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The correlation between tenascin-C expression, and formation of intestinal stricture

Emrullah Erdem[1], Koray Kochan[1], Nurcan Paker[2], Yasemin Gokden[1], Ayca Salturk Degirmenci[1], Fatin Kocak[1], Can Gonen[1]

[1]Haydarapasa Numune Training and Research Hospital, Istanbul, Turkey;
[2]Duzen Laboratory, Istanbul, Turkey

ABSTRACT

OBJECTIVE: A strong correlation exists between tenascin-C induction, and acute inflammation. Generally increased tenascin-C concentrations are correlated with various inflammatory, and infectious diseases. In patients with diagnosis of Inflammatory Bowel Disease (IBD) presence of tenascin-C in colonic mucosa demonstrates tissue repair, and its mucosal concentrations are correlated with local disease activity Therefore plasma levels of tenascin-C have been demonstrated to be a helpful indicator of the activity of inflammatory bowel diseases. In this study, firstly in the literature, we aimed to display the correlation between tenascin-C expression, and formation of intestinal stricture.

METHODS: A total of 43 patients (male, n=19; 44.2%; and female, n=24, 55.8%) aged between 19, and 63 years, with clinically, endoscopically, radiologically, and histopathologically confirmed definitive diagnosis of Crohn's disease who were examined, diagnosed, and treated in the Gastroenterology Clinic of Haydarpasa Numune Training and Research Hospital between January 2011, and April 2012 were investigated. Serum tenascin- C levels were measured using commercial sandwich enzyme-linked immunosorbent assay Human Tenascin-C Purified Protein kit (Chemicon, Millipore(R), USA). Study groups were categorized based on the type of the disease as inflammatory (n=17; 39.5%), obstructive (27.2%), and fistule formation (n=10; 23.3%) Crohn's disease. For statistical analysis SPSS (Statistical Package for Social Sciences) Statistics 15 program was used.

RESULTS: Median tenascin- C value in the obstructive group (6.57 ng/mL; range, 4.26-21.87 ng/mL) was statistically significantly higher than that detected in the inflammatory (1.74 ng/mL; range,1.29-3.16 ng/mL), and fistulizing (1.44 ng/mL; range, 0.74-2.47 ng/mL) groups (p=0.002).

CONCLUSION: Intestinal fibroblasts have an important role in the stricture formation process in CD. Transforming growth factor (TGF)-b1 cytokine is in the center of this process. A strong correlation exists between tenascin-C induction, and acute inflammation. As a known fact, serum tenascin-C levels can be used in the determination of activity of IBD. Starting from this point, serum tenascin-C levels can be useful in the categorization of the Crohn's disease without the need for invasive methods. In the future, studies with larger patient series investigating use of serum tenascin-C in the prediction of stricturing Crohn's disease should be conducted.

Key words: Intestinal stricture; tenascin C.

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Correspondence: Dr. Emrullah ERDEM. Haydarpasa Numune Egitim ve Arastirma Hastanesi, Istanbul, Turkey.
Tel: +90 216 - 542 32 32 e-mail: emrullah.erdem@gmail.com

Crohn's disease (CD) occurs as a result of complex interaction among genetic, immunological, and microbial factors. In more than one-third of the patients, fibrostenosing phenotype progressing with recurrent intestinal strictures develops [1]. Because of chronic, transmural inflammation, and dysregulated wound healing, strictures occur as a result of superfluous, and abnormal matrix accumulation [2]. This phenomenon induces reorganization of extracellular matrix, scar formation, tissue distortion, and finally intestinal obstruction [3].

Tenascin was discovered in 1983, and this protein is especially contained in the stroma of gliomas, and thus termed as glioma-mesenchymal extracellular matrix antigen [4]. Studies performed within years have demonstrated diffuse expression of tenascin-C in tendons, ligaments, and extracellular matrix of tumors [5]. Since tenascin-C modulates adhesion of cells on fibronectin, it has anti-adhesive or adhesive characteristics [6]. Apart from classical adhesion proteins, tenascin-C can be adhesive, and promigratory for one type of cells, while on the contrary it can be an inhibitor for the other types of cells. The effect of tenascin-C on a specific type of cells can change dependent on the contact of this cell with other extracellular matrix proteins [7].

