

Research Article

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## Comparison of Stool Microscopy between Young and Elderly Adults without Diarrhea in Rural Bangladesh

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### Abstract

**Background:** Aging declining immune response, and often compromised gastrointestinal tract functions result in altered digestion and/or malabsorption. These problems are manifested by the presence of abnormally increased amount of yeast and vegetable cells in their stool revealed by the simple microscopy.

**Methods:** We compared stool studies between young (15-59 years; n=101) and elderly ( $\geq 60$  years, n=99) adults for the presence of yeast and vegetable cells by stool microscopy in rural Bangladesh.

**Results:** Among the older subjects yeast (40% vs. 22%,  $p=0.038$ ) and vegetable cells (55% vs. 40%,  $p=0.023$ ) were most common in their stool specimens and the ova of *Ascaris lumbricoides* (5% vs. 21%,  $p=0.003$ ) were found less often than in the younger subjects.

**Conclusion:** Findings may have far reaching public health implications for formulating appropriate health care delivery for elderly population in rural Bangladesh.

**Keywords:** Elderly; Microscopy; Rural; Stool; Vegetable cells; Yeast

### Introduction

Ageing is a natural phenomenon; different systems are affected by the ageing process. The gastrointestinal system is mainly responsible for the mechanical and chemical break down of food products into components more easily absorbed by the system [1]. Generally, increasing age is associated with decreased food intake [2]. At least half of women aged  $\geq 60$  years are chronic energy deficient ( $BMI < 18.5$ ) [3]. After age 50, for decade increase in age there is a 10% decrease in calorie requirement and a gradual loss in lean muscle mass [1]. The stomach of an older adult cannot accommodate as much food as a younger adult due to decreased elasticity [1]. Simultaneously the surface area of the small intestine decreases due to shrinkage and broadening of the villi [2]. The mucosa and muscle layers of the colon become atrophied, leading to reduced peristaltic movement and increased transit time [4]. The pancreas usually generates a number of digestive enzymes, but there is decrease in production due to focal or diffuse fibrosis with advancing age. There is a reduction in the secretion of chymotrypsin and pancreatic lipase with age that may adversely affect the digestive ability of the small intestine [5]. The liver has at least 114 functions, but shrinks with age. The digestive system has a decrease in blood flow with age. There is also a reduction in gastric juice secretion, especially hydrochloric acid which can facilitate small bowel bacterial overgrowth leading to malabsorption [6]. These age related changes may manifest as an excessive presence of opportunistic pathogens, such as yeast, and the presence of undigested food particles, such as vegetable cells, in the stool [7-10]. Therefore, understanding age related findings on stool microscopy may have public health importance. Data of such comparative stool microscopy is limited. Therefore, we compared stool microscopy in elderly and young adults, with a special emphasis on yeast and vegetable cells.

### Materials and Methods

#### Study population

Mirzapur subdistrict, Tangail District, Bangladesh is located 60 km north of Dhaka, the capital, and comprises an area of 374 sq km.

The study area within the ongoing demographic surveillance system has a population of 234,949; residing in 55,196 households. Fifty nine percent individuals are 15-49 years; the elderly ( $\geq 60$  years old) people represent 10% of the total population. Ten percent of the total population is comprised of children aged less than 5 years. Average numbers of household member is 4.5. About 88% of the population are Muslim, 12% are Hindu,  $<1\%$  are Christian and  $<1\%$  are of other religion. Males comprise 51% of the population, and females comprise 49%. Average literacy rate is 37% (ASG Faruque, personal communication). Agriculture is the main occupation. The two main health facilities which serve the population in the study are: Kumudini Hospital (750 beds) and a sub-district government health facility (31 beds). According to interview and physical examination findings, our study population was apparently healthy and did not have any noticeable ailments.

#### Study design

We randomly selected 200 individuals from demographic surveillance system households in Mirzapur sub-district and obtained stool specimens from each of them; 99 subjects were aged  $\geq 60$  years, 101 subjects were added 15-59 years. Informed written consent was obtained from each participant prior to participation. A stool sampler was collected and kept in an ice box ( $4-8^{\circ}\text{C}$ ) for upto 6 hours prior to being sent for analysis at the Dhaka Laboratory of the International

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Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). We did not measure the nutritional status of the subjects but they were presumed to be healthy.

