Comparison of Fecal Calprotectin Levels in Iranian general population and army personnel

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ABSTRACT

Fecal Calprotectin is released in colon by activated neutrophils. Investigation of diagnostic application of determination of fecal Calprotectin levels is considered by many researchers to compare between different colorectal diseases like Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Diseases (IBD). Due to the effect of nutrition habits on the inflammatory processes of bowel, the main goal of this study was to evaluate the level of fecal Calprotectin in healthy subjects in Iranian army personnel that have specific and the same nutrition habits, in comparison with non-military population and also make this test an in-house method in Army laboratories.

In this study a prospective and sample collection method used available samples (Convenience sampling). We collected stool samples from 108 subjects from Army personnel and 108 samples from non-military Iranian population with corresponding data collection form. Determination of Calprotectin levels was done by specific third generation quantitative ELISA method and statistics were done using SPSS software.

The results showed that mean Calprotectin level in two included groups were 26.1 micrograms per gram in Army personnel group and 25.4 micrograms per gram in non-military control subjects. Fecal Calprotectin level in two studied groups wasn’t significantly different. Despite the little increase in Calprotectin levels in Army personnel group compared with control group this difference wasn’t statistically significant (P>0.05).

We can conclude that the nutrition habits of army personnel could cause effects on inflammatory processes in digestive system. Determination of fecal Calprotectin levels as a cost effective and non invasive test could be used by military physicians for early diagnosis of inflammation in personnel by routine tests subjects.

Keywords: Inflammatory Bowel Diseases; Irritable Bowel Syndrome; Calprotectin.

INTRODUCTION

Inflammation is one of the most common defense mechanisms of the human body and essentially is a part of non-specific immune response. General mechanism for regulating the inflammatory process can collide as the main cause of many chronic diseases including damages caused by digestive system autoimmune and inflammatory deficiencies [1-3].

When inflammation occurs, inflammatory cytokines are released by white blood cells or Affect on the surrounding tissues. These cytokines can increase blood pressure in that area and cause a leak of liquid containing cytokines into the tissues and cause swelling or edema.

Inflammatory process may stimulate nerves and cause pain[4].

Inflammatory bowel diseases (IBD) are the chronic conditions that are recurrent symptoms associated with repetitive episodes that occurred in the digestive tract including Crohn’s Disease (CD) and Ulcerative Colitis (UC). Patients with inflammatory bowel disease (IBD) are experience types of diarrhea, abdominal pain and bloating, indigestion, rectal bleeding and weight loss. The main cause of inflammatory bowel disease is still quite unknown. But it is likely that a series of immunological factors, genetic and environmental (including natural microbial flora of the intestine) can cause these symptoms [2, 4].
About two million people across Europe suffer from inflammatory bowel disease. Crohn's disease and Ulcerative Colitis are chronic and serious intestinal diseases that have severe, sudden and annoying effects on the patients’ quality of life. And finally will increase the possibility of malignant colon tumors in these individuals. When symptoms appear there are many similarities with the symptoms of functional bowel disorders such as IBS (irritable bowel syndrome); this makes accurate diagnosis very difficult for doctors [2, 4-7]. Inflammatory diseases, including diseases that are debilitating, especially moderate and severe forms can cause many problems for patients. One of the difficult diagnosis and complex digestive diseases that gastrointestinal specialists are constantly faced with is How to distinguish and differentiate patients with IBS from other intestinal disorders with Organ complications such as inflammatory bowel diseases and colorectal cancer [8, 9]. Between the two series of anomalies, there are common signs and symptoms such as abdominal pain, bloating and altered bowel habits and there are other clinical characteristics that lead the doctor suspected abnormalities of the organ origin including chronic bloody diarrhea and rectal bleeding. Although symptoms can guide doctors to diagnose diseases but most doctors follow a laboratory test to ensure they are having a more reliable diagnosis[10].

Among the tests that are done in order to include the total count of blood cells, Erythrocyte Sedimentation Rate and C - reactive protein test. By performing these tests can be largely ruled out the possibility of inflammatory diseases but the problem is that some organic bowel disease patients If only this measure be placed under test are wrongly diagnosed and this forced doctors to perform invasive procedures such as endoscopy and biopsy (biopsy) to ensure the absence of any organ complications in the intestine. Performing such invasive tests will impose many risks to the patients’ health and quality of life[11-14]. There is some evidence about contribution of atherogenesis in inflammation so dietary fats may be proinflammatory. The low-fat, high-carbohydrate diets applied bad effects on the inflammatory markers such as fecal Calprotectin [15]. In the present study, we aimed to explain whether the fixed and the similar diet of army personnel can adjust digestive system inflammation markers.

Few studies have examined the protein levels of fecal Calprotectin has been done on Iranian population [16]. Given the importance and benefits of this laboratory test and to generate more data for future studies, we performed the present study to compare the fecal Calprotectin among healthy personnel of the armed forces with healthy people outside of the Systems of the Armed Forces.

