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Original Article

Decreased neutrophil oxidative burst activity in children with failure to thrive - a pilot study

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Abstract: Introduction: Failure to thrive (FTT) refers to failure of expected weight gain, striking lack of well-being and inadequate physical growth in children. The causes vary with geographical and socio-economic factors. In developed countries, FTT is usually a symptom of an underlying disease, often a gastrointestinal or neurological disorder. However, in developing countries, FTT is often associated with inadequate caloric intake and malnutrition. Such children are at an increased risk of infections and infection-related mortality which may be related to altered immune responses. Rarely some Primary immunodeficiencies (PIDs) can manifest as FTT. Not much data regarding neutrophil functions in these children is available. Objectives: The present study aimed to analyse the functional activity of neutrophils in children with FTT using a highly sensitive and specific flow cytometry-based assay. Methods: 25 children with FTT (up to 5 years) and 25 healthy controls were assessed for haematological parameters and neutrophil oxidative burst activity by DHR Assay using Flow cytometry. Results: Compared to controls, the cases had significantly lower haemoglobin, hematocrit, RBC count and MCHC but a higher eosinophil count (*P<0.0001*). On flow cytometry, the Neutrophil Oxidative Index (NOI) was significantly reduced in cases (*P<0.0001*). 1 of 25 cases (4%) showed no change in neutrophil fluorescence after stimulation, suggesting the presence of CGD, which was later confirmed with molecular assay revealing a CYBB mutation. Conclusions: To conclude, children with FTT have a decreased Neutrophil Oxidative Burst, suggesting defective killing of pathogens by phagocytes. Also, the presence of CGD should be ruled out in such children.

Keywords: Failure to thrive, malnutrition, neutrophil functions, oxidative burst, DHR assay, chronic granulomatous disease

Introduction

Failure to thrive [FTT], a commonly encountered problem in pediatric practice is defined as a significant interruption in the expected rate of growth during early childhood. Such children are at an increased risk of infections and altered immune responses [1]. FTT may sometimes be an initial manifestation of underlying primary Immunodeficiencies (PIDs) [2]. A child is considered to have FTT when any of the following criteria are met on two or more occasions: Weight for age less than 5th percentile/ Weight deceleration crossing 2 major percentile lines/Weight velocity less than 5th percentile/Weight less than 75% of median weight for age/Weight less than 75% of median weight for length/Length for age less than 5th percentile/ BMI [Body Mass Index] less than 5th percentile for age and gender [3].

The causes of FTT vary according to geographical location and socio-economic status. In developed countries, FTT in childhood is usually a symptom of an underlying disease, often a gastrointestinal or neurological disease [1]. However, in developing countries, FTT is often associated with poor or inadequate caloric intake and childhood malnutrition. Poverty is known to be the greatest single risk factor for FTT in both developed as well as developing countries. Few studies found that children with FTT are four times more likely to be abused than children without FTT [4, 5].

The prevalence of FTT is highly variable across the world, depending on risks within popula-

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tions. In low-income settings, poverty, inadequate nutrition and infectious diseases are the primary risk factors, while preterm birth and family dysfunction constitute primary reasons for FTT in high-income settings. In the Western world, FTT is found among 8% of preschool children. The exact prevalence in India is not yet known but FTT is rampant in our country and commonly labelled as various grades of malnutrition [6].

FTT and malnutrition are closely related diseases. Children with FTT are often malnourished. Moreover, malnutrition can manifest as FTT. Such children are at an increased risk of infections and infection-related mortality. There is some data to prove that malnutrition is associated with disturbed immune function and hence, the same can be expected in children with FTT. The immunological alterations seen in these children may contribute to life-threatening complications and increased mortality. Moreover, infections can alter a child's nutritional status contributing to an immunodeficient state [2, 7].

