

## Determining the Role of Intestinal Microbiota Through Inflammatory Cytokines in the Occurrence of Constipation in the Elderly Referring to Hazrat Rasool Akram Hospital

Kiana Sohrabi<sup>1</sup>, Farshad Divsalar<sup>2\*</sup>, Raheleh Alimoradzadeh<sup>3</sup>

<sup>1</sup>School of Medicine, Iran University of Medical Sciences, Tehran, Iran

<sup>2</sup>Firoozgar General Hospital, Department of Infectious Disease, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

<sup>3</sup>MD, Geriatrician, Department of Geriatric, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

### Abstract

Constipation, which refers to difficult or incomplete and irregular bowel movements, is one of the common complaints of patients. According to the available articles, constipation is the most common digestive problem in the United States, which is more of a symptom than a disease. This problem is more common in women and adults over 65 years old. Considering that in the coming years, Iran's population will move towards old age, one of the biggest health problems of this population can be constipation. Currently, accurate statistics of constipation complaints are not available in Iran, and due to the nascent nature of the field of geriatric medicine in Iran, no study has been done specifically in this age group, but what is clearly known is that there are many patients with complaints. Constipation patients go to gastroenterology, internal medicine and surgery clinics and receive numerous drug treatments, and yet they still suffer from this problem. In all the studies conducted for the treatment of this disease, it is recommended to use a high-fiber diet, drink fluids, exercise, and change lifestyle. The results of the recent study showed that knowing the factors affecting the occurrence of constipation in the elderly population and measuring the frequency of these factors is very important and can be a guide to doctors and community health officers for appropriate and timely measures. In this study, which has been conducted for the first time in Iran, the high level of inflammatory cytokines in these people was an indication of the presence of more inflammation in the body of these people compared to people without constipation. In other words, elderly patients mostly have a decrease in activity and the above-mentioned reasons also increase the decrease in intestinal transit. It seems that this change has led to an increase in the growth of some bad intestinal microbial species, which themselves lead to the aggravation of constipation. The hypothesis of more supplementary studies is needed, which can be implemented in the continuation of this project, so that the group of prebiotics can be used in the treatment of functional constipation of the elderly.

**Keywords:** Intestinal Microbiota, Inflammatory Cytokines, Incidence of Constipation, Elderly

### Introduction

Today, there is no clear definition for constipation. Although definitions such as defecation less than three times a week or hard defecation accompanied by force or feeling of fullness and incomplete elimination can be included (1). According to the Rome criteria, having 2 or more of the following symptoms for at least 12 weeks within 12 months confirms the diagnosis of constipation:

Two or less than two bowel movements per week,  
Straining during defecation,  
Hard stools and  
Feeling of complete emptying.

In chronic constipation that lasts more than 12 weeks, the quality of life of the person is clearly affected and the person incurs significant treatment costs (2). Due to various treatments, most patients pay a lot of money and time, which affects their quality of life. This is despite the fact that sometimes this problem is not recognized in the elderly (3). Among the reasons that lead to not diagnosing constipation in the elderly, the following can be mentioned:

Inability to report the symptoms of constipation due to common cognitive disorders in the elderly (4);  
Having regular bowel movements despite having fecal accumulation in the intestine.  
Sensory disorders and lack of awareness of fecal accumulation,  
The presence of non-specific symptoms in the elderly related to constipation, such as delirium, anorexia and reduced performance (5).

Chronic constipation leaves complications such as fecal incontinence (6), hemorrhoids (2), anal fissure, organ prolapse (prolapse of the rectum and uterus or bladder can be seen after constipation), intestinal obstruction, agitation in patients with dementia, reducing the quality of life of the elderly and urinary retention are among them.

In the texts of geriatric medicine (7), there are some cases mentioned as risk factors for constipation in the elderly, which it seems necessary to investigate in each society according to the social structure and age pyramid of that society. Among these cases are the use of multiple drugs (more than 5 drugs), the use of anticholinergic drugs, the use of opium, non-steroidal anti-inflammatory drugs, calcium blockers, inability to move, living in a nursing home, neurological problems (dementia, Parkinson's, diabetes, stroke, autonomic neuropathy - spinal cord diseases), depression, dehydration, low dietary fiber, metabolic conditions (hypothyroid-hypercalcemia-hypokalemia-uremia-dialysis), mechanical obstruction, lack of privacy and inappropriate access to the toilet.

On the other hand, in elderly patients, the function of the immune system decreases, which is part of the well-known process related to the aging of the immune system and leads to local inflammation of low intensity in the intestine. On the other hand, the reduction of bacterial excretion following the slowing down of intestinal movement and constipation leads to bacterial overgrowth, which is an important factor for the production of various interleukins during the inflammatory response (8). The purpose of this study is to determine the inflammatory response caused by constipation by measuring cytokines such as IL6, IL1, and TNF in elderly people who refer to Hazrat Rasool Akram Hospital. In other words, the growth of intestinal microbiota caused by the reduction of intestinal transit in the elderly has been done indirectly through the measurement of inflammatory markers.

Colon microbiota plays a key role in host physiology and regulation of immune system and metabolism and provides natural defense against invading pathogens through the known process of clonisation resistance (9, 10). Improper nutrition is one of the main factors responsible for changes in the composition and metabolic activities of colon microbiota and immune senescence in elderly people (11, 12). Decreased colon motility due to low consumption of non-digestible carbohydrates such as non-starch polysaccharides and decrease in short-chain fatty acids can lead to constipation, which is a significant problem in the elderly (13, 14).

