-1- antitrypsin (reagent F1-Antitrypsin) was determined on a biochemical analyzer Architect C 8000 (Abbott) by the turbidimetry method. Pepsinogens I and II were determined by chemiluminescent immunoassay analysis on microparticles by Abbott reagents using immunological analyzer Architecr plus: 2000.

Statistical data processing was implemented within the Statistica 6 package by nonparametric statistics methods since the above-mentioned parameters had a large spread, and their empirical values did not correspond to the standard distribution law. Correlation analysis of enzyme parameters was carried out.

III. Results and Discussion

The aim of the research is the quantitative characteristics of the initial digestive potential of the alimentary tract in newborns and methods for its determination that includes the determination of digestive glands hydrolyzes in the newborn's blood serum, gastric aspirate, and amniotic fluids.

The amount of digestive glands hydrolases in human blood serum depends on the number and activity of glands producers granulocytes of the correlative enzymes [9]. In the blood serum of new mothers, the amount of hydrolases is higher (Table 2) than that in the blood serum of the newborns (Table 3).

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Table 2: Blood Serum Hydrolases in New Mothers

Enzymes	Average	Median Value	Minimum	Maximum	Lower Quartile	Upper Quartile	Shift direction Statistical significance
Lipase (U/l)	28,80 30,30	27,29 30,30	7,10 8,40	63,50 51,90	22,35 23,35	31,10 35,20	p > 0,10
Amylase (U/l)	52,97 52,28	52,97 52,28	4,00 3,00	82,00 78,00	48,50 41,00	61,50 65,50	p > 0,10
ALP (U/l)	182,5 120,8	181,0 118,5	94,0 56,0	441,0 235,0	137,0 87,0	185,5 136,0	↓p < 0,001
Pepsinogen I (ng/ml)	50,74 56,23	49,62 55,30	8,40 4,50	106,00 215,90	36,65 38,35	58,80 66,35	p > 0,10
Pepsinogen II (ng/ml)	8,88 7,79	8,74 7,55	2,70 1,70	33,10 17,50	4,90 5,65	10,70 8,75	p > 0,10
α-1-antitripsin (g/l)	0,30 0,47	0,30 0,30	0,30 0,30	0,30 2,38	0,30 0,30	0,30 0,38	p < 0,025

Note: ALP - alkaline phosphatase. (numerator – mature newborns, denominator - premature newborns)

It proves the incomplete development of the digestive glands enzymatic potential. Hydrolases differ in the initial level of their content in the blood serum that indicates that morphofunctional maturation of the enzyme systems of the fetus and the newborn is asynchronous. Producers of pepsinogen - the stomach glands (especially pepsinogen I) and producers of α-amylase - salivary and pancreas glands are most retarded. The antitrypsin activity of umbilical cord blood serum in the newborns was 4.5 times as high as that of the mother's.

Table 3: Hydrolases of the Umbilical Cord Blood Serum in Newborns

Enzymes	Average	Median Value	Minimum	Maximum	Lower Quartile	Upper Quartile	Shift direction Statistical significance
Lipase (U/l)	10,72 10,49	10,12 10,00	5,70 5,40	21,30 18,80	8,30 8,30	12,10 12,60	p > 0,10
Amylase (U/l)	9,00 4,72	8,50 4,00	1,00 0,00	52,00 16,00	5,00 2,00	9,00 6,00	↓p < 0,01
ALP (U/l)	157,5 166,5	157,5 171,0	97,0 11,0	243,0 274,0	119,0 134,0	181,5 200,5	p > 0, 10
Pepsinogen I (ng/ml)	10,92 4,82	8,45 4,20	3,40 1,10	55,10 12,20	6,75 2,60	10,92 6,10	↓p < 0,001
Pepsinogen II (ng/ml)	5,55 3,44	3,80 1,85	1,80 0,40	36,70 30,00	2,40 0,90	5,55 4,65	↓p < 0,001
α-1-antitripsin (g/l)	1,33 1,30	1,33 1,29	0,84 0,48	2,95 2,66	1,22 0,97	1,40 1,62	p > 0,10

Note: ALP - alkaline phosphatase. (numerator – mature newborns, denominator - premature newborns)

The new mothers that gave birth to both premature and mature newborns did not differ in the content of blood serum enzymes, except for alkaline phosphatase.

The concentration of amylase and pepsinogen (I, II) in the umbilical cord blood serum of the premature newborns was lower than that in the umbilical cord blood serum of mature newborns (Table 3). Reduced amyl lytic activity of the glands secrets in premature newborns can cause maldigestion in the case of mixed and artificial feeding of infants as most infant formula milk contains α-amylase- hydrolyzed polysaccharides. It is not contained in breast milk. Incomplete gestation reduces premature peptic potential of the fund-antro-duodenal producers of pepsinogens. It may affect the hydrolysis and protein metabolism in premature newborns, the formation of regulatory peptides (mainly breast milk casein) [5–13], and the process of proteolysis in the lacto trophy. This statement results from the postulate of the interaction of breast milk

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proteases and digestive glands excretions in the gastrointestinal tract [6, 7, 10], that have recently been confirmed by peptidomics and mass spectral chromatography. According to this fact the excretions proteinases (gastric aspirate) increase the hydrolytic effect of breast milk proteinases of lactating women by 1.5 -2.5 times [10]. The milk proteinases (like other hydrolases) have specific self-regulating dynamics during lactation [1, 14].

Premature birth did not affect the lipase content in the umbilical blood serum in the newborns. It speaks for the formation of the low initial level of lipase production by the digestive glands of the fetus during earlier gestational periods than other considered enzymes.

The reduced content of three hydrolases (α-amylase, pepsinogen I and II) in the umbilical cord blood serum of the newborn proved immature initial digestive potential in preterm pregnancy. No significant data concerning other hydrolases were found. It is explained by the fact that enzymatic homeostasis in the blood is provided not only by transporting the corresponding enzymes and zymogens using increment and resorption but removing the same enzymes from the bloodstream by different mechanisms. It has been the subject of quite a number of experimental and clinical research (Review: [5, 16]). Therefore, the relatively constant content of hydrolases at one or another level is the result of the balance of the given complex multidirectional regulated processes. In newborns we observed three low hydrolases content in the blood and their severe vibrations, that prove low enzyme potential, its variability, and, consequently, limited diagnostic information value.