Haematological parameters are usually significantly lower in children with FTT as compared to healthy controls which may be due to many factors such as dietary iron intake, prevalence of parasitic and infectious diseases and socioeconomic status. A study by Nodoshan et al. and a few other studies conducted in Nepal and India have reported a higher prevalence of anaemia among malnourished children. Anaemia, as well as mild leucocytosis, are both common features of PEM as frequently reported by previous studies. Polymorphonuclear neutrophils are important phagocyte cells providing the host with first line of defence against acute bacterial and fungal diseases. Neutrophils generate reactive oxygen species [ROS] during phagocytosis in response to soluble agonists termed as neutrophil oxidative burst. Chronic Granulomatous Disease (CGD) is a primary immunodeficiency characterized by repeated bacterial and fungal infections as well as formation of granulomas in tissues. It results due to a defective NADPH (nicotinamide adenine dinucleotide phosphate) oxidase system, leading to an inability of the phagocyte to generate superoxide, which causes defective killing of pathogenic organisms. FTT is one of the most common manifestations of CGD. Testing for CGD is done by nitroblue tetrazolium (NBT) dye slide test and flow cytometry based Dihydrorhodamine 123 (DHR) assay. The first method depends on the reduction of cytochrome c assessed by photometry, while the second method relies on changes in the fluorescence properties of dihydrorhodamine 123 assessed by flow cytometry. DHR assay is an analytically sensitive test performed in routine clinical laboratories and is now considered the preferred screening tool due to its higher sensitivity and faster ability to detect X-linked carriers in CGD. Apart from inherited defects in neutrophil functions such as CGD, it is now known that neutrophil functions may be defective in several acquired disorders leading to a high risk of infection-related morbidity and mortality in these children [8]. Several studies prove that neutrophil functions are affected in subjects with Iron deficiency and malnutrition [9-14]. However, not much data is available regarding neutrophil functions in children with FTT. Thus, the present study was conducted to analyse the functional activity of neutrophils in children with FTT using a highly sensitive and specific flow cytometry based assay.

Methods

This case-control study was carried out in the Departments of Pathology and Pediatrics at a 1500-bed tertiary care hospital between January 2021 and April 2022. The study was approved by the Ethics Committee of the Institution of Human Research [IECHR/2020/PG/46/17] and informed consent was obtained from the parents of all the selected children (Annexure 1, 2).

Case selection

The study included 25 randomly selected children up to 5 years of age clinically diagnosed with FTT as per either of the following criteria met on two or more occasions: Weight for age less than 5th percentile/Weight deceleration crossing 2 major percentile lines/Weight velocity less than 5th percentile/Weight less than 75% of median weight for age/Weight less than 75% of median weight for length/Length for age less than 5th percentile/BMI [Body Mass Index] less than 5th percentile for age and gender [3]. 25 healthy age and sex-matched controls visiting the immunization clinic were also included in the study after obtaining appropriate consent and assent. Children with known

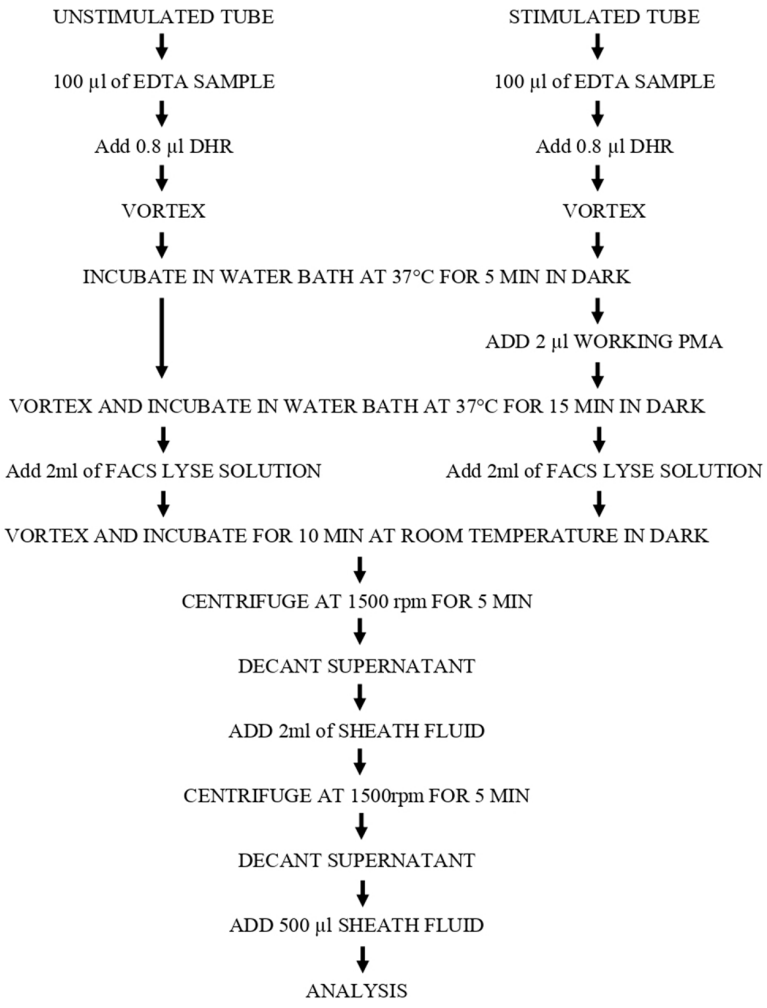

Figure 1. Flow diagram - DHR assay methodology for all subjects (cases + controls).

immunodeficiencies [known cases of HIV], children on immunosuppressants [oral methotrexate and/or chemotherapy] and children on steroids for more than 4 weeks were excluded from the study.

