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"A CROSS-SECTIONAL STUDY OF FAT DROPLETS IN STOOLS AS A PREDICTOR OF ROTAVIRAL DIARRHOEA IN CHILDREN AGE < 5 YEARS OF AGE."

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ABSTRACT **Introduction:** This study evaluates stool fat droplet microscopy as a simple, low-cost tool for early diagnosis of rotavirus diarrhoea in children. **Methodology:** This prospective observational cross-sectional study enrolled 88 children under 5 years with acute diarrhoea, using stool microscopy (Sudan III staining) and rotavirus antigen ELISA for diagnosis. Data were analyzed with SPSS v22, applying chi-square tests for associations. **Results:** Rotavirus antigen was detected in 30.7% of children, with a significant association between fat droplet presence and infection ($p<0.001$). Stool fat droplet microscopy showed 70.4% sensitivity and 85.2% specificity for rotavirus detection. **Conclusion:** Stool fat droplet microscopy using Sudan III is a simple, affordable, and fairly accurate screening tool for early identification of rotavirus diarrhoea in children.

KEYWORDS : Rotavirus, Diarrhoea, Stool Microscopy, Sudan III Staining

INTRODUCTION:

Acute diarrhoea, defined as the passage of three or more loose stools per day lasting less than 14 days, remains a major public health problem globally, especially among infants and children.[1] Despite advances in healthcare, diarrhoeal diseases continue to contribute significantly to morbidity and mortality in developing nations like India, where poor sanitation, unsafe water, and limited healthcare access persist. Worldwide, diarrhoea is among the top three infectious causes of death, mostly in children under five. The primary cause of death is dehydration due to rapid fluid and electrolyte loss, which is particularly dangerous in infants.[2]

Rotavirus is recognized as the leading cause of severe childhood diarrhoea, responsible for nearly 2.3 million hospitalizations and about 600,000 deaths globally each year, highest in developing regions.[3] Rotavirus infection impairs intestinal absorptive functions, particularly of amino acids and fatty acids, contributing to malabsorption and nutrient deficiencies.6–8 Clinically, this is often reflected as steatorrhea-pale, oily stools rich in unabsorbed fat-which correlates with mucosal damage.[4]

Detection of steatorrhea through stool microscopy provides a simple and inexpensive diagnostic clue in rotavirus diarrhoea. The use of Sudan III staining enhances visualization of fat droplets in stool samples, making this method particularly valuable in resource-limited settings. Although stool rotavirus antigen tests are accurate, their high cost and limited availability restrict their use in rural areas.13 Correlating fat droplet detection with antigen positivity could therefore establish stool microscopy as a practical alternative for early diagnosis.[5,6]

Early recognition of rotavirus diarrhoea is crucial to initiate timely rehydration therapy and supportive measures such as zinc supplementation, which reduces the duration and severity of diarrhoeal episodes.14 Preventive strategies, including rotavirus vaccination, improved sanitation, and health education, are essential to reduce the burden.[6] This study was planned considering the paucity of research on stool fat droplet sensitivity and specificity in diagnosing rotaviral diarrhoea, which could strengthen diagnostic and management strategies.

METHODOLOGY:

Study Setting And Duration:

The study was conducted in the Department of Paediatrics, Tirath Ram Shah Hospital, New Delhi, from June 2023 to December 2024.

Study Design: An observational, cross-sectional, prospective study.

Study Population And Sampling: Children under 5 years of age presenting with acute diarrhoea (<14 days) were considered. A consecutive sampling technique was used, and only those meeting the inclusion and exclusion criteria and whose parents provided written informed consent were recruited.

Inclusion Criteria:

1. Children of either sex <60 months.
2. Diarrhoea of <14 days duration.
3. Informed written consent from parents.

Exclusion Criteria:

1. Children with shock, sepsis, or systemic illness.
2. Dysentery (blood in stool).
3. Prior medication (except zinc/ORS).
4. On special formulas or therapeutic diets.
5. Diarrhoea persisting beyond 14 days (confirmed telephonically on day 15).

Study Methods:

After consent, socio-demographic and clinical data were collected using a pretested questionnaire. The first stool sample post-admission was collected for routine and microscopic examination, including Sudan III staining for fat droplets, and rotavirus antigen ELISA card testing.

Results were recorded as:

- Normal: ≤100 fat droplets (1–2 µm/HPF)
- Slightly increased: >100 droplets (1–5 µm/HPF)
- Significantly increased: >100 droplets (6–75 µm/HPF).

Outcome Measures:

Primary outcomes were prevalence of rotaviral diarrhoea and its association with stool fat droplets.

Sample Size:

Based on Kumar et al.[7] (p = 36.5%), with 10% precision and 95% confidence, the required sample size was calculated as 88.

Data Management And Analysis:

Data were entered in Microsoft Excel and analyzed using SPSS v22. Continuous variables were expressed as mean ± SD; categorical data as percentages. Chi-square test was applied for associations, with $p < 0.05$ considered significant.

Ethical Considerations:

Ethical clearance was obtained. Written informed consent was taken, and confidentiality was maintained through anonymized, password-protected data storage.