The Digestive Glands Hydrolases in Amniotic Fluids

The volume and composition of amniotic fluids have been studied under normal and pathological conditions by lots of researchers at different times. The presence of enzymes in the amniotic fluids, including digestive gland hydrolases, has been established. However, the informational hydrolases criteria concerning the enzyme potential of the glands have not been studied in full, especially the mechanisms of origin of this group of enzymes in the amniotic fluids [15]. In different periods of gestation, hydrolases in the amniotic fluid are of different origin, but at the end of the gestation they come mainly from the digestive glands of the fetus. We cannot deny participation of hydrolases of amniotic fluid and placenta in the genesis [17], as well as the transport of hydrolases from the blood of a pregnant woman [15, 17]. They seem to be additional sources of enzymes in the amniotic fluids. These problems have recently been under our consideration [14].

In the amniotic fluids of new mothers with full-term gestation, the composition of hydrolases (see Table 4) differs from that in the blood serum of newborns (Table 3) and their new mothers (Table 2). The concentration of α- amylase, α-1-antitrypsin, pepsinogen I and especially pepsinogen II in the amniotic fluids is much higher than that in the blood serum of new mothers. What concerns alkaline phosphatase the differences are insignificant. The lipase composition in the amniotic fluid is five times as low as in the umbilical cord blood serum and even 15 times as low as the average blood serum index of the new mother.

Table 4: Hydrolases of delivery waters

Enzymes	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, Statistical significance
Lipase (U/l)	2,00 4,64	1,90 4,05	0,90 0,90	4,90 33,40	1,45 2,10	2,35 4,64	↑p< 0,01
Amylase (U/l)	182,73 67,40	146,50 65,70	37,00 16,00	537,00 162,00	92,00 47,00	229,00 84,50	↓p< 0,01
ALP (U/l)	182,94 38,74	132,50 23,50	18,00 0,00	999,00 396,00	74,00 14,00	167,50 44,00	↓p< 0,01
Pepsinog en I (ng/ml)	33,95 19,28	29,85 17,90	10,40 7,00	106,00 63,10	24,85 13,40	38,00 19,90	↓p< 0,01
Pepsinog en II (ng/ml)	545,79 254,47	493,05 124,40	100,00 14,10	1635,90 1631,60	320,80 57,15	733,70 254,47	↓p< 0,01
α-1-antitrypsi n (g/l)	4,43 1,21	0,30 0,30	0,30 0,30	30,00 30,00	0,30 0,30	0,30 0,39	↑p< 0,005

Note: ALP – alkaline phosphatase (numerator – full-term newborns, denominator – premature newborns)

We cannot but mention the moderate statistically significant correlation between the hydrolase content in amniotic fluids and blood serum of umbilical cord: for α-amylase r=0,63; for pepsinogen I r=0,68; for pepsinogen II r=0,50; for alkaline phosphatase r=0,52. Hence, the hydrolases of amniotic fluids are informative

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concerning the individual morphofunctional immaturity of digestive glands in both fetuses and newborns.

In incomplete pregnancy, the amniotic fluids contain all types of hydrolases except lipase in a less concentration than in full-term pregnancy. The decreased content of hydrolases is statistically highly significant ($p<0, 01$).

High concentration of pepsinogen II in amniotic fluids that differ greatly from pepsinogen I prove the differences in development mechanisms of hydrolases of digestive glands in the systemic bloodstream of newborns, their mothers, and amniotic fluids. This phenomenon can be explained by the early development of enteric enzyme producers in the fetus [3, 18-20]. Pepsinogen II is mostly synthesized by pyloric and duodenal glands. That is why the concentration of this isoproenzyme in amniotic fluids in incomplete pregnancy as well. So, the regurgitated stomach content is transported to the amniotic fluids as the result of the duodenal, gastric and oral reflux which is common for both fetuses and newborns, while in their stomach content we found out higher concentration of pepsinogen II in comparison with pepsinogen I. Evidences of these differences are given in Table 5.

The high hydrolytic activity of amniotic fluids, the high volume of their transfer into the digestive tract of the fetus by swallowing, breathing and inhaling makes it possible to conclude that hydrolases of amniotic fluids take part in the hydrolysis of nutrients of the gastrointestinal tract which provides the amniotic trophism with its specific autolytic and self-digestion. It is necessary for the nutrition of the digestive tract mucous coat structures.

Hydrolases of Aspirated Stomach Content of Newborns

Fasting stomach content of a newborn is a mixture of gastric glands secretions, duodenal contents (pancreas secretion, duodenal secretions, and bile secretions), swallowed oral liquid (secretions of salivary glands and crevicular fluids) and amniotic fluids. Due to the absence of recurring activities of the digestive system in neonates [1], the volume and composition of the aspirated stomach content are relatively stable and demonstrate the total secretory activity of the above mentioned digestive glands, including their enzyme production.

Table 5: Hydrolases of stomach content in newborns

Enzymes	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, statistical significance
Lipase (U/l)	43,68 40,47	10,65 20,50	0,30 0,10	270,00 244,10	2,5 4,45	48,75 41,94	p> 0,10
Amylase (U/l)	278,03 92,03	204,00 92,03	12,00 3,00	1289,00 237,00	140,00 44,00	340,00 103,00	↓p< 0,001
ALP (U/l)	423,23 55,31	70,00 46,00	21,00 3,00	4988,00 397,00	32,00 19,50	389,00 55,31	↓p< 0,001
Pepsino gen I (ng/ml)	133,74 42,70	90,85 42,70	4,10 0,00	907,50 150,10	44,60 19,80	133,75 58,15	↓p< 0,001
Pepsino gen II (ng/ml)	1125,03 573,64	1108,96 388,15	100,60 0,00	3087,80 2648,70	679,25 148,55	1761,10 861,00	↓p< 0,001
α-1-antitryps in (g/l)	0,32 0,31	0,30 0,30	0,30 0,30	0,45 0,48	0,30 0,30	0,32 0,31	p> 0,10

Note: ALP – alkaline phosphatase (numerator – full-term newborns, denominator – premature newborns)

Judging by the data given in Table 5, polysecretion aspirated from the stomach possessed the high concentration of α-amylase, lipase and pepsinogens, especially pepsinogen II; all 76 samples of stomach content demonstrated the higher level of pepsinogen II than pepsinogen I. The same result was received after the analysis of amniotic fluids. The level of similar hydrolases both in the gastric aspirate and amniotic fluids had moderate statistically significant correlation coefficients: for amylase $r=0,57$; for pepsinogen II $r=0,60$. We registered a strong correlation among five enzymes of amniotic fluids and gastric aspirate: the index of canonical correlation was $R_{pcc}=0,82$ (that characterizes the stage and interaction force between two variable lists). The results of these findings prove the above- formulated discovery of one more physiological mechanism, namely the development of digestive glands hydrolases of high concentration in amniotic fluids: duodenogastrooral regurgitation (reflux) into the amnion.