Clinical details

A baseline assessment including clinical, immunization and birth history was done along with general and anthropometric examination. Demographic details including age, sex, address, contact number and date of enrolment were recorded.

Laboratory assessment

After all universal precautions, 2 ml of peripheral venous blood was drawn under aseptic conditions and collected in a pediatric EDTA vial for CBC and DHR assay. CBC was performed on a six-part differential hematology analyser [Mindray ltd]. Hb, TLC, DLC and Platelet count were recorded for all cases and controls. Flow cytometry was performed on DxFLEX-13 color 3 laser equipment by Beckman Coulter Ltd. for neutrophil oxidative burst activity.

Principle

NADPH oxidase produces a respiratory burst in neutrophils which is assessed by in-vitro stimulation with PMA [Phorbol Myristate Acetate]. The oxidation of DHR [Non-fluorescent 123-dihydrorhodamine] to fluorescent rhodamine 123 is measured by flow cytometry. A normal neutrophil oxidative burst is demonstrated by a complete shift in the stimulation histogram.

Methodology

First, a stock solution of DHR was prepared by adding 200 µL of dimethyl sulfoxide [DMSO] to 2 mg DHR-123 and that of phorbol 12-myristate 13-acetate [PMA] was made by adding 1 mg PMA to 1 mL of DMSO, which were stored at -20°C. The stock solutions were stored as 5 µL aliquots; each aliquot was diluted with 45 µL of normal saline before usage. For each case, two tubes were prepared in duplicate - the stimulated tube (with PMA added) and the unstimulated tube. **Figure 1** describes further methodology.

Acquisition and analysis

Flow cytometric analysis was carried out on a 13-colour Beckman Coulter DxFLEX Flow Cytometer using CytExpert for DxFlex 2.0 software. After excluding the debris and doublets, neutrophils were gated based on forward scatter and side scatter characteristics. Care was

Table 1. Comparison of Haematological parameters between cases and controls

Haematological parameters (Mean Values)	Controls (N=25)	Cases (N=25)	Statistical significance [*p* Value]
Haemoglobin (g/dl)	10.2 ± 1.53	7.78 ± 2.17	*<0.0001*
RBC count (10^6/μL)	4.38 ± 0.54	3.29 ± 1.05	*<0.0001*
Haematocrit (%)	32.38 ± 4.01	24.82 ± 6.48	*<0.0001*
MCV (fL)	73.32 ± 6.73	75.69 ± 11.72	0.38
MCH (pg)	22.94 ± 2.77	21.36 ± 5.52	0.20
MCHC (g/dl)	30.65 ± 1.38	25.86 ± 5.36	*<0.0001*
TLC (10^3/μL)	9.56 ± 2.94	10.55 ± 3.41	0.27
Neutrophils (%)	41.36 ± 14.85	38.4 ± 20.8	0.56
Lymphocytes (%)	51.04 ± 15.29	53.84 ± 20.7	0.58
Monocytes (%)	4.84 ± 2.01	5.12 ± 2.57	0.66
Eosinophils (%)	1.88 ± 1.12	5.12 ± 2.57	*<0.0001*
Basophils (%)	0.52 ± 0.5	0.56 ± 0.5	0.77
Platelet count (lacs)	3.14 ± 0.9	3.05 ± 1.4	0.78

Table 2. Comparison of DHR Assay between cases and controls

DHR-Assay Parameters	Controls (N=25)	Cases (N=25)	Statistical significance [*p* Value]
Stimulated Neutrophils (%)	92.7 ± 8.18	87.51 ± 20.05	0.23
Mfi-Unstimulated (×10^5)	12.1 ± 5.5	15.6 ± 6.8	0.06
Mfi-Stimulated (×10^7)	12.7 ± 4.4	11.2 ± 2.4	0.14
Mfi Index	132.76 ± 86.14	71.9 ± 37.9	*<0.0001*

taken to include only neutrophils by drawing an appropriate gate around the population of interest. DHR flow histograms on this gated population were then recorded both with and without stimulation with PMA for all the study subjects. The following parameters were recorded directly from the histogram statistics: Percentage of neutrophils which underwent stimulation, Mean fluorescence Intensity [MFI] of unstimulated neutrophils and Mean fluorescence Intensity [MFI] of stimulated neutrophils after stimulation with PMA. In addition, a Neutrophil oxidative index [NOI] [or stimulation index] was calculated using the formula = MFI stimulated tube/ MFI unstimulated tube.