Many studies have confirmed the difference between the intestinal microbiota in the elderly compared to young adults, that the increase of enterobacters and clostridia was accompanied by the decrease of beneficial intestinal bacteria such as bifidobacteria and lactobacillus (15) and that this change in the intestinal microbiota of the elderly is due to reasons such as the aging process and diet change. food and lifestyle and reducing the function of the immune system. These changes in the composition and structure of the intestinal microbiota are related to specific aging conditions such as frailty, immune senescence, metabolic syndrome, diabetes, and sarcopenia. On the other hand, aging causes a decrease in the TH1:TH2 ratio and an increase in the production of inflammatory cytokines including TNF and IL6 (16). These changes lead to greater vulnerability to diseases such as gastroenteritis and reduced vaccination efficiency and increased vulnerability to Clostridium difficile infections (17).

Age-related decline in the function of the immune system (18), which is characterized by a chronic low-grade inflammatory state. In the intestine of a healthy person, the microbiota and the immune system dependent on the homeostatic intestine create a balanced dynamic (19), which weakens the inflammaging process of this balance and leads to changes in the structure and composition of the intestinal microbiota.

Due to the aforementioned changes, sometimes prebiotics have been used to maintain and regulate intestinal microbiota and the immune system and improve intestinal function (20), but there are very few studies in this field in the elderly and studies focused on microbiota changes. The gut that occurs in the aging process and its consequences on the health of the elderly are still limited.

The importance of this change is that intestinal dysbiosis, which is defined by the increase of undesirable bacteria and the decrease of beneficial bacteria, changes the motility of the colon by changing the metabolic environment caused by the increase in PH produced by undesirable bacteria, which is caused by the production of toxic substances such as phenol and ammonium. It reduces procarcinogen (2). Normal intestinal products, such as short-chain fatty acids, regulate normal intestinal motility by exerting trophic effects on epithelial cells and increasing regional blood flow, which is disturbed in dysbiosis. (21) On the other hand, the relationship between constipation and the GUT-brain axis is vital in controlling homeostasis, and the imbalance of this axis

leads to behavioral changes (11), which is important in the elderly due to their cognitive and behavioral disorders.

Beneficial microbiota is bifidobacterium and lactobacillus, which promote bowel movements through the fermentation of indigestible carbohydrates into short-chain fatty acids. But *Escherichia* species inhibit motility (3). On the other hand, dysbiosis is associated with the increase of some inflammatory cytokines such as IL6-IL8-IL1-TNF, which has been investigated in limited studies (22). Since these inflammatory factors play a role in frailty and sarcopenia. In this study, the hypothesis of the role of intestinal microbiota in the development of functional constipation in the elderly was carried out by examining the traces of inflammation caused by it in the serum. This study has been conducted for the first time in Iran, and further studies are needed to confirm the results obtained in this study, such as the investigation of microbiota.

## Background research

There is a hypothesis that maintaining the structure of normal intestinal flora during aging can be effective in delaying or preventing the inflammatory process associated with aging. Different amounts of native bifidobacterium in the intestines of elderly people have a negative relationship with serum levels of inflammatory cytokines TNF and IL10 regulation (3). In the study conducted in 2008, the hypothesis was proposed that the inflammatory process can be caused by an abnormally activated immune response against intestinal microbiota compositions, which is caused by a decrease in mucosal tolerance or age-related changes in microbiota compositions or both (23).

In 2009, it was proposed that nutritional deficiency and tissue weakness caused by age and mucosal injuries are related to the stimulation of pathological inflammatory response in the presence of beneficial intestinal symbiotic bacteria. (24) An important determinant in the production of interleukin during the inflammatory response is the reduction of bacterial excretion caused by slow intestinal transit and constipation, which was proposed in 2008 (25). In several studies, several bacterial species such as *Lactobacillus* and *Bifidobacterium* have been known to be able to reduce the inflammatory response in the intestinal epithelial surface (3). In 2008, a negative relationship related to the level of the inflammatory cytokine TNF with intestinal bifidobacterium in the elderly has been suggested (2).

In another study, a deeper view of the relationship between the composition of intestinal microbiota and the levels of serum inflammatory markers was provided. In this study, which was conducted in 2010, with the use of human intestinal tract chip (HIT chip), quantitative PCR, age-dependent differences in the composition of intestinal microbiota between three groups of young and elderly adults over 65 years old and the group over 100 years old were investigated. The results showed the similarity of the intestinal microbiota of the first two groups and a significant difference with the group over 100 years old, which was the preference of anaerobic species and the increase of inflammatory markers in the group over 100 years old, and the relationship between *Escherichia*, *Haemophilus*, *Klebsiella* and *Pseudomonas* with IL6 and IL8 have been associated with *Proteus* and *Leminorella*, *Alcaligene* (26). In this study, the inflammatory cytokines IL6 and IL8 have been reported to be increased in the group over 100 years old, and the levels of IL2 and IL12, which play an important role in activating the immune response, have decreased, all of which indicate a higher inflammatory state in the group over 100 years old.