High enzyme activity of gastrointestinal contents provided by the fetal enzymes of both digestive glands

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and enterocytes performs the cavitary, parietal, and intracellular digestion of the fetus, including its amniotic trophism. In neonatal and subsequent stages of the child's development the hydrolases of his digestive tract that made up his initial digestive potential provide (together with the breast milk hydrolases) the lacto trophy with its peculiar proper and autolytic types (including the induced subtype) of digestion.

Saliva proteases increase the activity of casein by pepsins and trypsin in vitro. Similar interaction of proteinases in lacto trophy takes place in the stomach and small intestine under appropriate conditions (pH of the medium) [1]. In several recent works devoted to enzyme peptidomics, the summing up of the proteolysis produced by secretory proteases in the baby's stomach and similar proteases of mother's milk incubated in the stomach (2 h) by nano-chromatographic identification of peptides formed mainly during hydrolysis of β casein was established. At the same time, the effects of plasmin did not change, or they reduced by 1.3 times. Cathepsin D actions increased by 2.3 times, of pepsin by times, of elastase by 1.6 times, of chymotrypsin by 2.5 times, and those of prolineendopeptidases by 1.5 times. Hence, milk autoproteolysis was increased twice as much by secretory proteases in the stomach of the infant by the proteases [16]. The authors verified the relevance of intragastric proteolysis in the formation of regulatory peptides, most of which have acknowledged effects.

Pediatricians take an interest in the lipolytic activity of milk and its lipids, which play energetic, plastic, nutritional and protective role in the lacto trophy of the child. The lipolysis technology is multistage: it is performed by lipases of saliva and gastric secretion in the stomach cavity, then by lipases of milk and pancreatic secretion in the small intestine with the participation of bile salts inducers (promoting milk lipase) and colipase (promoting the effect of pancreatic lipase) [15]. Triglycerides are released from milk fat globules in the stomach by hydrophobic lipases of saliva and gastric secretion, that act as inducers of lingual and gastric lipases of the infant as well. The material of the globules membranes is recognized as a valuable product for the infant and has recently been added to milk mixtures. By the way, during the period of lactation, the lipolytic activity of milk is reducing more slowly than the content of other hydrolases in milk [15].

In human breast milk, there is no substrate for α-amylase, but its activity is high in the gastric aspirate. It is significant for the polysaccharide hydrolysis in complementary foods in the case of mixed and artificial feeding of infants. Hydrolysis of the principal carbohydrate of lactose milk is carried out by milk lactases and the small intestinal mucosa. Lots of researchers have lately focused their attention on these enzymes. Lactase is one of the disaccharides of enteric membrane digestion it was not included in the secretory potential and was not found in the gastric aspirate. The results shown in Table 5 indicate a significant decrease in hydrolases content (except lipase and antitrypsin) in the gastric aspirate of premature newborns if compared to full-term newborns. These data are extremely informative about the secretory digestive potential of newborns.

IV. Summary

The technology of lacto trophy makes it possible to conclude that the secretory hydrolases of the digestive glands, which form the digestive potential of the newborn, are of fundamental importance for its implementation. In this regard, its quantitative characteristic should be taken into account not only in incomplete gestation periods, but also in normal ones.

It is all the more important because hydrolase levels proved to be higher than the average in gastric aspirate, amniotic fluid, and umbilical cord blood serum in the group of premature infants, who had mainly a reduced digestive potential, while in infants of the group with standard gestational age enzymatic indicators of three bio liquids were reduced in comparison with average values. This phenomenon took place at the gestational borderline. Such results make it possible to acknowledge the digestive potential of newborns during childbirth the diagnostic test in the trophological prognosis of the development of newborns. Due to its digital variability and quantitative insufficiency, the material obtained does not allow determining the reference enzyme parameters of the standard initial digestive potential. That is why further fact-finding inquiry is necessary. At the current state of knowledge only a sharp decrease in the quantitative initial digestive potential of hydrolases in amniotic fluids and in umbilical cord blood serum can serve a reliable prognostic sign of trophological dysfunction in a newborn.

V. Conclusions

1. Hydrolases of the secrets of the digestive glands and small intestine of newborns make up the prenatally formed initial digestive potential of their digestive system.
2. The digestive potential characterized by the enzymes of the cord blood serum of the infant is significantly lower than that of the mother's venous blood serum and proves the incompleteness of the digestive potential in the antenatal period.
3. Low enzymatic activity of this potential requires proper and autolytic digestion of breast milk hydrolases to participate in lacto trophy.
4. Morphofunctional maturation of producers of different digestive system hydrolases of the fetus and the child are asynchronous: the digestive system of the small intestine matures earlier than the others, next come to the lipase producing

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glands followed by fetal zymogenic proteases and α-amylase.

5. The adequate initial digestive potential of the digestive system of newborns is marked by the content of hydrolases in gastric aspirate; the content of hydrolases in amniotic fluid and umbilical cord blood serum are less informative.
6. It is recommended to characterize the initial digestive potential of newborns by the parallel with the results of determination of several hydrolases and zymogens mentioned above that were obtained from gastric aspirate at the end of the delivery as well as in the umbilical cord blood serum and amniotic fluids.
7. In immature gestation, the digestive potential of the digestive system turns out to be reduced differently in different hydrolase systems, but not in the lipase system.
8. The determination of the initial digestive potential of the digestive system of newborns is promising for justifying the management of their natural, mixed, and artificial feeding.

Gratitude

The authors of the article thank the administration of the Regional Clinical No. 2 and the Perinatal Center for the cooperation in carrying out the research.

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Global Journal of Medical Research: F
Diseases
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Gastrointestinal Protozoan Infections and Associated Factors among Children under 5 Years with Diarrhea in Kisii County, Kenya

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Abstract- Globally every year, it is projected that approximately two million infections related to diarrhea occur among children who have not reached their fifth birth anniversary. In Kenya, gastro intestinal protozoan infection is a major problem primarily due to fecal contamination of food and water causing high morbidity. The aim of this study was to establish the predisposing factors associated with gastro intestinal protozoan infections in children under five years with diarrhea in Kisii county, Kenya.