Statistical analysis

Analysis was done using Stata software [Stata Incorporation, USA; version 13]. Continuous variables were reported as mean [± standard deviation] or median [± inter-quartile range] as appropriate. The categorical variables were reported as numbers [percentages]. Chi-square test was used to compare proportions between groups, while Student's t-test was used to compare the means. A *p*-value <0.05 was taken as significant. Z-score was measured in terms of standard deviations from the mean.

Results

The study included 25 cases of FTT along with 25 age and sex-matched controls.

*Comparison of hematological parameters (**Table 1**)*

Children with FTT had significantly lower red cell indices including haemoglobin, haematocrit, RBC and MCHC as compared to healthy age/sex-matched controls [*P<0.0001*]. A higher eosinophil count was seen in cases as compared to the controls [*P<0.0001*].

*Comparison of neutrophil oxidative burst activity (**Table 2; Figure 2**)*

The differences between mean values of percentages of stimulated neutrophils, unstimulated MFI and MFI after PMA stimulation were insignificant on comparison between cases and controls. However, on comparing the MFI index among the controls and cases [mean ± SD = 132.76 ± 86.14 and 71.9 ± 37.9 respec-

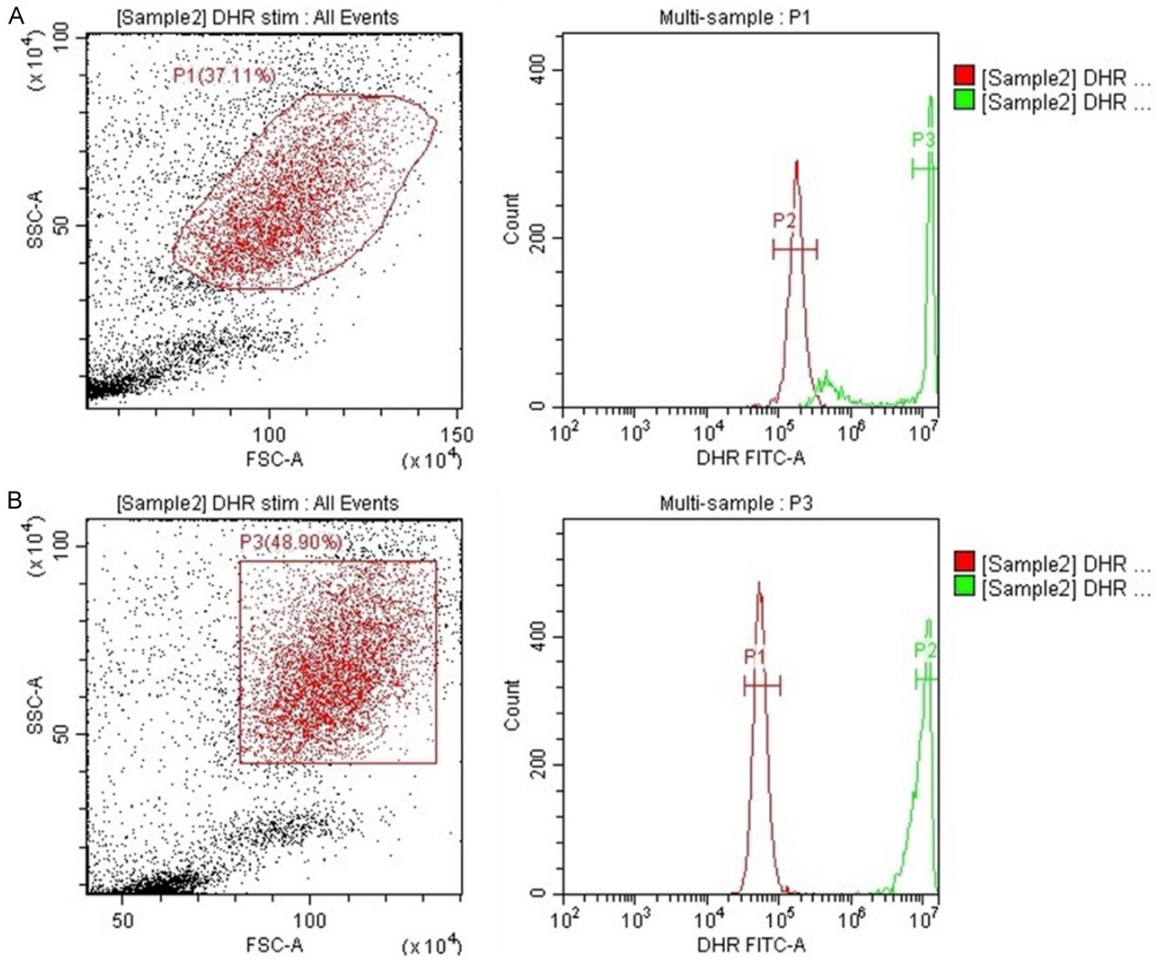

Figure 2. MFI overlay histograms in control (A) and case (B) *[Red - Unstimulated, Green - Stimulated].*

tively] a significant statistical difference was noted [*p-value ≤0.0001*] i.e. the MFI index was significantly lower in cases as compared to controls.

