Short Communication

Association Between Nutritional Status And Mucosal Gut Immune System In Elementary School In Manado

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Received on: 26-Nov-2019 Accepted for Publication: 20-Jan-2020

ABSTRACT

Backgorund: In recent years, there has been increasing appreciation on nutritional imbalances, both on obesity and malnutrition that affect our immune response, especially in mucosal gut immunity. Alterations in cellular metabolism influence immune cell function. Fecal calprotectin (FC) is a biomarker protein that works as an innate immune system in the mucosal gut. This study aimed to evaluate the association between nutritional status and mucosal gut immune system in elementary school using FC concentration.

Method: Healthy children aged 6-12 years in Elementary School in Manado were enrolled in this study. The subjects were consecutively recruited from February to April 2019. Children's stool samples were collected, analyzed with FC concentration. The children's weights and lengths were measured. Nutritional status was based on Body Mass Index (BMI). Data were analyzed by Gamma correlation using SPSS version 25 software.

Result: In total 38 children were recruited; 22 boys (57.9%) and 16 girls (42.1%). According to BMI, the subjects were divided into 4 groups: 3 children underweight (7.9%), 23 children normal (60.5%), 6 children overweight (15.8%) and 6 children obesity (15.8%). From 38 children, only 8 children whom FC concentration were high (> 50mcg/g); 6 children were normal and 2 children were overweight. There were no correlation found between nutritional status and FC concentration (rG = -0.108; p = 0.713).

Conclusion: Our research found that there were no association found between nutritional status and mucosal gut immune system.

Keywords: fecal calprotectin, overweight, obesity, underweight, immune system

INTRODUCTION

Fecal calprotectin is a non-glycosylated calcium- and zinc-binding protein with antimicrobic, immunomodulatory, and antiproliferative properties. It is present in the mucous membrane squamous epithelium but not in the normal intestinal mucosa [1]. Calprotectin is secreted extracellularly from stimulated neutrophils, eosinophils and monocytes and is expressed in some mucosal epithelial cells. It is also released during cell disruption and death. When bound to calcium, calprotectin has a high heat resistance and is stable in stool samples for up to one week at room temperature. These properties allow calprotectin to be eliminated intact in the feces and give it an advantage as a noninvasive biochemical marker for the screening of intestinal inflammation, compared with other markers that are currently used (lactoferrin, neutrophil elastase, and leukocyte esterase) [2].

It is well-known that inflammation, either locally in the gastrointestinal (GI) tract or systemically, is a key factor that adversely affects nutrition and metabolic outcomes in patients [3]. Inflammation has often been identified as one of the root-causes of several chronic disease affecting patients nowadays [3]. The endoscope remains the gold standard method for assessing intestinal inflammation; however, because endoscopic examinations are invasive, expensive and uncomfortable, a non-invasive, inexpensive, simple and sensitive maker for detecting and monitoring the occurrence and development of intestinal inflammation diseases is greatly needed [4].

Our study was to find the association between nutritional status and mucosal gut immune system in elementary school using Fecal Calprotectin concentration (FC).

METHODS

In the present study, fecal samples were obtained from 38 healthy children from February to April 2019 in elementary school. Subjects were divided into 4 group according to BMI (Body Mass Index) : 3 subjects underweight, 23 normal, 6 overweight, and 6 obese subjects. Diagnosis nutritional status was based on the BMI for age percentile according to CDC growth chart for children. The subjects in the study met the exclusion criteria, based on a study of factors affecting levels of FC 5, which are as follows: no history of colorectal or systemic inflammatory condition, proton pump inhibitor use, regular (≥4 doses per week) use of nonsteroidal anti-inflammatory drugs, smoking or alcohol.

Subjects provided a single fecal sample for calprotectin measurement. The FC concentration was determined after being homogenized, using a commercial enzyme linked immunoassay. The results are expressed as mcg/g stool.

The association between nutritional status and FC concentration in children was analyzed with Gamma correlation analyses using SPSS version 25 software.

RESULTS

Based on the BMI percentiles threshold, 3 of the 38 (7.9%) enrolled patients were underweight, 23 (60.5%) normal, 6 (15.8%) overweight and 6 (15.8%) obese. The clinical characteristics data of the normal-weight and obese subjects are reported in Table 1. From 38 subjects only 8 subjects had high FC concentration which is 6 (15.8%) subjects in normal weight and 2 (5.2%) subjects in overweight.

The association between nutritional status and FC concentration was analyzed using Gamma correlation because all data variable was in ordinal categorized. The analyzed results is rG = -0.108 with p = 0.713. No significant association between patients with nutritional status and FC concentration (p=0.713) .