The age-related increase in the intestinal amount of enterobacteriaceae and other gram-negative bacteria may lead to an increase in endotoxin, which is a CHALLENGE for the weak intestinal barrier of the elderly, which was proposed in 2009 (27). Some studies have shown the difference in the intestinal microbiota between people with and without constipation, that an increase in clostridia and enterobacteriaceae has been reported in patients with constipation, and the origin of this dysbiosis is unclear in this study, whether it is secondary to constipation and or dysbiosis is the cause of constipation, of course, this study was conducted in 42 children with an average age of 8 years (14). Clinical trial studies that have used prebiotics in the treatment of constipation in the elderly are very limited and have been limited to residents of nursing homes, which definitely cannot be a reflection of the effect of prebiotics in the elderly living in the community (4). In Finnish elderly living in nursing homes, two species of bifidobacterium *LONGUM* and *LACTIS* have been reported to be useful for increasing the frequency of bowel movements.

In a 2009 study that was conducted to evaluate prebiotic treatment in elderly people with dementia in Sweden, there were no promising results and *Lactobacillus* and *Lactococcus* species did not have significant effects on constipation (28). In 2010, contrary to the above study, it was shown that the prebiotic combination of *Lactobacillus acidophilus pedicus* and *Bacillus longum* had a significant improvement on the excretion status in

Korean nursing home residents (6). In 2015, in patients with functional constipation, the number of Bifidobacterium and Bacteroidetes bacteria in feces was reported to be lower, which was checked by PCR (7). In 2012, the effects of prebiotics, probiotics and symbiotics in the treatment of constipation were investigated, which had similar effects to the above studies (4). In another study in 2015, changes in the composition of the intestinal microbiota in functional constipation were conducted, which evaluated the short-term effects of treatment with VSL, but the results of this study, as there was no control group, should be interpreted with caution (29).

In the next study, focusing on the elderly hospitalized population, it was shown that the level of the inflammatory cytokine TNF decreased in response to the supplement of fermented milk containing the probiotic Lactobacillus species, and a decrease in the incidence of infection was also noted in this group. A significant increase in phagocytosis and NK cell activity has been reported following the administration of FOS and GOS supplements in the elderly, which was accompanied by a decrease in IL6, IL, and TNF cytokines (5).

In 2011, a study was conducted by Dr. Arnold Wald in the United States entitled Quality of life in children and adults with constipation. According to this study, there are various tools to assess the quality of life in functional constipation in children, and it is very difficult to compare each of them. This questionnaire contains 28 items, including: 11 items related to worry and anxiety, 4 items related to physical discomfort, 8 items related to mental discomfort, and 5 items related to satisfaction (13).

In a 2007 study by Leung and colleagues titled "Etiological Factors of Chronic Constipation - A Review of the Scientific Evidence", the researchers concluded that in the absence of planned studies there is sufficient evidence-based information to support the role of low fiber intake. Lack of fluid intake, reduced physical activity, side effects of drugs, hypothyroidism, sex hormones, and obstruction caused by cancer do not exist as an important etiological factor in the development of chronic constipation. Researchers interested in studying constipation should re-evaluate the etiological role of the mentioned factors in the development of constipation using modern techniques and methods (14).

In 2005, a study titled "Effect of regular physical activity on defecation pattern in middle-aged patients with chronic constipation" was conducted by De Schryver and his colleagues, 43 patients with chronic constipation were divided into two groups. Group A patients had their normal life for 12 weeks and then did physical activity for 12 weeks. In group B, patients were physically active for 12 weeks from the beginning of the study. Physical activity consisted of 30 minutes of brisk walking and 11 minutes of exercise at home. At the end of this study, the researchers stated that in inactive middle-aged people with symptoms of chronic constipation, having regular daily physical activity will improve the pattern of defecation and also the overall transit time of the recto sigmoid (5).

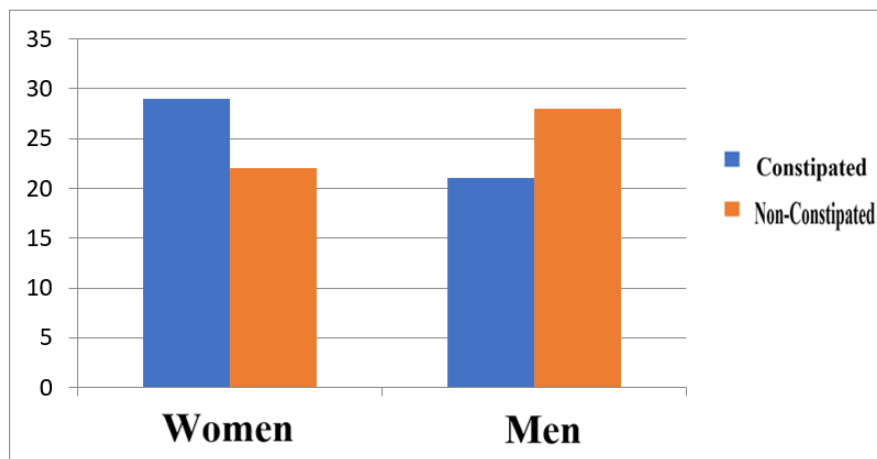
**How to measure the samples:** IL1, IL6 and TNF values from the peripheral blood of elderly patients were measured through a laboratory kit as follows, and the results of the two groups were compared. Serum IL-1 $\beta$  and IL-6 levels were measured by ELISA method (IEMA) with DIACLONE kit made in France, and TNF- $\alpha$  level was measured by ELISA (Sandwich) method and with R&D SYSTEMS kit made in USA. The above measurements were made by Mindray MR-96A Elisa Reader device made in Germany.