Out of 25 cases, one case demonstrated no change in neutrophil fluorescence after stimulation (**Figure 3**). The MFI before stimulation in this case was 160729 and that after stimulation with PMA was 174902. The MFI Index was calculated to be 1.1 which was extremely low. Hence, *X-linked chronic granulomatous disease [CGD]* was suspected in this child, which was later confirmed with molecular assay for CYBB mutation.

Discussion

Failure to thrive [FTT] is a common problem in paediatric practice. Like malnourished children, children with FTT may also have phagocytic dysfunction as they are more prone to infections as compared to well-nourished children. In this study, the authors attempted to evaluate the neutrophil oxidative burst activity in children with FTT and compare the results with healthy age and sex-matched controls.

Neutrophils play an important role in first line of defence. Defects in neutrophil functions can result in increased acute bacterial and fungal infections. Apart from inherited disorders like CGD, it is increasingly recognised that neutrophil functions may be defective in common ailments like malnutrition and Iron deficiency anaemia also. Traditionally, neutrophil functions have been assessed using the NBT [Nitroblue Tetrazolium] dye test. Since NBT test is semi-quantitative and relies on light microscopy to provide a mostly qualitative determination of phagocytic NADPH oxidase activity, DHR Assay using flow cytometry is being increasingly

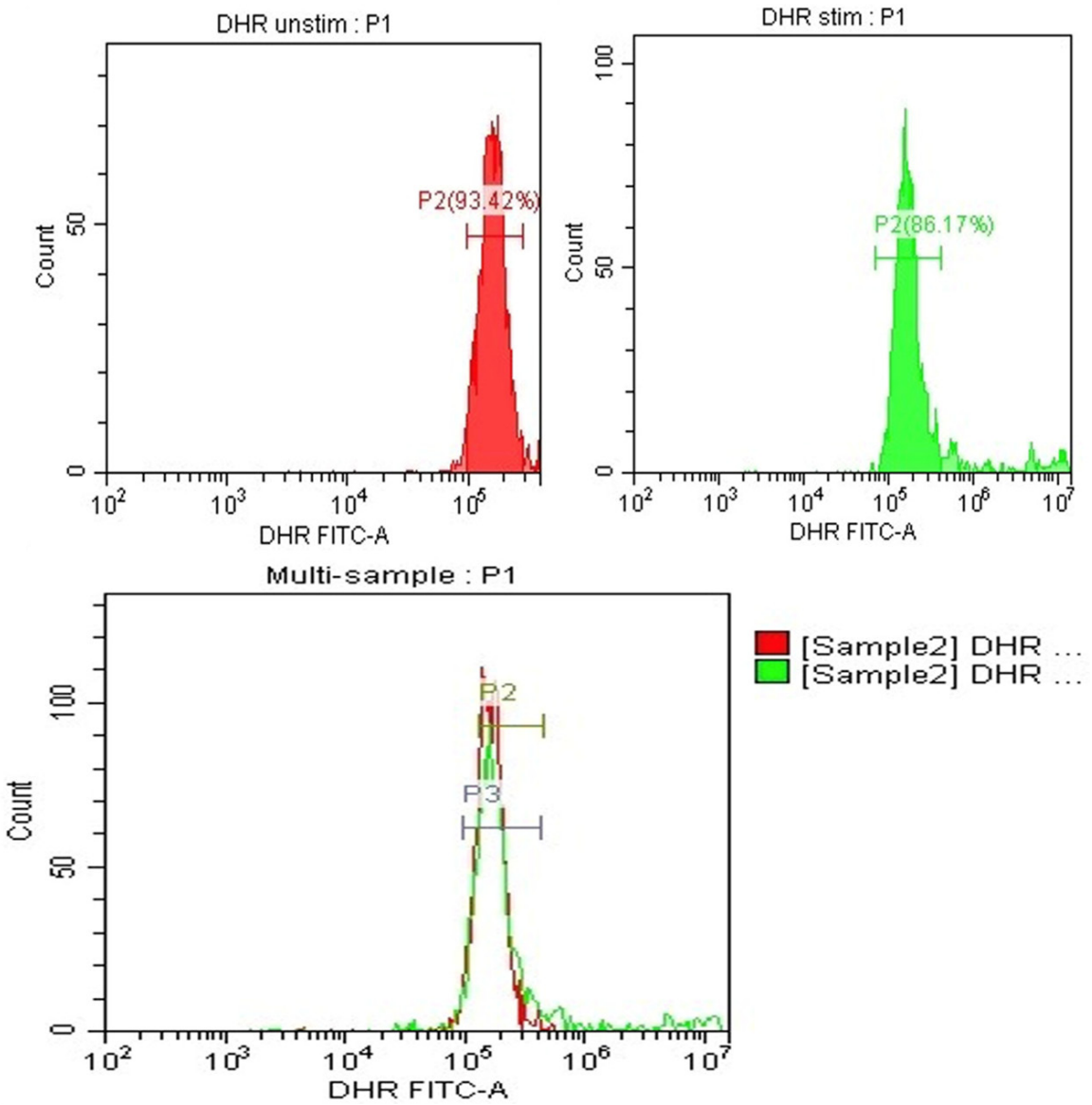

Figure 3. Overlapping unstimulated and stimulated MFI peaks in a case of suspected X-linked CGD *[Red - Unstimulated, Green - Stimulated].*

used to quantitatively evaluate the functional capabilities of neutrophils. It provides a rapid screen for abnormalities of neutrophil function and reflects more accurately their behaviour in vivo [8].

Haemoglobin, RBC count, Haematocrit and MCHC of children with FTT were significantly lower than the healthy controls which may be due to many factors such as dietary iron intake, prevalence of parasitic and infectious diseases and socioeconomic status. A study by Nodoshan et al. and a few other studies conducted in Nepal and India have reported a higher prevalence of anaemia among malnourished children [15-18]. An elevated eosinophil count may be associated with many disorders of immune deficiency or dysregulation. Some PIDs can also show eosinophilia hence, must be considered as a differential diagnosis [19]. However, not much literature could be found regarding the significance of higher eosinophil counts in children with malnutrition or FTT. Anaemia, as well as mild leucocytosis, are both common features of PEM as frequently reported by previous studies [20, 21].