### Laboratory investigations

The stool specimens were prepared for routine microscopy following standard laboratory procedures [11] to reveal the presence of white blood cells, red blood cells, yeast, vegetable cells, protozoa, macrophages helminthes and to determine pH.

### Data analysis

Laboratory data were collected, checked visually for errors and entered into the computer. SPSS (Statistical Package for the Social Sciences) for Windows (SPSS version 12.0, Chicago, IL) was used for analysis. We validated the data through a series of logic and range checks. Differences in proportions were compared with the  $\chi^2$  test. A probability of <0.05 was considered statistically significant. Strength of association was determined by calculating the odds ratio (OR) and their 95% confidence intervals (CI).

### Ethical consideration

This stool microscopy analysis of the subjects were the healthy

controls (without diarrhea within last 14 days) of a diarrheal disease etiology and burden study who were enrolled between January 2010 to March 2011, and the study was approved by the Research Review Committee and the Ethical Review Committee of ICDDR,B.

### Results

The data from the two groups are shown in the Table 1. Yeast was found in elderly subjects more often than in younger subjects (40% vs. 22%, p=0.038) as were vegetable cells (55% vs. 40%, p=0.023). *Ascaris lumbricoides* ova were found in younger subjects more often than in elderly subjects (21% vs. 5%, p=0.003). The presence of protozoa (7% vs. 9%, p=0.675), RBC (1% vs. 2%, p=0.578), pus cells (1% vs. 3%, p=0.331), and macrophages (2% vs. 1%, p=0.555) were not significantly different between the elderly group and the younger group, respectively.

### Discussion

Yeast was more common among the stool samples of elderly subjects. Aging may be a consequence of oxidation that damages DNA, protein, carbohydrates and lipids and leads to degenerative disease due to a disruption of cellular homeostasis [12]. Simultaneously, inadequate intake attributable to a lack of appetite or difficulty in preparing food, depression, isolation, low income and reduced sense

Parameters	60+ years n=99 (%)	15-59 years n=101(%)	Odds Ratio (95% CI)	p value
<b>Stool appearance</b>				
<b>Color</b>				
Brown	99 (100)	101 (100)	-	-
<b>Consistency</b>				
Soft	57 (58)	50 (51)	1.16 (0.71-1.91)	0.528
Loose	41 (41)	49 (49)	0.85 (0.50-1.45)	0.534
<b>Blood</b>	1 (1)	0	-	0.313
<b>Mucus</b>	94 (95)	81 (80)	1.18 (0.77-1.81)	0.415
<b>Worm</b>	0	0		
<b>pH</b>				
<b>Acidic</b>	85 (86)	91 (90)	0.95 (0.62-1.46)	0.815
<b>Alkaline</b>	14 (14)	10 (10)	1.43 (0.56-3.66)	0.413
<b>Microscopic examination</b>				
<b>Protozoa/HPF</b>	7 (7)	9 (9)	0.79 (0.25-2.44)	0.675
<i>Giardia lamblia</i>	3 (7)	3 (3)	1.02 (0.16-6.51)	0.980
<i>E. coli</i>	4 (4)	2 (2)	2.04 (0.3-16.44)	0.407
<i>Endolimax nana</i>	0	2 (2)	0.00 (0.00-4.25)	0.163
<i>T. hominis</i>	0	1 (1)	0.00 (0.00-17.97)	0.323
<i>E. hominis</i>	0	1 (1)	0.00 (0.00-17.97)	0.323
<i>Blastocystis hominis</i>	17 (17)	17 (17)	1.02 (0.46-2.24)	0.957
<b>Helminth/HPF</b>	7 (7)	21 (21)	0.34 (0.12-0.89)	0.014*
<i>Ascaris lumbricoides</i>	5 (5)	21 (21)	0.24 (0.08-0.72)	0.003*
<i>Trichuris trichura</i>	1 (1)	00	-	0.313
<i>E. vermicularis</i>	1 (1)	00	-	0.313
<b>Cell/HPF</b>				
<b>RBC (1-50)</b>	1 (1)	2 (2)	0.51 (0.02-7.32)	0.578
<b>Pus Cells (11-50)</b>	1 (1)	3 (3)	0.34 (0.01-375)	0.331
<b>Macrophage (1-20)</b>	2 (2)	1 (1)	2.04 (0.14-57.79)	0.555
<b>Others</b>				
<b>Vegetable cells</b>	55 (56)	40 (40)	1.91 (1.05-3.84)	0.023*
<b>Yeasts</b>	40 (40)	22 (22)	1.85 (0.99-3.49)	0.038*
<b>Neutral Fat</b>	13 (13)	9 (9)	1.47 (0.56- 3.94)	0.393
<b>C. L. Crystal</b>	1 (1)	0	-	0.313
<b>Starch</b>	2 (2)	6 (6)	0.34 (0.05-1.92)	0.173