RESULTS:

We studied total 88 cases in our study. In our study, the maximum cases of acute diarrhoea were observed in children aged 13–24 months (31.8%), followed by 37–48 months (25%) and ≤12 months (22.7%). The proportion declined with age, with the lowest cases seen in 49–60 months (5.7%). We had 61.4% males and 38.6% females. The most commonly reported symptom besides diarrhea was fever (15.9%), followed by vomiting (14.8%), abdominal distention (11.4%), lethargy (8%), and oliguria (3.4%).

Table 1: Diarrhea Related Characteristics Distribution Of Patients (n=88)

Characteristics	Mean	SD
Duration (days)	4.7	1.4

Frequency of stool (/day)	11.4	4.7

The mean duration of diarrhea at presentation among the patients was 4.7 days (SD: 1.4), with an average stool frequency of 11.4 times per day (SD: 4.7).

Table 2. Fecal Fat Droplets Distribution Of Patients (n=88)

Fat droplets in stool	Frequency	Percentage
Negative	60	68.2
Slightly increased	7	8
Significantly Increased	21	23.8
Total	88	100

Among the patients, 68.2% had no fat droplets in their stool, whereas 8% had a slight increase and 23.8% had significantly increased fat droplets.

Rotavirus antigen was detected in 30.7% of the children, while 69.3% tested negative.

A majority of the patients (68.2%, n=60) required hospitalization, while 31.8% (n=28) were managed on an outpatient basis.

Table 3. Factors Associated With Rotavirus In Stool Distribution Of Patients (n=88)

	Rotavirus negative (n=61)		Rotavirus positive (n=27)	
Fat Cells	**Frequency / mean**	**Percentage / SD**	**Frequency / mean**	**Percentage / SD**
Negative	52	85.2	8	29.6
Slightly increased	1	1.6	6	22.2
Significantly Increased	8	13.1	13	48.2

*Statistically significant, p *<0.001**

The presence of fat droplets in stool was significantly associated with rotavirus infection (p<0.001), with 48.1% of rotavirus-positive cases showing increased fat droplets compared to only 13.1% in rotavirus-negative cases.

Table 4. Diagnostic Accuracy Of Fat Droplets In Identifying Rotaviral Infections (n=88)

Fat droplets	Rotavirus positive (n=27)		Rotavirus negative (n=61)	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Present	19	30.4	9	14.7
None	8	29.6	52	85.2

The presence of fat droplets in stool had a sensitivity, specificity, PPV, and NPV of 70.4%, 85.2%, 67.9% and 86.7% in identifying rotaviral infections.

DISCUSSION:

The present study investigated the association between fat droplets in stool and rotavirus infection among 88 children under five years presenting with acute diarrhea. The majority of cases occurred in the 13–24 months age group (31.8%), followed by 37–48 months (25%) and ≤12 months (22.7%). This aligns with Kumar et al.[7], who reported that 41.8% of rotavirus cases were in children aged 6–23 months, reflecting waning maternal antibodies and incomplete immunization (40).

A male predominance was observed (61.4%), consistent with Karadag et al.[8], who noted 40.8% of boys compared to 31.8% of girls tested positive for rotavirus (38). Rural residence was strongly associated with rotavirus positivity (81.5%, p=0.001), similar to Kumar et al.[7], who highlighted higher prevalence in rural regions of India.

The mean diarrhea duration was 4.7 days, and mean stool frequency was 11.4/day, supporting Martinez et al.[9], who described altered lipid metabolism and gut motility aggravating severity.

Rotavirus antigen was positive in 30.7% of children, comparable to Kumar et al.[7] (36.5%) and Ali et al. (40.3%)[10]. Among the 88 children, 23.8% had significantly increased fat droplets, 8% slight increase, and 68.2% none. Notably, 48.1% of rotavirus-positive cases had significantly increased fat droplets and 22.2% slight increase, compared to 13.1% and 1.6% among rotavirus-negative cases (p<0.001). This strongly mirrors Rahman et al.[11], who showed 70% positivity with fat droplets, with sensitivity 66% and specificity 85%. The present study showed higher sensitivity (70.4%) and equivalent specificity (85.2%).

Chowdhury et al.[12] found fat droplets in 58% of 13,000 diarrheal patients, especially with rotavirus and ETEC, though specificity was lower in malnourished children under 2 years. Martinez et al. showed lipid droplets aid viral replication[9], supporting the pathophysiological basis of steatorrhea in rotavirus.

The Sudan III stain showed diagnostic accuracy consistent with Masamune et al. (72% accuracy, 6% false negatives)[13]. In this study, PPV was 67.9% and NPV 86.7%, supporting its utility as a simple, affordable tool in resource-limited settings.

Finally, rotavirus ELISA antigen testing remains the diagnostic gold standard, with Hung et al. reporting pooled sensitivity 89.2% and specificity 93.2% [14]. However, given cost and infrastructure limitations, stool microscopy with Sudan III provides a practical alternative with reasonable sensitivity and specificity.

CONCLUSION:

This study found a strong association between stool fat droplets and rotavirus infection in children under five with acute diarrhea. Nearly half of rotavirus-positive cases showed steatorrhea, with stool microscopy demonstrating 70.4% sensitivity and 85.2% specificity. Given its simplicity, affordability, and reasonable accuracy, Sudan III staining can serve as a useful screening tool in low-resource settings. While ELISA remains the standard, fat droplet detection provides a rapid, practical aid in identifying rotavirus-related diarrhea and guiding timely management.

Conflict Of Interest: None

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