Keywords: *gastro intestinal protozoans, diarrhea, children under five years.*

GJMR-F Classification: *NLMC Code: WI 407*

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Gastrointestinal Protozoan Infections and Associated Factors among Children under 5 Years with Diarrhea in Kisii County, Kenya

Caleb Okeri Ondara [α], Benson Omweri Nyachong'I [σ], Wycliffe Nyamwancha Mogoa [ρ] & Vincent Obino Orucho [ω]

Abstract- Globally every year, it is projected that approximately two million infections related to diarrhea occur among children who have not reached their fifth birth anniversary. In Kenya, gastro intestinal protozoan infection is a major problem primarily due to fecal contamination of food and water causing high morbidity. The aim of this study was to establish the pre disposing factors associated with gastro intestinal protozoan infections in children under five years with diarrhea in Kisii county, Kenya.

Methodology: one hundred and twenty stool samples of children under five years with diarrhea were screened for gastro intestinal protozoan infections between 1st April and 30th November 2017. The stool samples were processed using direct fecal smear and formol ether concentration procedures and the identification of the parasites was based on the morphological differences of their cysts and trophozoites under microscopy. Chi-square test and multi logistic regression was used to establish the association between the possible predisposing factors and gastro intestinal protozoan infections with the differences considered statistically significant at $P < 0.05$.

Results: Out of the 120 stool samples examined, 34(28.3%) were infected where 28 (23.3%) were single case infections of either Entamoeba *histolytica* or *Giardia lamblia* and 6(5%) were mixed infection cases of both *Entamoeba histolytica* and Giardia *lamblia*. The source of water for drinking was a major determinant for the risk of infections ($P=0.030$). Hygienic practices like hand washing before meals, and use of toilets/latrines by a single household highly reduced the risk of infections whereas unhygienic practices like finger sucking increased the risk of infection ($P < 0.05$). The economic status of caregivers and the practice of fruit washing did not have statistical significance ($P>0.05$). Infections of gastro intestinal protozoan infections generally decreased with the advance in age having the peakat 6-11 months, though not statistically different ($P=0.337$). There were high parasite densities among mixed cases of infections relative to single case infections.

Conclusion: The prevalence was comparably higher in children with diarrhea in the region. Use of treated tap water, washing hands before meals and not sharing latrines/toilets greatly reduced infections while finger sucking increased the risk of infections among children under five years.

Keywords: gastro intestinal protozoans, diarrhea, children under five years.

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I. Background of the Study

Globally every year, it is projected that approximately 2 million illnesses related to diarrhea occur among children who have not reached their fifth birth anniversary from which slightly almost 50% prevalence is recorded in Africa, South Asia (38%), East Asia (9%) and Pacific (7%) respectively. The bulk of gastrointestinal related illnesses are self-limited and very specific, However, certain possible causal factors that include nutritional deficiency, immunity suppression, and early years of age prompt the development of tenacious diarrhea. Diarrhea remains a major problem among the leading causes of death in children who have not reached their fifth birth anniversary globally out of which, Kenya records 27,400 deaths among children under five years associated to diarrhea and other diarrheal illnesses (Bryce, Boschi-Pinto, Shibuya, Black, & Group, 2005).

As much as the gastro intestinal protozoa appear to have less impact on humans than the other diseases like AIDS and tuberculosis, which has been put to priority while neglecting it among many other tropical diseases, they are an immense problem and set back in tropical regions and should be put to consideration in the aid of reducing the child death rate and generally improve child health in the struggle to meet the sustainable development goals. The effects of Intestinal parasites cause noteworthy ill- health and death across the world which has made it a global problem, principally in unindustrialized countries where a larger population has other related ill- health conditions. These effects result to a tremendous effect on socio-economic aspects in terms of high treatment and hospitalization costs (Utzinger, N'goran, Marti, Tanner, & Lengeler, 1999). Recent studies that have been done in the area shows that *Entamoeba histolytica* and *Giardia lamblia* are prevalent and are commonly spread through contaminated water, (Nyarango, Aloo, Kabiru, & Nyanchongi, 2008), However, there is not much documentation on the diarrhea and other related diseases in children under five years caused by parasitic infections in Kisii County and the surrounding areas. For this reason, this study was is intended to carry out a study to determine the pre disposing factors

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associated with protozoan infections among children with diarrhea under five years of age brought for medication at the facility (KTRH). The research findings will serve as an imperative tool in allocating limited public health resources, help in achieving government development goals, and the vision 2030 of Kenya.

II. Methodology

The study was carried out at Kisii teaching and referral Hospital, which is the main referral hospital in Kisii county and surrounding counties with the major population being low- income earners. One hundred and twenty children aged five years and below that were presented with diarrheic symptoms seeking medication and subsequent treatment were included in the study. Fecal samples were collected in clean dry fecal containers from each patient, all the fecal samples were observed macroscopically for color, odor, consistency, presence of mucus and blood stains. Subsequently, a microscopic examination was done to examine the trophozoites/cysts of various gastro intestinal protozoan parasites presumed to be the causal agents for diarrhea in children. Direct wet mount preparation and formol-ether stool concentration methods were used in the microscopic examination and identity of the suspected organisms in the stool sample within the first 30 minutes to give accurate and reliable results. Socioeconomic factors were assessed using a structured questionnaire that was filled by the caregiver guided by the research assistant.

Percentages were used to describe the characteristics of the study population, including the occurrence of gastro intestinal protozoa identified among the study population. Chi-squares test (χ^2) was used to check on the associations between the variables. All variables that were significantly associated with the profile of *E. histolytica* and *Giardia lamblia* or both were included in a logistic regression analysis to ascertain the predisposing causal factors for *E. histolytica* and *Giardia lamblia* infections. For each statistically significant factor, 95% confidence interval (CI) was computed by the univariate and multinomial logistic regression analyses, and level of statistical significance determined at $P<0.05$.

III. Results

a) Prevalence and distribution of gastro intestinal protozoa per age among children under five years with diarrhea examined at the Kisii County referral hospital (KTRH)

In this study, children aged between 6-11 months had significantly highest proportion (47.6 %) of children infected with gastro intestinal protozoans than other age cohorts, but it generally decreased with the advance in age ($p<0.005$) (see table 1).