In patients with diagnosis of Inflammatory Bowel Disease (IBD) tenascin-C in the colonic mucosa indicates tissue repair, and their mucosal concentrations are associated with the activity of the local disease. Therefore plasma levels of tenascin-C have been demonstrated to be a beneficial indicator of the activity of the inflammatory bowel diseases [8].

In this study, first time in the literature, we aimed to demonstrate the association between tenascin-C expression, and intestinal stricture formation.

MATERIALS AND METHODS

A total of 43 patients (male, n=19; 44.2%;, and female, n=24, 55.8%) aged between 19, and 63 years, with clinically, endoscopically, radiologically, and histopathologically confirmed definitive diagnosis of Crohn's disease who were examined, diagnosed, and treated in the Gastroenterology Clinic of Haydarpasa Numune Training and Research Hospital between January 2011, and April 2012 were investigated.

After an overnight fasting of 10-12 hours- to eliminate possible lipemic interference- during morning hours, venous blood samples from the patient group

Table 1. Demographic distribution, and laboratory data of the cases included in the study

	Inflammatory	Obstructive	Fistulizing	P value
Patients, n (%)	17 (39.5)	16 (37.2)	10 (23.3)	0.368*
Age (mean±SD) years	35.1±12.1	40.0±10.7	31.5±11.7	0.182**
Gender				
Male, n (%)	9 (47.1)	8 (50.0)	2 (20.0)	0.210*
Female, n (%)	8 (52.9)	8 (50.0)	8 (80.0)	
Laboratory data				
ESR (mm/s),mean±SD	38.3±27.2	36.5±17.7	30.9±21.2	0.735**
CRP (mg/L), median (IQR)	1.56 (0.43-3.3)	1.44 (1.00-2.75)	2.65 (0.5213.00)	0.560***
WBC(K/mm^3), mean±SD	7.7±2.1	7.6±1.8	6.7±2.3	0.473**
Hemoglobin (g/dL), mean±SD	11.7±1.9	10.9±2.1	12.0±2.0	0.380**
Platelet (K/mm^3), mean±SD	327±98	332±136	342±109	0.950**
Tenascin C (ng/mL), median (IQR)	1.74 (1.29-3.16)	6.57 (4.26-21.87)	1.44 (0.74-2.47)	0.002***

*Chi-squared test; **Oneway Anova; ***Kruskal Wallis; IQR, interquartile range; SD, Standard deviations ESH, Erythrocyte Sedimentation Rate; CRP, C-reactive protein.

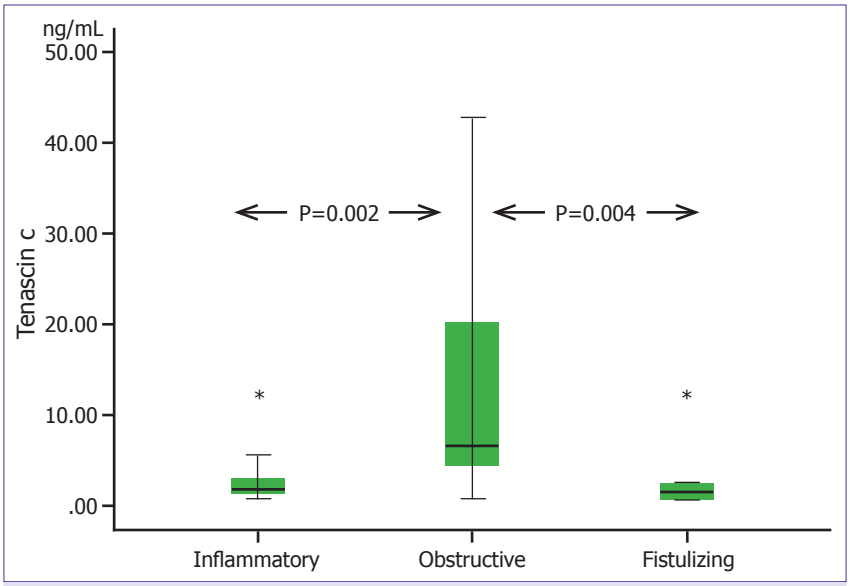

FIGURE 1. Distribution of tenascin-C levels in inflammatory, obstructive, and fistulizng type groups.

were drawn into tubes containing EDTA, sodium citrate, and gel (Becton Dickinson, USA)

Tubes with gel were left aside for 30 minutes, then centrifuged at 3500 rpm (1300 g) for 10 minutes. After an overnight fasting of 10-12 hours- to eliminate possible lipemic interference- during morning hours, venous blood samples from the patient, and the control groups were drawn into tubes containing EDTA, sodium citrate, and gel (Becton Dickinson, USA) Blood counts, and measurements of CRP, and ESR were performed without delay. Blood samples of all patients, and the control group in EDTA containing tubes were pipetted out for blood counts, while blood samples preserved in sodium citrate solution were analyzed for ESR measurements using Westergreen method, and Sed Rate Screener 100 (SRS 100, Greiner Bio-one GmbH, Austria) device. CRP measurements were performed from serum samples using nephelometric methods (Immage, Beckmann Coulter, USA).