#### Sample size

According to the estimation of diagnostic accuracy equivalent to 96% of similar studies and considering the maximum effect size of 3% in the formula, the sample size was calculated as 50 people.

#### Findings

In this study, 100 people were evaluated, 50 of them were constipated. In the patients with constipation, 29 people (58%) were women and 21 people (42%) were men, and in the group without constipation, 22 people (44%) were women and 28 people (56%) were men. Data analysis using Chi-square statistical test showed that the gender difference of the two groups is not significant ( $P=0.161$ ) (Chart 1).



**Chart 1. Frequency of gender of two groups of people with/without constipation**

In people with constipation, 38 people (76%) had diabetes and in people without constipation, 18 people (36%) had diabetes. Data analysis using Chi-square statistical test showed that the difference between the two groups was significant ( $P=0.0001$ ).

In people with constipation, 30 people (60%) and in people without constipation, 19 people (38%) used several drugs. Data analysis using Chi-square statistical test showed that the difference between the two groups was significant ( $P=0.028$ ). Regarding the use of calcium channel blocker drugs, 25 people (50%) in the constipated group and 10 people (20%) in the other group used this category of drugs. Data analysis with Chi-square statistical test showed that the difference between the two groups was significant ( $P=0.002$ ).

Twenty people (40%) of people with constipation and 8 people (16%) of people without constipation were using anticholinergic drugs, and data analysis using chi square test showed that the difference between the two groups is significant ( $P=0.008$ ).

The prevalence of depression in people with constipation was 20% (10 people) and in people without constipation it was 8% (4 people). Data analysis using chi-square test showed that the difference between the two groups was not significant in terms of the prevalence of depression ( $P=0.084$ ).

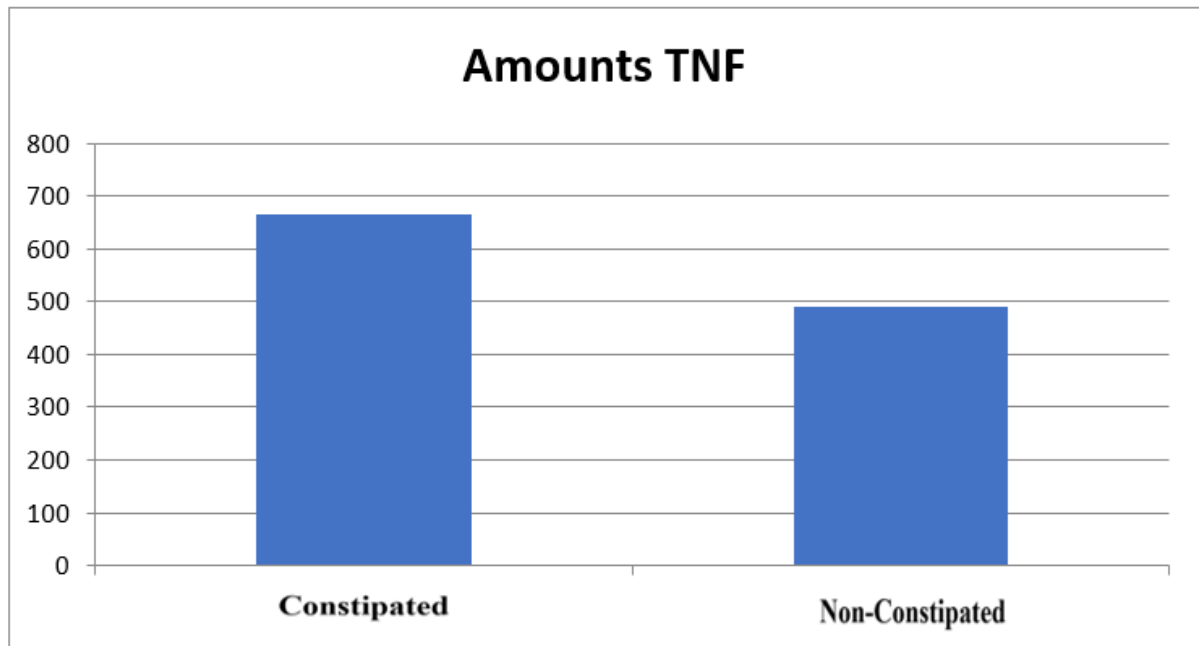
In the group with constipation, 26 people (52%) and in the group without constipation, 8 people (16%) were immobile. Data analysis using chi-square test showed that the difference between the two groups was significant in terms of immobility ( $P=0.0001$ ).

The prevalence of hypothyroidism in people with constipation was 20% (10 people) and 4% (2 people) in other people, and data analysis using chi square test showed that the difference between the two groups was significant in this regard ( $P=0.014$ ).

In the surveys, it was also found that 34 people (68%) of people with constipation and 10 people (20%) of people without constipation did not receive enough fluids during the day. Data analysis using chi-square test showed that the two groups have a significant difference in the use of fluids ( $P=0.0001$ ). One of the things that was evaluated in the patients was the amount of fiber intake in the daily diet. Based on this, 32 people (64%) in the constipated group and 18 people (36%) in the non-constipated group received small amounts of fiber in their diet. Data analysis showed that there is a significant difference between the two groups in terms of fiber intake from food ( $P=0.005$ ). In the elderly with constipation, 8 people (16%) and in the elderly without constipation, 2 people (4%) had a history of stroke, which Chi-square analysis showed a significant difference between the two groups in this regard ( $P=0.046$ ). In people with constipation, 8 people (16%) and in people without this problem, 1 person (2%) had a history of malignancy, which data analysis showed a significant difference between the two groups ( $P=0.014$ ). In the patients with constipation, 8 people (16%) and in other people 1 person (2%) were undergoing dialysis. Data analysis using chi-square statistical test showed that there is a significant difference between the two groups in the field of dialysis ( $P=0.014$ ). In people with constipation, 12 people (24%) were addicted to drugs, while in the other group, 6 people (12%) had this problem. Data analysis using Chi-square statistical test showed no significant difference between the two groups in this field ( $P=0.118$ ).