The present study used flow cytometry based DHR assay to assess the oxidative burst in neutrophils and on comparing the percentages of stimulated neutrophils and Mean Fluorescent Intensities [MFI] before and after stimulation of the controls with the cases, the authors found no statistical significance between the two groups. However, on comparing the MFI Index or NOI [Neutrophil Oxidative Index], a significantly higher value was observed in healthy age/sex-matched children than in children with FTT. According to the available literature, changes in the immunological status of children with severe malnutrition include a decrease in neutrophil microbicidal activity [7, 22]. Not enough studies were found describing the neutrophil oxidative burst activity in children, particularly with FTT, however, a few studies were found regarding the same in children with malnutrition in India as well as worldwide. In a study conducted by Rosen et al., chemotaxis of granulocytes was observed to be reduced in malnourished children. The microbicidal activity of granulocytes was also found to be reduced in malnourished children in several studies [14, 23, 24]. An Indian study conducted by Goyal et al. discussed the diminished ability of granulocytes to adhere to foreign material in malnourished children [11]. A study by Salimonu et al. found a significant lack of function in phagocytes of malnourished children [using NBT reduction method], which may be a factor responsible for the impaired bactericidal activity and hence, one of the causes of a high incidence of infections in such children [13]. On the contrary, a few studies conducted to analyse the macrophage function in kwashiorkor and protein energy malnutrition found a normal microbicidal activity of neutrophils in similar children [25, 26].

Primary immunodeficiencies are rare diseases associated with defective innate or adaptive immune response resulting in various developmental disorders as well as life-threatening bacterial, fungal or viral infections. Failure to thrive can sometimes be the initial presentation of these disorders [2]. Given the low index of suspicion and lack of investigative support, most of these go undiagnosed resulting in high morbidity, mortality and burden on the health care system due to the unnecessary hospital visits and battery of investigations. In a pediatric report by Leung et al., three young children with common variable immunodeficiency who presented as FTT had abnormal humoral and cellular immune functions as well as altered functions of granulocytes and natural killer cells. The oldest boy died from severe pneumonia whereas the other two patients were free from serious infection on follow-up. Although common variable immunodeficiency typically affects young adults, this diagnosis should also be considered in infants and young children who suffer from atypical or recurrent infections. Hence, thorough immunological investigations need to be performed on these children presenting as FTT [27]. A retrospective study by Eugenia et al. evaluated the records of 18 CGD patients who clinically manifested as FTT [28]. In the present study, one child out of 25 cases [0.4%] showed no change in neutrophil fluorescence after stimulation, suggesting the presence of X-linked chronic granulomatous disease [CGD]. On further clinical workup, some of his symptoms of frequent aerodigestive and genitourinary infections corroborated with the diagnosis. The child also had a positive family history in one of the siblings. Molecular confirmation was done which revealed a CYBB gene mutation.