Table 1: Prevalence and distribution of gastro intestinal protozoa in children under age of five years in Kisii County

Age in months	Number of patients	Parasite species	Number infected (%) per cohort	P value
6-11	21	*Entamoeba histolytica* *Giardia lamblia* Both *(E. histolytica and G. lamblia)*	6(28.6) 2(9.5) 2(9.5)	.337
			10 (47.6)*	
12-23	50	*Entamoeba histolytica* *Giardia lamblia* Both (*E. histolytica and G. lamblia*)	10(20) 2(4) 1(2) **13(26)**	
24-35	24	*Entamoeba histolytica* *Giardia lamblia* Both *(E. histolytica and G. lamblia)*	2(8.3) 0(0.0) 2(8.3) **4(16.7)**	
36-47	12	*Entamoeba histolytica* *Giardia lamblia* Both *(E. histolytica and G. lamblia)*	2(16.7) 0(0.0) 1(8.3) **3(25)**	
48-60	13	*Entamoeba histolytica* *Giardia lamblia* Both (*E. histolytica and G. lamblia)*	2(15.4) 2(15.4) 0(0.0) **4(30.8)**	
Sub total	120	*Entamoeba histolytica* *Giardia lamblia* Both *(E. histolytica and G. lamblia)*	22(18.4) 6(5) 6(5)	
Total	120		34(28.3)	

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Additionally, *Entamoeba histolytica* was the most prevalent among the patients accounting for 64.7 % of the parasite infections in the children, while the rest were *G. lamblia* infections. Remarkably, there were 6 cases of mixed infections of *E. histolytica* and *G. lamblia* accounting for 5.0% of the infections.

Out of the 120 sampled tested for protozoal infections 71 (59.1%) were males while 49 (40.1%) were females. Thirty-four cases out of the 120tested positive for either or both *Entamoeba histolytica* and *Giardia lamblia* infections, of which 23 (67.6%) males and 11 (32.4 %) female.

Twenty-two of the positive cases (64.7%) were *Entamoeba histolytica*, 6 (17.6 %) *Giardia lamblia*, while 6 (17.6%) cases had mixed infections of *G. lamblia* and *E. histolytica* infections. The densities were classified as: rare (3 organisms per 22 mm square cover slip), few (1 organism per 8 high power fields (40x), moderate (2 organisms per high power field to as few as 1 organism per 2 high power fields.) and many (over 3 organisms in every high power field.) in that order.

The distribution of the parasite densities for *Entamoeba histolytica* was significantly higher by proportion in a category identified as few (38.2 %) as compared to rare (14.8 %), moderate (11.8 %) and finally many (0.0%) being the lowest. The same trend was realised in *Giardia lamblia*, where few had the highest frequency, followed by rare, moderate, and eventually many with the following percentage proportion, 8.8 %, 5.9 %, 2.9 %, and 0.0%, respectively. Nonetheless, in mixed infections, all cases had very high numbers of each parasite species that were categorized as many. The gastro intestinal protozoal densities for all single case infections and mixed infections were significantly lower (P = 0.000.)

b) Predisposing factors to gastrointestinal protozoan infections

In this study 25 (74%) of children who had a habit of sucking the fingers were infected by gastro intestinal protozoan infections while only 9 (10%) of the children who did not suck fingers were infected P =0.000, therefore indicating that this practice increased risk of infection see Table 2.

Table 2: Effect of hygienic practices on gastro intestinal protozoan infections among diarrheal children under age five years in Kisii County, Kenya

Hygienic practice	Practice presence/absence	Parasite identified	Number infected (%)	χ^2	P value
Hand washing before eating meals	No 32	*Entamoeba histolytica*	14(44)		
		Giardia lamblia	5(16)		
		Both (*E. histolytica and G.lmblia*)	3(9)		
	Yes 88	*Entamoeba histolytica*	8(9)	34.789	0.000**
		Giardia lamblia	1(1)		
		Both (E. *histolytica and G.lmblia*)	3(6)		
Fruit washing before eating	No 50	*Entamoeba histolytica*	11(22)		
		Giardia lamblia	5(10)		
		Both (E. *histolytica and G.lmblia*)	5(10)		
	Yes 70	*Entamoeba histolytica*	11(16)	11.758	0.508
		Giardia lamblia	1(1)		
		Both (E. *histolytica and G.lmblia*)	1(1)		
Waste disposal	Single 66	*Entamoeba histolytica*	4(6)		
		Giardia lamblia	1(2)		
		Both (*E . histolytica and G.lmblia*)	1(2)		
	Multiple 54		18(33)	28.072	0.000**
		Entamoeba histolytica	5(9)		
		Giardia lamblia	5(9)		
		Both(*E. histolytica and G.lmblia*)			
Finger sucking	No 86	*Entamoeba histolytica*	7(8)		
		Giardia lamblia	1(1)		
		Both (*E. histolytica and G.lmblia*)	1(1)		
	Yes 34	*Entamoeba histolytica*	15(44)	47.071	0.000**
		Giardia lamblia	5(15)		
		Both *(E. histolytica and G.lmblia)*	5(15)		
Water source for drinking	Streams/rivers	19	12(63)	18.479	0.030*
	Unprotected springs	19	6(32)		

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	Tap Bore hole Others	34 26 22	1(3) 10 (38) 7(32)		
Economic status of caregiver(s).	Low income High or middle income	102 18	27(23%) 7(39)	3.395	0.758

Additionally, there were 32 out of 120 children who did not regularly practice hand washing before eating meals, out of which 22(67%) were found to be infected by gastrointestinal protozoans while for the 88 children that regularly washed their hands before eating meals only 12(14%) were infected (see Table 2). Therefore, hand washing before meals significantly reduced the risk of infection, P= 0.000.

The study found out that households either used one unit of disposal per home 66 (55%) while others shared one disposal unit for many families 54 (45%). We found out that 28 (52%) children of those who shared a single disposal unit per multiple households were infected while only 6 (9%) of the children from households that did not share disposal units were infected (see Table 2). Therefore, the use of a single unit per household significantly reduced the risk of infection (P = 0.000).

Water from rivers/streams had the highest protozoan infections, with 12 (35%) children infected; others included, borehole 7 (21%) children, rainwater 7 (21%) children, springs 6 (18%) children and tap water with 1 (3%) child. Fruit washing was also seen to be a factor in the infections, with fifty children (42%) not regularly practicing fruit washing before eating, and 70 children (58%) often washed the fruits before eating. Among the 34 children that were infected with gastro intestinal protozoa, 21(62%) who did not regularly wash the fruits before eating were infected while 12(35%) who always practiced fruit washing before eating tested positive, though this factor was not seen to statistically significant.

The study also found out that 102(85%) of the caregiver(s) were in low- income level and 18(15%) of those caregiver(s) that were classified as high / middle - income level. Out of the 34 children that tested positive of gastro intestinal protozoa, 27(79%) of the infected children came from households where caregivers were of low income, while only 7 (21%) came from homes where caregiver(s) belonged to high /middle level of income (P=0.758).