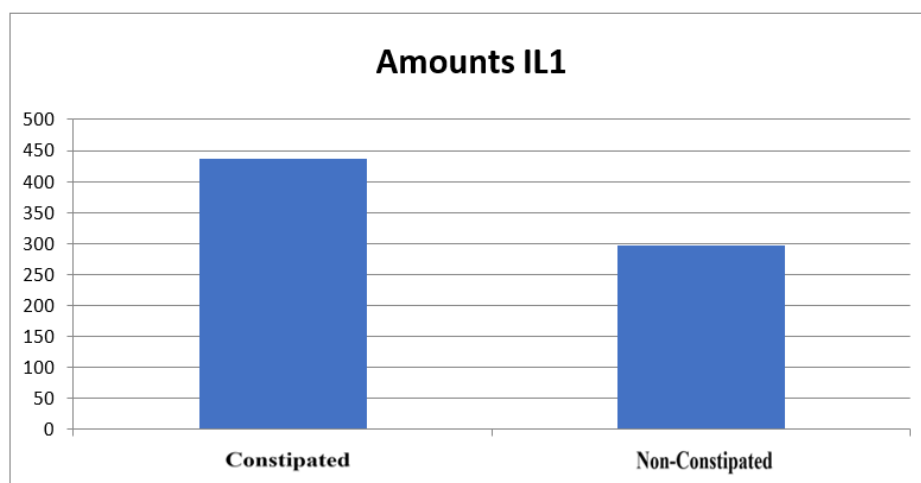
Six (12%) of the elderly with constipation and one (2%) of the elderly without constipation suffered from Parkinson's disease, which data analysis showed that the two groups had a significant difference in this case ( $P=0.050$ ).

In the study, the amounts of inflammatory factors such as TNF, IL1 and IL6 were measured in all subjects. Therefore, the average values of TNF in the group of patients with constipation were equal to  $666.80 \pm 101.40$  and in those without constipation it was equal to  $489.20 \pm 53.68$ . Data analysis using independent T-test showed that there is a significant difference between TNF values in two groups ( $P=0.0001$ ) (Chart 2).



**Chart 2. Mean TNF serum levels in two groups of subjects with/without constipation**

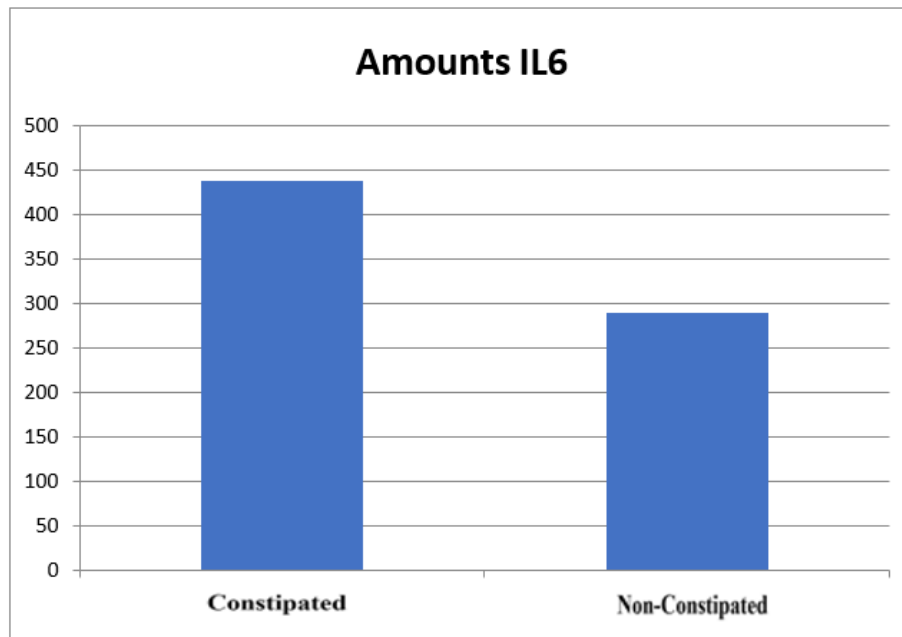
The mean IL1 serum values in constipated subjects were  $435.96 \pm 52.31$  and in non-constipated subjects it was  $296.44 \pm 45.50$ . Data analysis using the independent T-test showed that the mean values of IL1 in the two groups were significantly different ( $P=0.0001$ ) (Chart 3).



**Chart 3. Mean IL1 serum levels in two groups of subjects with/without constipation**

Finally, the mean IL6 serum levels in the elderly with constipation was  $438.18 \pm 59.572$  and in the elderly without constipation it was  $290.14 \pm 36.39$ . Data analysis using the independent t-test showed that the mean values of IL6 in the two groups were significantly different ( $P=0.0001$ ) (Chart 4).