\*p value<0.05

Table 1: Characteristics of stool microscopy in elderly individuals and young adults.

of smell, taste, drug-nutrient interaction and reduction in nutrient absorption contributes to malnourishment among the elderly [13]. Achlorhydria or hypochlorhydria, a common feature in malnourished individuals enhances infection by opportunistic pathogens including yeasts [14-17]. Reduced secretion of lipase, chymotrypsin and bicarbonate in pancreatic juice occurring with aging [18] may contribute to the survival of yeast even in the upper gastrointestinal tract. Compromised function of the gastrointestinal tract contributes to maldigestion and malabsorption which may increase the survival of yeast in the gastrointestinal tract. This might explain our findings. Previous studies found a wide variation in the presence of yeast in stool [19-22] without significant difference by age group [20]. This may be due to the differences in nutritional status, immune status and genetics. A similar study to ours found an increased prevalence of yeast in the stool with increasing age [23] similar to our findings.

Vegetable cells were more common among the elderly in this study. Vegetable fibers which are not completely hydrolysed in the stomach or small intestine reach the large intestine where they are partially or completely fermented by the intestinal flora [24]. Age may be associated with a change in intestinal microflora, such as a decrease in anaerobes and bifidobacteria and an increase in enterobacter [25], reducing intestinal immunity favoring gastrointestinal infection [25]. This may explain the presence of undigested vegetable cells in the stool of elderly subjects in our study.

*Ascaris lumbricoides* ova were found more often among younger subjects than older subjects in our study. Young adults work primarily in the agricultural field in rural Bangladesh. Young adults are more likely to be exposed to contaminated soil. The infection may be transmitted directly through the skin, by contaminated food due to poor hand hygiene [25] or by ingesting raw vegetables [26]. This may explain why the younger subjects in our study population were more likely to be infected with *Ascaris lumbricoides* [27,28].

We observed no significant differences in protozoa, red blood cells, white blood cells or macrophages on stool microscopy between the two groups. We found no asymptomatic bacterial or parasitic infections among the elderly group in our study. We found no inflammatory markers in the stool specimens of those with fungi present on microscopy. We did not ask about the use of anti-parasitic drugs or diarrhea among the subjects in this study.

A lack of dietary data among our subjects was also a major limitation of our study which could influence our findings. We did not exclude the hepatic function with other chronic disorders such as hypertension, diabetic mellitus or hepatitis which may alter the normal dietary habit and other metabolic functions. However, we were very much careful to select the apparently healthy control asking them having any previous history of such chronic illnesses and we performed physical examinations too. Moreover, the present study was conducted only in a single site in rural Bangladesh. Thus, the findings may not be generalizable.

## Conclusion

In conclusion, yeast which can be opportunistic pathogens and vegetable cells which are undigested or partially digested particles of foods, were more frequently present in the stool of elderly individuals. We speculate the gut of elderly individuals may have a compromised immune status and reduced digestive activity compared to young adults. Well designed immunological and physiological studies are to further explore these findings and their public health importance.

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