IV. Discussion

a) *Prevalence and distribution of gastro intestinal protozoa among children under age five years with diarrhea*

Out of a total of 120 screened stool samples, 34 (28.3%) tested positive for gastrointestinal protozoans with *Entamoeba histolytica, Giardia lamblia, or* both (co-infections) accounting for 18.0 %, 5.0 % and 5.0 % respectively. This prevalence is higher than other sites in Kenya, which include, a study in Kitui County that reported a prevalence 12.6%, of intestinal protozoa (Nguhiu et al., 2009). The high rates observed were comparable to other findings in Mukuru informal settlement in Nairobi, that reported the infections of protozoa at 25.6%, (Mbae *et al*, (2013) and in Kitui County 38.6% (Kisavi, 2015). Similarly, our findings showed a relatively higher numbers than other countries including, Mozambique 16% (Kneel. J. *et al*, 2018) but were comparable to those in Nigeria 36.52% (Firdu *et al,* 2014) and Tanzania 29.6% (Ngoso. B.E. *et al*, (2015). The high infections warrant attention and institution of measures to control and treat infected individuals.

We found out that the increase in age was correlated to a decrease in the prevalence of infections, with the peak being at children aged between 6-11 months (47.6 %). Findings in Tanzania differs from this study finding as it showed that the highest infection of gastro intestinal infections was at (34.6%) in the age groups of 12-24 months, followed by 24-36 months (15.6%), 6-12 months (8%) and finally least among children 0-5 months (2.4%), (Ngoso. B.E *et al (2015)*. The study carried in south Ethiopia on infectious protozoa diseases of poverty also demonstrated that children of the age group between 2 -3 years were most infected, while the age group of less than one or equal to one year were least infected, (Mulatu, Zeynudin, Zemene, Debalke, & Beyene, 2015). This study also differs from another study done by De Souza *et al*. (2007), who found that "Intestinal parasitism inclines to be less predominant among children under one year of age, afterward reaching a prevalence plateau around 50%. The reason for this age group (less than 12 months old) vulnerability in this study might be explained by milk bottles contamination and crawling on contaminated grounds and accessing filthy material into their mouths (Adnan et al, 2008). Also, these age group children use diaper, which may allow the transmission via hand to mouth contamination if not used properly.

We found that there was a significantly higher number of males infected 23 (67.6%) as compared to females 11 (32.4%). Therefore, a male child was 1.5 times more likely to be infected with GI protozoacompared to a female child. These findings are similar to other studies done in Nakuru Kenya (Chabalala H. P and Mamo H, (2001), Nigeria (Anosike et al., 2004; Adeyeba & Akinlabi, 2002) and South Korea (Nkengazong, Njiokou, Teukeng, Enyong, & Wanji, 2009). However, some studies have reported higher infections in females than males (Chukwuma et al., 2009). Higher infection rates in males could be due to

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differences in behavioral factors (Coutsoudis et al., 2001), males in general show reduced immune responses and increased intensity of infection compared to females (Stanley, 2003). These disparities usually attributed to ecological factors including differential exposure to pathogens because of sex-specific behavioral or morphological patterns (Stanley, 2003).

a) *Effect of predisposing factors to gastrointestinal protozoan infections*

Various hygienic factors were seen to have contributed to the risk of infections in children under the age of 5 years. These included hand washing before eating meals, finger sucking, waste disposal practices, the main source of drinking water, fruit washing before eating, and economic status of caregivers. There were 32 (26.7%) out of 120 children in the study who did not regularly practice hand washing before eating meals, out of which 22(67%) were found to be infected by gastrointestinal protozoans while for the 88 children that regularly washed their hands before eating meals only 12(14%) were infected. Hand washing before meals was found to significantly reduce the infection of gastro intestinal protozoa infections among the study population, P= 0. 000. This finding is similar to other studies in Kilifi, Kenya, (Njuguna et al., 2016), Benue, Nigeria, (Ojiaku, Pena, Belanger, Chan, & Dennie, 2014),Malawi (Morse et al, 2008) and later in Nigeria (Strunz et al., 2013), where all of them showed that hand hygiene greatly reduced the infection by significantly reducing the fecal contamination and improving health. Therefore, the practice of hand washing before eating meals elementarily reducing the infection may be because contaminated hands play a major role in the fecal -oral route of transmissions in humans and therefore we advocate for high standards of hand hygiene for all as a measure of reducing the intestinal protozoa infections. We further found that there were 86(71.7%) children who did not suck the fingers while 34(28 .3%) practiced finger sucking. Interestingly we established that 25 (74%) of children who had a habit of sucking the fingers were infected by gastro intestinal protozoan infections while only 9 (10%) of the children who did not suck fingers were infected hence indicating that this practice increased risk of infection, P= 0.000.This agrees with the study in Sri Lanka on habits of nail- biting and sucking fingers (Lahiru S. 2016), a study in Nepal that both nail- biting and sucking fingers are significantly associated factors in school children (Sah R.B et al ,2014). However, in Benue, Nigeria, hand eating was negatively associated with diarrhea and intestinal infections (Ojiaku, Pena, Belanger, Chan, & Dennie, 2014). Therefore, health education on the practice of finger sucking aout the risk of intestinal protozoa infections should be embraced among the children in Kisii County.

Water sources for drinking was also a prominent risk with rivers/streams being the greatest with 12(35%) children out of the 34 infected, others included borehole 7 (21%) children, rain water 7 (21%), springs 6 (18%) and tap water1 (3%) child was infected. These findings are similar to one done in Nepal, Nigeria, where water from the river/streams had higher infections compared to other water sources (RB.Sah et al 2016).

The study also found out that households that were using a single unit of disposal per household decreased the chances of infections (P = 0.000). This findings are similar to (Adamu, Endeshaw, Teka, Kifle, & Petros, 2006); Noor Azian et al., 2007; Atukorala & Lanerolle, 1999) who found that intestinal parasitic infections have a global distribution with high prevalence registered in people with poor living conditions characterized by overcrowding, poor environmental sanitation, in appropriate waste disposal and unhealthy usage of pit/ latrine.

V. Conclusion

Gastro intestinal protozoa infections among children under the age five years with diarrhea in Kisii County are high. A male child under age five years in Kisii County is 1.5 times more likely to be infected by gastro intestinal protozoa compared to a female child. The parasite densities for each species was highest in mixed infection cases compared to single infection cases among children of age under five years with diarrhea in Kisii County. Hygienic practices like hand washing before meals and the use of single human waste disposal units per household highly reduced the risk of infection, while unhygienic practices like finger sucking increased the risk of infection. The source of water for drinking was a major determinant of risks of infections where treated tap water highly reduced probability of infection, but the use of water from streams and rivers for drinking was positively correlated with infections.