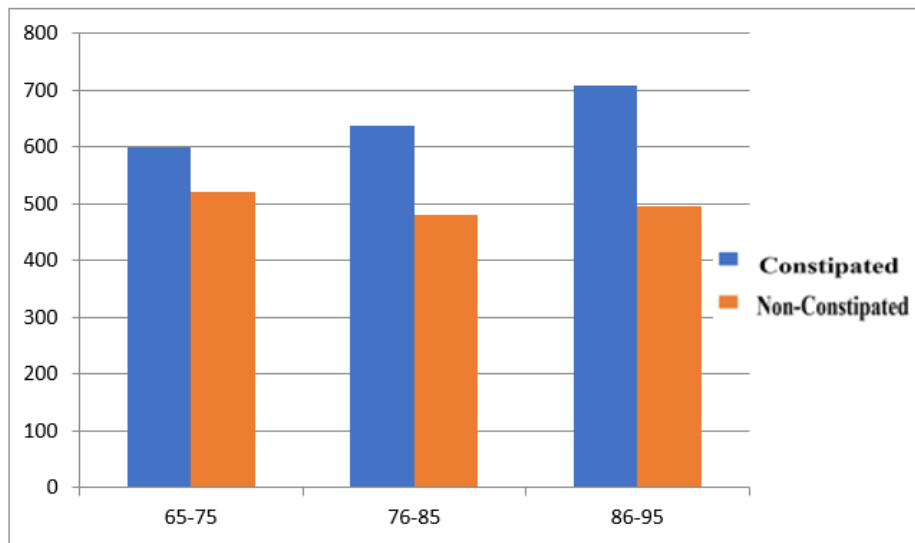


**Chart 4. Mean IL6 serum levels in two groups of subjects with/without constipation**

In another part of the data analysis related to this study, the amounts of inflammatory cytokines in three different age decades were also compared. These decades included 65-75, 76-85 and 86-95 years. Statistical comparison using T-test in two groups showed that there is a significant difference between the group of patients with constipation in the age group of 65 to 75 years (P=0.001). Also, a significant difference can be seen between these two groups in the age groups of 76 to 85 years and 86 to 95 years (P=0.0001) (Table 1).

**Table 1. Serum levels of TNF in three decades of age of study patients**

P-value	Mean ± standard deviation	Number	Group	Age decade
0/001*	599/70±89/7	20	Constipation	65-75
	521/06±19/8	16	No constipation	
0/0001*	636/71±87/9	14	Constipation	76-85
	481/75±54/7	20	No constipation	
0/0001*	708/43±111/9	16	Constipation	86-95
	495/64±51/6	14	No constipation	

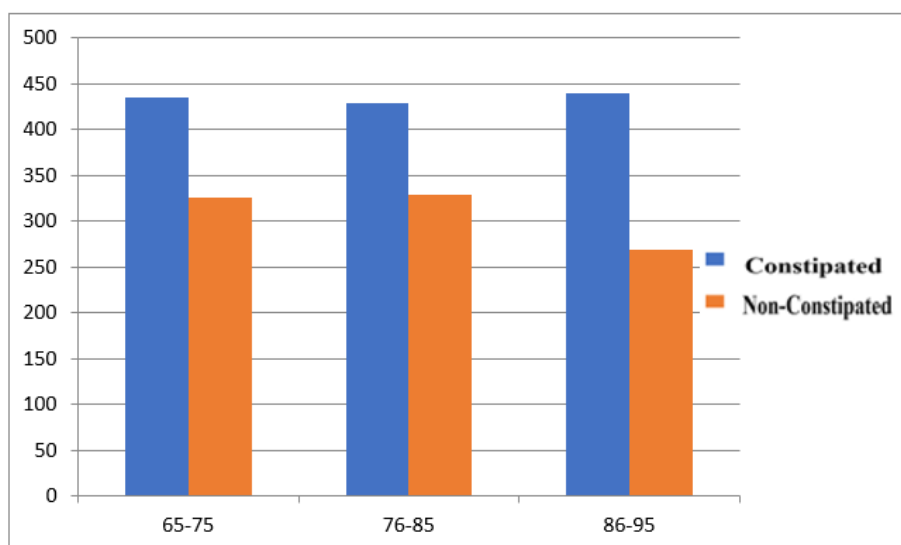


**Chart 5. Mean TNF serum values in three different age decades**

Statistical comparison using T-test in two groups showed that there is a significant difference between the group of patients with constipation in the age group of 65 to 75 years (P=0.0001). Also, a significant difference can be seen between these two groups in the age groups of 76 to 85 years and also 86 to 95 years (P=0.0001) (Table 2).

**Table 2. Serum levels of IL-1 in three decades of age of study patients**

P-value	Mean ± standard deviation	Number	Group	Age decade
0/0001*	435/50±50/3	20	Constipation	65-75
	326/06±28/1	16	No constipation	
0/0001*	428/71±61/8	14	Constipation	76-85
	328/70±63/0	20	No constipation	
0/0001*	439/69±54/6	16	Constipation	86-95
	268/86±48/4	14	No constipation	