However, it has also been noted that complete myeloperoxidase [MPO] deficiency may occasionally lead to a strongly decreased DHR signal on flowcytometric DHR assay which can give a false positive result for CGD. In a study conducted by Mauch et al. to differentiate MPO deficiency [false positive for CGD] and NADPH oxidase abnormalities [true CGD], the authors found that eosinophils in MPO-deficient individuals retain eosinophilic peroxidase and therefore can generate a normal DHR signal. The addition of recombinant human MPO to MPO-deficient cells showed an enhanced response, while no response was seen when added to NADPH-oxidase-deficient [CGD] cells [29, 30].

The authors' main challenge during the study was a limited sample size. Also, due to economic constraints, other immunological workup could not be carried out for these patients. Hence, the authors recommend more studies with larger sample sizes and long term follow-up for describing in detail these parameters in children with FTT in order to enhance the anticipatory care and outcome of the children affected.

Conclusion

Laboratory evaluation of children with Failure to Thrive is complex but essential for the care and treatment of such patients. FTT is a condition that constantly modifies and alters the affected child's body defence mechanisms and immunological profile at all levels, therefore, stepwise and systematic testing for immunodeficiency and phagocytic activity in children is important. A decrease in the Neutrophil Oxidative Index in these children depicts an impaired neutrophil oxidative burst and a poor innate response for pathogens like Staphylococcus, Salmonella, Aspergillus and Candida species. Also, the presence of PID should be suspected in children with FTT and minimum baseline investigations to rule out PIDs should be conducted.

Acknowledgements

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Disclosure of conflict of interest

None.

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ANNEXURE-I

Informed consent including Participant Information Sheet (English)

UNIVERSITY COLLEGE OF MEDICAL SCIENCES
(UNIVERSITY OF DELHI)
DILSHAD GARDEN, DELHI-110095
011-22586262, Fax: 0091-11-22590495

Informed Consent form for Research Work Entitled 'Decreased neutrophil oxidative burst activity in children with failure to thrive - A pilot study'.

This informed consent form is for parents of failure to thrive children who are coming to OPD and emergency in the Department of Paediatrics, University College of Medical Sciences & GTB Hospital, Dilshad Garden, Delhi-110095.

Principal Investigator: Dr. Saumya Jindal, MD student, Department of Pathology
UCMS & GTB University, Delhi -110095 Phone No- 8800472445
Email id - saumyajindal1802@gmail.com

Organization: UCMS & GTB Hospital, Delhi University, Delhi -110095 TB Hospital, Delhi.

Name of Proposal: Decreased neutrophil oxidative burst activity in children with failure to thrive - A pilot study

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part) **You will be given a copy of the full Informed Consent Form**

PART I:
Information Sheet Introduction

Introduction

I am Dr. Saumya Jindal, an MD student in Department of Pathology, UCMS & GTB Hospital, Dilshad Garden, Delhi -110095. I invite your child to participate in the research study on immune profile in failure to thrive children. Research work on this condition is expected to help in expanding our current knowledge about this disease. Your consent for your child's participation as a study subject is required as a mandatory requirement for research involving human subjects. You are free to talk to anyone you feel comfortable talking with about the research. You can decide whether you want to participate or not. If you do not understand some of the words or concepts, they will be explained to you.

Purpose of the research

Role of neutrophils in FTT children is not fully investigated. Such research could help us to discover immunotherapies in future for FTT patients.

Participant selection

We are inviting all the patients of Failure To Thrive who are coming to our hospital.

Voluntary Participation

Your child's participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive in this hospital will continue and nothing will change. If you choose not to participate in this research project, you will offered treatment that is routinely offered in this hospital. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

After obtaining consent from the patient, a detailed history and examination will be done. Information will be obtained as per the case record form. Apart from blood sampling for routine investigations, *3 ml of extra blood* will be taken for the study (total 5 ml). The blood sample of the patient will be collected & processed within 24 hrs. The sample will be evaluated for Neutrophil function over and above the samples for routine work-up of Failure to thrive. Routine investigations for workup includes CBC.

Expected duration of the subject participation

Once, at the time of submission of the sample for evaluation.

Risks

Sampling will be done by experts under all aseptic precautions and will pose a negligible risk.