Authors Contributions

Caleb Okeri Ondara designed, performed sampling, data collection, data analysis and participated in manuscript preparation. Benson Omweri Nyanchongi did the research planning, data analysis and preparation of the manuscript. Mogoa Nyamwancha Wycliffe assisted in proposal development, research planning and making findings and Vincent Obino Orucho, participated in data analysis, discussion of the results and development of the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical Approval and consent to participate

The study obtained approval from the Kisii teaching and referral hospital ethical committee (KTRH)

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and the National Commission for Science and technology (NACOSTI). Parents/caregivers of all the participants in the study signed a written consent before being incorporated in the study.

Funding
The authors did not receive any funding

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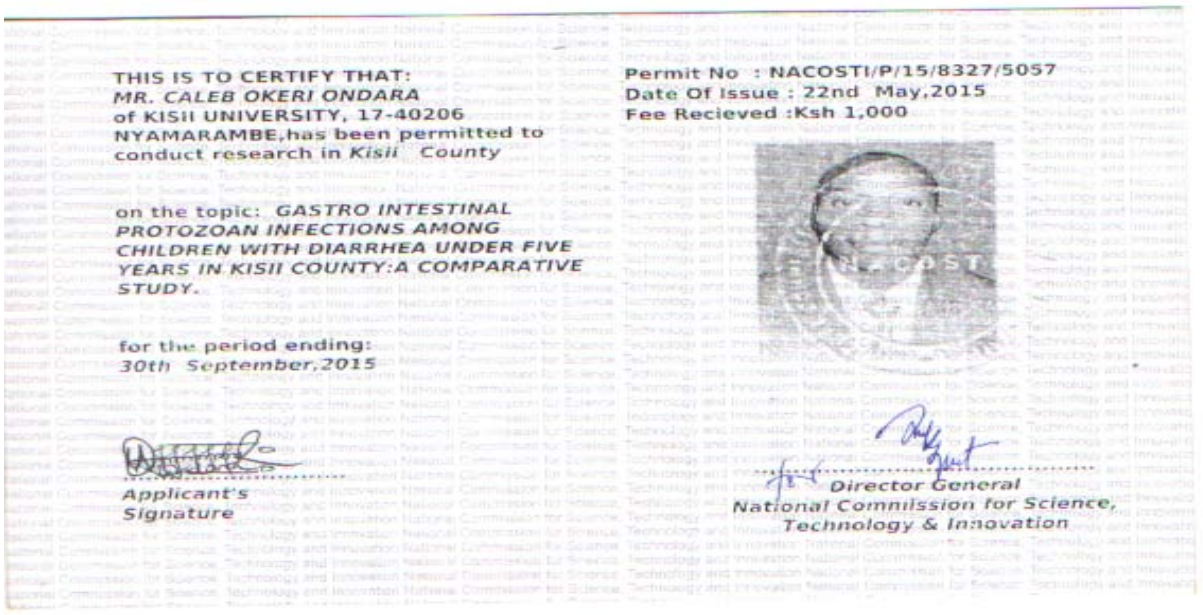
Appendix I: Research Permit



THIS IS TO CERTIFY THAT:
MR. CALEB OKERI ONDARA
of KISII UNIVERSITY, 17-40206
NYAMARAMBE,has been permitted to
conduct research in *Kisii County*

on the topic: *GASTRO INTESTINAL PROTOZOAN INFECTIONS AMONG CHILDREN WITH DIARRHEA UNDER FIVE YEARS IN KISII COUNTY:A COMPARATIVE STUDY.*

for the period ending:
30th September,2015

Applicant's Signature

Permit No : NACOSTI/P/15/8327/5057
Date Of Issue : 22nd May,2015
Fee Recieved :Ksh 1,000

Director General
National Commission for Science, Technology & Innovation

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Appendix II: Research Authorization Letter

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471, 2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date:

22nd May, 2015

NACOSTI/P/15/8327/5057

Caleb Okeri Ondara
Kisii University
P.O. Box 402-40800
KISII.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Gastro intestinal protozoan infections among children with diarrhea under five years in kisii county: A comparative study,"*** I am pleased to inform you that you have been authorized to undertake research in **Kisii County** for a period ending **30th September, 2015.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Coordinator of Health, Kisii County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. S. K. LANGAT, OGW
FOR: DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Kisii County.

The County Director of Education
Kisii County.

COUNTY COMMISSIONER
KISII COUNTY

COUNTY DIRECTOR OF EDUCATION
P.O. Box 402-40200 KISII

25/05/2015

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Appendix III: Kisii Teaching and Referral Hospital Authorization Letter

MINISTRY OF HEALTH

Telegramme "medical" Kisii
Telephone: (058) 31310 Kisii
Email:kisiihospital@gmail.com
Web: www.kisiihospital.org.ke

DEPARTMENT OF RESEARCH
THE KISII TEACHING & REFERRAL HOSPITAL
P.O. BOX 92
KISII

REF. NO. DATE: 12th July, 2015

ONDARA CALEB

RE: RESEARCH AUTHORIZATION

This is to inform you that you have been authorized to extend your data collection on "Gastro-intestinal protozoan infection among children under 5 years with diarrhea attending OPD at Kisii Teaching and Referral Hospital' for 3 weeks with effect of 13th august 2015.

THE KISII TEACHING AND REFERRAL HOSPITAL
13 AUG 2015
P. O. BOX 72, KISII
SIGN.....

DR. E.B. MASANTA -MBCHB(UoN),MPH(Epidem),(JOOUST), PGDPM(KIM),Applied Epidem & Bio (UoN)