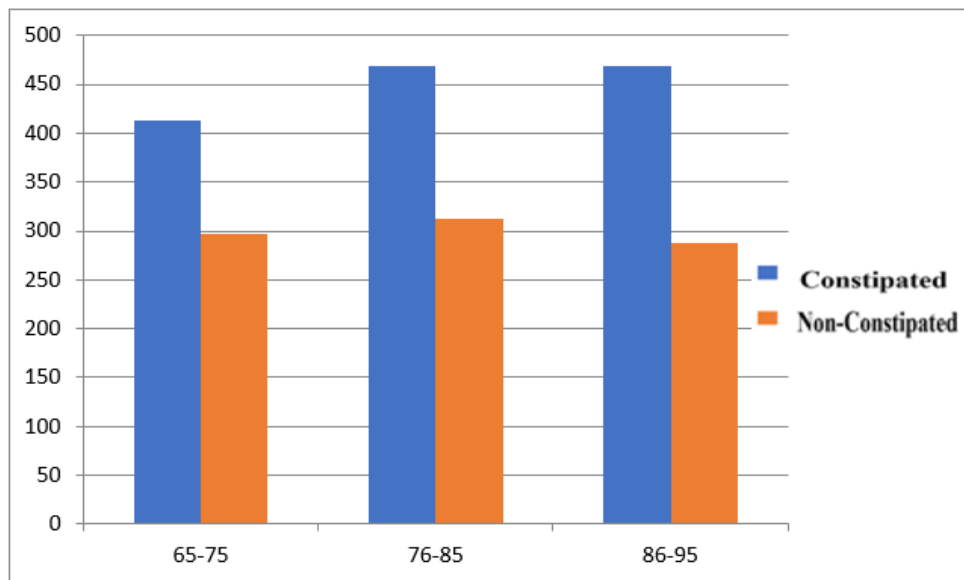
**Chart 6. Average IL-1 serum values in three decades of different ages**



Statistical comparison using T-test in two groups showed that there is a significant difference between the group of patients with constipation in the age group of 65 to 75 years (P=0.0001). Also, there is a significant difference between these two groups in the age groups of 76 to 85 years and 86 to 95 years (P=0.0001).

**Table 3. Serum levels of IL-6 in three decades of age of study patients**

P-value	Mean ± standard deviation	Number	Group	Age decade
0/0001*	412/80±63/5	20	Constipation	65-75
	296/81±44/3	16	No constipation	
0/0001*	468/57±41/8	14	Constipation	76-85
	312/6±51/2	20	No constipation	
0/0001*	469/25±53/5	16	Constipation	86-95
	288/14±40/9	14	No constipation	



**Chart 7. Mean IL-6 serum values in three decades of different ages**

**Table 4. Parameters investigated in subjects with/without constipation**

P-value	No constipation	Constipated	
0/0001*	18	38	Diabetes
0/028*	19	30	Taking multiple medications
0/002*	10	25	Taking calcium blockers
0/008*	8	20	Taking anticholinergics
0/084	4	10	Depression
0/0001*	8	26	Immobility
0/014*	2	10	Hypothyroidism
0/0001*	10	34	Dehydration
0/005*	18	32	Low fiber diet
0/046*	2	8	Stroke
0/014*	1	8	Malignancy
0/014*	1	8	Dialysis
0/118	6	12	Addiction
0/050*	1	6	Parkinson

\*Values P<0.05

**Table 5. Mean serum levels of inflammatory cytokines in subjects with/without constipation**

P-value	No constipation	Constipated	
0/0001*	489/20±53/68	666/80±101/40	TNF
0/0001*	296/44±45/50	435/96±52/31	IL1
0/0001*	290/14±36/39	438/18±59/572	IL6

\*Values P<0.05

## Discuss

Serological studies also showed that the levels of inflammatory factors such as TNF, IL1 and IL6 in people with constipation are significantly higher than other people, which indicates the existence of an inflammatory process in these people. Constipation is usually considered a natural part of aging, but it is actually a disorder that is not caused by aging itself. Although the changes that occur in the digestive system with age make a person susceptible to constipation. But this disorder usually has a multifactorial etiology and may involve a person throughout his life. The results of the study by Zahedi and his colleagues in Kerman showed that the prevalence of constipation increases with age (29). This issue has been confirmed in other studies as well (30). The reason for the increase in the prevalence of constipation in the elderly can be due to the decrease in physical activity and food intake, the analysis of abdominal muscles, the higher prevalence of mental disorders and the use of various drugs in this age group. Also, the prevalence of secondary causes of constipation increases in the elderly (31). There are various causes that can cause constipation in the elderly. In the present study, not all factors, but the most important of these factors were evaluated and their frequency was investigated in the elderly with constipation and also in the elderly without this problem. Below we discuss these factors and discuss the cause of constipation by them.

## Diet

Compared to developing countries, Western lifestyle and diet have the highest prevalence of constipation. In the present study, 64% of constipated patients and 36% of healthy people used low-fiber diets, and there was a significant difference between them. The composition of the diet, especially its fiber content, is one of the most important causes of constipation. Not getting enough fiber in the diet causes feces that have a small volume, contain less water, and are more difficult to excrete (23). In societies such as West Africa, where an average of 35 grams of insoluble fiber is consumed per day, people experience between 2-3 bowel movements per day, and reports of constipation are also uncommon in these areas (32). In the United States of America, the average amount of dietary fiber is 12 grams per day, and the prevalence of constipation in this country is between 3 and 5% (33). Insoluble fiber in the diet draws water to the intestinal lumen and leads to the formation of bulky, soft and large stools that contain large amounts of water. Peristaltic movements of the colon are also stimulated due to the distention of the colon.