**MATERIALS AND METHODS**

For this Descriptive study, samples were collected from two study populations including 108 healthy employees of the armed forces from one military unit with fixed and similar diet and 108 age and sex matched healthy people outside of the armed forces with normal and homemade diet (volunteers from the general population of the country). A questionnaire based on Rome III Criteria was filled for each person. Exclusion criteria for both groups was existence of any digestive system disorders such as inflammatory bowel disease, irritable bowel syndrome, chronic constipation and diarrhea and Other complications that are listed in the Rome III criteria. Fresh stool samples were collected of each person in special containers and were maintained until performing the tests at -70 °C. Fecal Calprotectin levels were determined using third generation specific ELISA kits (Hycult biotech, Netherlands) according to manufacturer's instructions. Shortly before the ELISA test, stool samples prepared using extraction solution according to protocol proposed by kit manufacturer. Statistical data analysis was performed using SPSS software version 13 and Student’s T test.

**RESULTS**

In coordination with the military hospital laboratory, stool samples were obtained from personnel referring for their periodic tests. Fecal Calprotectin tests were performed on all samples to detect the average amount of protein in two
studied groups. 108 army personnel were considered for sampling and to compare the results we prepared stool samples from one hundred and eight, age and sex matched Iranian healthy individuals.

Results of laboratory tests and characteristics of study subjects are summarized in Table 1. As the data summarized in the table show, despite the amount of fecal Calprotectin was slightly higher in the group of army personnel in comparison with healthy individuals outside the military system, but this difference was not statistically significant. In order to achieve a better understanding of the statistical output, such as better hand out and see the many dimensions of data, data analysis diagram as in diagram (1) can be seen, would be useful. In this chart, the minimum and maximum values obtained for each study group is written in the side of the mean values. From this chart we can see that the scattering data on fecal Calprotectin levels of army personnel is greater than the general population’s values. Also maximum calculated amount of fecal Calprotectin equal to 1.38 micrograms per gram, and the minimum value equal to 1.14 micrograms per gram, was corresponded to army personnel group.

Table 1. Comparison of Fecal Calprotectin level in the general population with army personnel in both genders.

<table>
<thead>
<tr>
<th>Variable</th>
<th>General Population</th>
<th>Army Personnel</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD )</td>
<td>35 ± 6.6</td>
<td>36 ± 14.2</td>
<td>0.293</td>
</tr>
<tr>
<td>Male / Female (n %)</td>
<td>68 (63%)/ 40 (37%)</td>
<td>68 (63%)/ 40 (37%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Mean Calprotectin Level</td>
<td>25.4 ± 3.3</td>
<td>26.1 ± 12</td>
<td>0.116</td>
</tr>
</tbody>
</table>

Figure 1. Comparison of minimum, maximum and average fecal Calprotectin in the two study groups.

DISCUSSION AND CONCLUSION

Many studies have been done on application of fecal Calprotectin level determination in different gastrointestinal diseases with similar symptoms. This study was the first report of such comparison among Iranian population. A study by Rezaei Tavirani and colleagues in 2008 has been done on comparison of the inhibitory effect of killing the cells (Cytotoxicity) of Calprotectin on two types of fibroblast cell lines. In this study selected cell lines were affected by different concentrations of purified Calprotectin; And the rate of cell
proliferation in the presence of this active ingredient, in different time periods were measured using the MTT assay. The very important result of this study was despite all the tested cells were fibroblast but their differential differences makes them to show different results in Calprotectin cytotoxicity at the same concentration and exposure time [16]. Erbayrak and colleagues in 2009 were performed a study in Turkish population on the role of fecal Calprotectin levels in inflammatory bowel disease. They examined a total of 65 patients with inflammatory bowel disease and 20 healthy controls. The survey results showed that fecal Calprotectin levels in all controls were lower than the Cut-Off point (50 micrograms per gram of stool) Resulting in fecal Calprotectin can be strongly correlated with inflammation in the colon and rectum. The researchers were introduced the fecal Calprotectin test as a non-invasive method for evaluation and diagnosis process of the transition of macrophages into the colon lumen space and suggested that this test can be used to measure patient response to therapy and predict recurrence of inflammation in IBD patients [10]. Kallel and colleagues in 2010, in Turkey, a study conducted on 53 patients with Crohn's disease. They aimed to use the measurement of fecal Calprotectin as a diagnostic predictor for individuals suffered from Crohn's disease in a one-year Follow up. Relapse of inflammation was observed in 10 patients among total 53 studied individuals. Fecal Calprotectin levels in the relapse group compared with non-relapse group was significantly higher. Average amount of fecal Calprotectin in relapse group was 380.5 micrograms per gram and in the non-relapse group was 155 micrograms per gram. Obtained p-value was less than 0.001 and shows a high significance level. According to this study presence of fecal Calprotectin more than 340 micrograms per gram of stool, raises the chance of relapse up to 18 times. Fecal Calprotectin can be introduced as an independent factor to predict relapse of inflammation in patients with Crohn's disease [17]. People with the same dietary, hygienic behaviors and health habits, such as army personnel have the same chance for the incidence of gastrointestinal diseases with unknown causes. Given the high importance of their professional duties the importance of rapid and accurate diagnosis of the diseases is more important. According to the results of the present study Calprotectin as a marker of intestinal inflammation in the army personnel in comparison with the general population of Iran was slightly higher. To achieve more reliable and applicable results this phenomenon needs further investigations on different populations.

REFERENCES