DEPARTMENT OF RESEARCH

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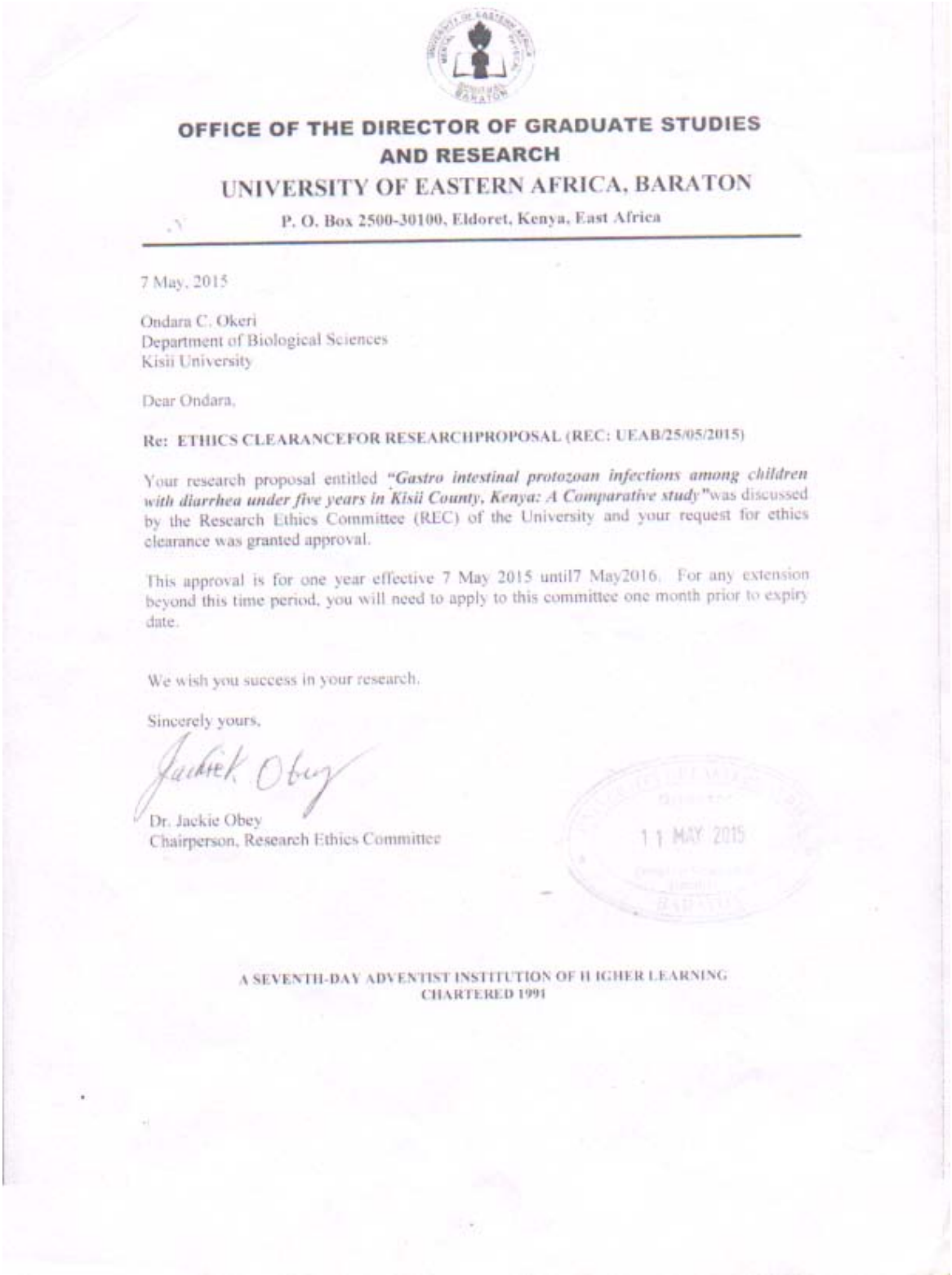
Appendix IV: Ethical Clearance Certificate.



OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

P. O. Box 2500-30100, Eldoret, Kenya, East Africa

7 May, 2015

Ondara C. Okeri
Department of Biological Sciences
Kisii University

Dear Ondara,

Re: ETHICS CLEARANCEFOR RESEARCHPROPOSAL (REC: UEAB/25/05/2015)

Your research proposal entitled ***"Gastro intestinal protozoan infections among children with diarrhea under five years in Kisii County, Kenya: A Comparative study"*** was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective 7 May 2015 until7 May2016. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date.

We wish you success in your research.

Sincerely yours,

Jackie Obey

Dr. Jackie Obey
Chairperson, Research Ethics Committee

11 MAY 2015

A SEVENTH-DAY ADVENTIST INSTITUTION OF H IGHER LEARNING
CHARTERED 1991

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Appendix V. Informed Consent form

Kisii University, post graduate studies section, school of pure and applied sciences in the department of biological sciences.

Title: Prevalence and predisposing factors associated with gastrointestinal protozoa in children under age five with diarrhea, Kisii County.

Patient identity no._____

Consent to participate in this study

I greet you, I am …….. Working on this research with an objective of determining the factors associated with gastro intestinal protozoa in diarrheal children under age five. We plan to examine 120 diarrheal children under age five attending outpatient department of Kisii Teaching and Referral Hospital. We are therefore asking you to be part in this study since you are a patient having a visit at this clinic. You have been randomly selected. We would like you to understand the intention of this study and your part so that you may take decision if you would like to join us in this study. If you accept to join, we will then ask you to sign for us this paper (or if you cannot read/ write, make your mark in front of a witness). Please ask us to explain any information that you may have not understood.

Information about the research

If you accept to participate we will interview you. We will ask you about your background and brief history of your illness. The interview will last at maximum 20 minutes. After the interview, we shall collect fresh stool sample from you for examination.

In case of the possible risks, we shall do our best to safeguard your privacy and study records. This interview shall be private. However, it is possible that others may learn that you have joined the research. Because of this, others may treat you dishonorably.

The interview questions may make you have some anxiety. You can reject to answer any question. You may also end the interview at any time without notice.

For the Possible benefits, this study has no one on one benefit but the findings of this study will help to improve interventions against diarrhea, gastro intestinal protozoa infections and other related illnesses. We do not provide any incentive for preventing or curing diarrhea and gastro intestinal protozoa if any but the interview may offer a good advice to you on how you can perhaps live diarrhea and gastro intestinal protozoa infections free life. If you decide not to be in the research.

You are free to decide if you want to take part in this research or not.

Confidentiality

We will do our best to protect information about you and your role in this research. We will interview you in a private place. We will not write your name on the interview form. We will use your form number to connect your interview response to our stool testing laboratory. You will not be named in any reports. Only the study staff and investigators will know your responses to the questions.

Compensation

You will not receive any cash by joining this study.

Leaving the research study

You may leave the research at any time. If you leave, it will not change the health attention you receive here. If you choose to take part, you can change your mind at any time and pull out. If so, please tell the research interviewer why you wish to leave.

Your rights as a participant: This research has been reviewed and approved by the Kisii University research and extension unit and NACOSTI.

If in case you have questions about this study, you should contact the Coordinator or the Principal Investigator ONDARA CALEB OKERI, Kisii University School of pure and applied sciences. P.O BOX, 408-40200.

Signature: ……

Do you agree?

Participant Agrees

Participants disagree

I ---------- have read and understood the matters in this form. I agree to participate in this study.

Participant signature ----------

Signature of witness (if can't read) ---------- Signature of research assistant ----------

-Date of signed consent ----------

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Fellows/Associates of Medical Research Council

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- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

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a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
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f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
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Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

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k) There ought to be references in the conventional format. Global Journals recommends APA format.

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The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

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ISSN 9755896

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