## Medicines

Several drugs such as anticholinergics, antidepressants, and opioids may cause iatrogenic constipation by inhibiting neuronal signals and disrupting the coordination of colon muscles. The initial treatment of constipation with a specific pharmacological cause includes stopping the drug and, if possible, replacing it with a drug that does not cause constipation. Mental disorders such as depression, psychosis and anorexia nervosa, in addition to drug treatment, may contribute to the worsening of constipation. The categories of drugs that because constipation is given in table 4. In the present study, the use of several drugs, the use of calcium blockers, and the use of anticholinergics in people with constipation were significantly higher than in the control group, but the frequency of use of narcotics and antidepressants was not significantly different between the two groups. Meanwhile, according to previous studies conducted in Iran, the prevalence of constipation among opium addicts is higher than other people (34). The effect of opium in the digestive system is due to mu-opioid receptors, whose stimulation leads to a decrease in the movements and secretions of the digestive system, the stool remains in the colon for a longer period of time, becomes hard and comes out harder (35).

## Malignancies

Neoplasms of the colon, rectum, and anus may cause gradual changes in excretion as they grow larger and block the passage of feces. These lesions are usually easily recognized by radiographic and colonoscopy examinations, and clinical suspicion of malignancy often occurs due to the patient's clinical condition and symptoms. In this study, 16% of the patients with constipation had malignancy, while only 2% of the patients without constipation suffered from this problem.

## Systemic diseases

Systemic diseases such as diabetes mellitus, multiple sclerosis, hypothyroidism, hypohes and porphyria may cause or aggravate constipation. For example, in diabetes, with the passage of more time than the onset of the disease, the patient develops autonomic neuropathy, which itself can cause digestive dysfunction and reduce the movements of the digestive system. The results of the present study also showed that the frequency of diabetes and hypothyroidism is significantly higher in people with constipation. Neurological disorders such as neoplasms of the brain and spinal cord, trauma to the central nervous system (CNS) and Parkinson's disease are all associated with constipation and significantly affect the patient's quality of life.

### Immune system in the elderly

In normal physiological conditions, the human immune system consists of different parts and pathways that will trigger an immune response after creating an external signal (36). Among these multifaceted components of the immune system, anti-inflammatory cytokines have been the target of extensive studies for years. The study of the regulation of inflammation by these cytokine inhibitors is very complicated because for the proper understanding and detailed analysis of the effect of these cytokines, several external factors must be considered. It is assumed that factors such as the release time of the cytokine, the local environment in which it acts, the presence of competing or synergistic elements, the nature of the target cells, the availability and concentration of cytokine-specific receptors, and the tissue-specific response to each cytokine, determine the final effect and specifies its purity (37). In addition, the discovery of various pro-inflammatory cytokines has led to further complexity of the pathways that occur during the immune response. The total findings of extensive research on cytokines show that the net effect of the inflammatory response is determined by the delicate balance between pro-inflammatory and anti-inflammatory cytokines (2). Disturbance in this balance can lead the immune response of the host to chronic inflammation or to recovery. Until now, various anti-inflammatory cytokines have been investigated, including IL-1, IL-4, IL-6, IL-10, IL-11, IL-13, TGF- $\beta$  and TNF- $\alpha$ .

Although it is known that the immune system becomes old with age, the changes in intestinal immune function have not been widely studied (8, 23). Studies show that inflammation plays an important role in chronic diseases related to age and disability and is related to nutritional problems such as malnutrition and obesity (4). Several studies have investigated the value of IL-1, IL-6 and TNF- $\alpha$  in elderly people as inflammatory markers related to cardiovascular diseases (25, 26), movement limitation (27) and old age (28). Among the inflammatory markers that are frequently evaluated for elderly studies, TNF- $\alpha$  is mainly produced by macrophages (29) and is involved in the production of IL-1 (30) and IL-6 (31); Therefore, it directs a chronic inflammatory process in the body (32). The level of inflammatory cytokines IL-1, IL-6 and TNF- $\alpha$  was measured in our study, and the results of this test showed a significant and significant increase of these cytokines in the elderly with constipation.

TNF- $\alpha$  is a major adipocytokine that is produced in adipose tissue, and when adipose tissue becomes large, it causes mild chronic inflammation and insulin resistance (33). An increase in the level of inflammatory cytokines in the plasma is related to a decrease in physical performance and independence in the elderly (34). TNF- $\alpha$  is a pro-inflammatory cytokine with strong catabolic effects on muscles (35). The results of the study by Pereira and his colleagues showed that the increase in the serum levels of TNF- $\alpha$  and IL-6 causes more physical breakdown and a decrease in the mobility of the elderly (36). On the other hand, Cordova and his colleagues confirmed that long-term resistance training in elderly women reduces the serum level of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6, and thus reduces the occurrence of disorders related to physical inactivity and aging (37).

### Conclusion

Knowing the effective factors in the occurrence of constipation in the elderly population and measuring the frequency of these factors is very important and can be a guide to doctors and community health officials for appropriate and timely measures. In this study, which has been conducted for the first time in Iran, the high level of inflammatory cytokines in these people was an indication of the presence of more inflammation in the body of these people compared to people without constipation. In other words, elderly patients mostly have a decrease in activity and the above-mentioned reasons also increase the decrease in intestinal transit. It seems that this change has led to an increase in the growth of some bad intestinal microbial species, which themselves lead to the aggravation of the constipation process. Certainly, in order to confirm this hypothesis, more supplementary studies are needed, which can be implemented in the continuation of this project, so that the group of prebiotics can be used in the treatment of functional constipation in the elderly.

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