Benefits

This research study will evaluate neutrophil oxidative burst in failure to thrive children under 5 years of age and will provide data for community benefits.

Reimbursements

Your child will not be given any money or gifts to take part in this research.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Sharing the Results

We intend to publish the results of this research in order that other interested people may learn from our research. Confidential information will not be shared.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Student: Dr. Saumya Jindal (8800472445)

Post Graduate Student
Department of Pathology, UCMS & GTB Hospital

Supervisor: Dr. Richa Gupta (9910790101)

Professor
Department of Pathology
UCMS and GTB Hospital

This proposal has been reviewed and approved by Institutional Ethics Committee- Human Research (IEC-HR), University College of Medical Sciences, University of Delhi. It is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find more about the committee.

Contact: Institutional Ethics Committee - Human Research
University College of Medical Sciences
Delhi 110095, India
Phone: +911122595974
Email: iechrucms@gmail.com

You can ask me any more questions about any part of the research study, if you wish to.

PART II
CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____
Signature of Participant/Parent*/Guardian*_____
Date_____(day/month/year)
(*)In case of minors/children

For the illiterate patient

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____AND Thumb print of participant
Signature of witness_____
Date_____(Day/month/year)

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Venous blood sampling

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____
Signature of Researcher/person taking the consent_____
Date_____
Day/month/year

ANNEXURE-II

Informed consent including Participant Information Sheet (Controls) (English)

UNIVERSITY COLLEGE OF MEDICAL SCIENCES
(UNIVERSITY OF DELHI)
DILSHAD GARDEN, DELHI-110095
011- 22586262, Fax : 0091-11-22590495

Informed Consent form for Research Work Entitled 'Decreased neutrophil oxidative burst activity in children with failure to thrive - A pilot study'

This informed consent form is for parents children not having failure to thrive who are coming to OPD and emergency in Department of Paediatrics, University College of Medical Sciences & GTB Hospital, Dilshad Garden, Delhi-110095.

Principal Investigator: Dr. Saumya Jindal, MD student, Department of Pathology
UCMS & GTB University, Delhi -110095
Phone No- 8800472445
Email id - saumyajindal1802@gmail.com

Organization: UCMS & GTB Hospital, Delhi University, Delhi -110095 TB Hospital, Delhi.

Name of Proposal: Decreased neutrophil oxidative burst activity in children with failure to thrive - A pilot study

This Informed Consent Form has two parts

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part) **You will be given a copy of the full Informed Consent Form**

PART I:
Information Sheet Introduction

Introduction

I am Dr. Saumya Jindal, an MD student in Department of Pathology, UCMS & GTB Hospital, Dilshad Garden, Delhi -110095. I invite your child to participate in the research study on immune profile in failure to thrive children. Research work on this condition is expected to help in expanding our current knowledge about this disease. Your consent for your child's participation as a study subject is required as a mandatory requirement for research involving human subjects. You are free to talk to anyone you feel comfortable talking with about the research. You can decide whether you want to participate or not. If you do not understand some of the words or concepts, they will be explained to you.

Purpose of the research

Role of neutrophils in FTT children is not fully investigated. Such research could help us to discover immunotherapies in future for FTT patients.

Participant selection

We are inviting children who are coming to our hospital who do not have Failure To Thrive and would serve as controls for our study.

Voluntary Participation

Your child's participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive in this hospital will continue and nothing will change. If you choose not to participate in this research project, you will offered treatment that is routinely offered in this hospital. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

After obtaining consent from the patient, a detailed history and examination will be done. Information will be obtained as per the case record form. Apart from blood sampling for routine investigations, for the study, 3 *ml of extra blood* will be taken (total 5 ml). The blood sample of the patient will be collected & processed within 24 hrs. Sample will be evaluated for Neutrophil function over and above the samples for routine work-up of Failure to thrive. Routine investigations for workup includes serum CBC.

Expected duration of the subject participation

Once, at the time of submission of the sample for evaluation.

Risks

Sampling will be done by experts under all aseptic precautions and will pose a negligible risk.

Benefits

This research study will evaluate neutrophil oxidative burst in failure to thrive children under 5 years of age and will provide data for community benefits.

Reimbursements

Your child will not be given any money or gifts to take part in this research.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Sharing the Results

We intend to publish the results of this research so that other interested people may learn from our research. Confidential information will not be shared.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Student: Dr. Saumya Jindal (8800472445)

Post Graduate Student
Department of Pathology, UCMS & GTB Hospital

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For the illiterate patient

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Date_____(Day/month/year)

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I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____
Signature of Researcher/person taking the consent_____
Date_____
Day/month/year