Table 1. Characteristics Subjects (n=38)

Characteristics (n=38)	Value (%)
Sex	
Male	22 (57.9)
Female	16 (42.1)
BMI status	
Underweight	3 (7.9)
Normal	23 (60.5)
Overweight	6 (15.8)
Obesity	6 (15.8)
FC Concentration High (>50μg/g)	
Underweight	0 (0)
Normal	6 (15.8)
Overweight	2 (5.2)
Obesity	0 (0)

DISCUSSION

The innate immune system of the gut comprises of multiple elements, such as mucous layer, intestinal epithelial cell layer, intestinal motility, osteoprotegerin, defensins, lysozyme, angiogenins, calprotectin, gastric acid, lactoferrin, intestinal microflora, cathelicidins, immune cells and secretory IgA. Each of which contributes to the fine balance between tolerance to commensal bacteria and response to potential pathogens. The gastrointestinal epithelium in particular, is constantly exposed to a large amount of intestinal microflora yet is able to maintain a physical barrier to exogenous stimuli while allowing the selective entry of essential nutrients [6]. .It has been widely

hypothesized that this resultant dysbiosis can lead to gradual bacterial invasion, inflammation, and a loss of tolerance to gut bacteria [7].

Fecal Calprotectin (FC) is a calcium- and zinc-binding protein of the S100/calgranulin family 8. FC have a proinflammatory role in innate immunity and are part of a group called damage-associated molecular pattern molecules (DAMPs), due to their release by activated or damaged cells under conditions of cellular stress 6. FC is an objective and non-invasive test reflecting various pathological processes occurring in the mucosa of pediatric patients [8].

In accordance with their pro-inflammatory role, FC and S100A12 are significantly overexpressed at sites of inflammation, and there is a strong correlation of their serum concentrations to inflammation [6]. The secretion of calprotectin by phagocytes is induced when phagocytes come into contact with inflamed endothelium. One mechanism that calprotectin is thought to promote inflammation is via induction of proinflammatory chemokines, adhesion molecules (e.g., VCAM1 and ICAM-1) and β2-integrin, thereby mediating leukocyte recruitment, adhesion, and trans endothelial migration to inflamed tissue [9]. S100A12 has also been shown to mediate inflammation via the induction of similar adhesion molecules to calprotectin and it also upregulates the production of pro-inflammatory cytokines by macrophages, including TNF-α and IL-1β [6,9].

Fecal calprotectin is resistant to bacterial degradation during passage through the GI tract and can be easily be measured, rendering it a surrogate marker of neutrophils into the bowel lumen. Fecal calprotectin was evaluated as an index of inflammation. Fecal excretion of calprotectin significantly correlated with the finding of inflammation at endoscopy and histology [1].

Normal values for FC in different age groups have been investigated in high-income countries [10]. Fagerberg et al, have documented that healthy children aged between 4 and 17 years apparently exhibit a similar pattern of fecal calprotectin excretion as in adults. The conclusion is that the suggested cutoff level for adults (<50 mcg/g) can be used for children aged from 4 to 17 years regardless of sex [11].

Fecal calprotetin is secreted extracellularly from stimulated neutrophils and the FC levels of healthy children exhibit a downward trend with increasing age and reach the adult level by the age of four [2,12]. Growing research suggests that obesity is not a simple phenomenon form improper diet and lifestyle, but a multi-factorial medical condition beyond individuals control [13]. Among the factors, growing evidence suggests inflammatory links with obesity. Tumor necrosis factor (TNF)-α expression in the adipose tissue was reported to be increased in obese humans several decades ago [14].

Elevated BMI, which has been shown to be associated with increased gut permeability in both animal and human studies could be associated through a variety of mechanisms, including altered bowel flora, the effects of circulating inflammatory cells and markers or through direct effects of dietary fats on local cytokine production [15,16]. Supporting the idea that altered bowel flora in the obese could mediate obesity associated elevations on FC, is a study in subjects with a variety of BMIs [5].

Malnutrition is associated with structural and immunologic changes in the small intestine, such as villous atrophy, increased permeability of the intraepithelial layer, and local inflammation including infiltration of lymphocytes 17,18 and Th1-mediated upregulation of interferon g (IFNg) [17]. Malnutrition is also associated with altered systemic immune responses, such as abnormal proinflammatory priming [19,20] decreased regulatory cytokines, and impaired immune activation [21]. Attia et al, described that fecal calprotectin was elevated in malnutrition because malnutrition have high degrees of intestinal inflammation [21].

In the current study, we found no association between fecal calprotectin and nutritional status. These results may be explained due to limitedly small sample size and only 6 subjects with high fecal calprotectin. Nutritional status was not equally distributed, only 3 subjects with underweight, 6 overweight, 6 obesity and no malnutrition. Also, subjects were not checked for further examination to rule out other disease that can cause fecal calprotectin increased, such as inflammatory bowel disease (IBD).

CONCLUSIONS

There were no association found between nutritional status and mucosal gut immune system. Nevertheless, further research still needed to evaluate these results, as there were many limitations in this study.

ACKNOWLEDGMENT
The author would like to thank the reviewers for this review to manuscript.

DISCLOSURE
The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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