Serum samples were divided into two portions for the future measurement of tenascin-C levels and awaited at -70°C. Frozen samples were thawed just befors the analysis, and then analyzed. Recurrent freezing, and thawing cycles were avoided. Serum tenascin- C levels were measured using a commercial Human Tenascin-C Purified Protein kit (Chemicon (Millipore, USA).

Based on the types of the Crohn's disease the study groups were divided into inflammatory (n=17; 39.5%), obstructive (n=16; 37.2%), and fistulizing (n=19; 23.3%) categories. For statistical analyses of data obtained from this study, SPSS (Statistical Package for Social Sciences) Statistics 15 program was used. The results were evaluated within 95% confidence interval at a statistically significant level of $p < 0.05$.

RESULTS

Groups were not different as for the distribution of age, and gender. Demographic characteristics, and laboratory results are indicated in Table 1. Median tenascin- C value in the obstructive group (6.57 ng/mL;4.26-21.87) was statistically significantly higher than that detected in the inflammatory (1.74 ng/mL; 1.29-3.16), and fistulizing (1.44 ng/mL; 0.74-2.47) groups (p=0.002) (Figure 1). Any statistically significant intergroup difference was not detected regarding ESR, CRP, leukocyte, hemoglobin, and platelet values (Table 1)

DISCUSSION

Ulcerative colitis (UC), and CD which are classified as inflammatory bowel diseases discriminate themselves from other many inflammatory diseases which might affect colon in that they reflect a chronic process, and they are characterized by periods of exacerbations, and remissions during their natural courses [9]. Three various phenotypes of Crohn's disease have been detected. Patients manifest non-structural, and non-penetrating type (70%) (disease progression only with inflammation), and stricturing type (17-20%) (progressing with fistulas, and abscess formations) [10]. Anatomic location of CD in the gastrointestinal system does not change with time, however its phenotype can alter. The disease usually starts with a inflammatory phenotype, and intractable inflammation transforms with time into a stricturing or penetrating form [10].

Intestinal fibroblasts have a critical role in the development of strictures in CH. Fibroblast migration is a very important component of fibrosis. Therefore, interest in the role of this phenomenon in the healing process of intestinal wounds, and formation of strictures has increased [11]. During the process of stricture formation in CD, histologically, excessive accumulation of mesenchymal cells in muscularis mucosa, submucosa, and muscularis propria is observed.

Intestinal fibroblasts do not proliferate as a result of stimulation by TGF-b1, but they demonstrate supermigration. Therefore, these mesenchymal cells possibly migrate from seromuscular layer. [12]. Expression of TGF-b isoforms in intestinal fibroblasts changes dependent on the structure of the affected tissue. Fibroblastas both in normal, and inflamed CD mucosa express both TGF-b1, and TGF-b3 isoforms. Expression of fibroblasts in the fibrotic tissue decreases, but they promote increases in the production of TGF-b1, and TGF-b2 [13].

Tenascin-C is the firstly discovered growth factor which reportedly upregulates mRNA of growth factor TGF-b1. increases proliferation, and production of tenascin-C by osteoprogenitor cells [13].

S.Tanaka et al. indicated that presence of tenascin-C is associated with inflammation rather than the disease itself. In their study, they stated the presence of a strong correlation between induction of tenascin-C, and acute inflammation, and reported the existence of a correlation between increased levels of circulatory tenascin-C concentrations, and various inflammatory, and infectious disease. [14].

Riedl S. et al. reported that tenasscin-C is induced by various pro-, and anti-inflammatory cytokines, and *de novo* expression of tenascin-C is a reliable marker for acute inflammation. Starting from that phenomenon, they indicated that trnascin-C levels can be used in the determination of disease activity of IBD [15].

Intestinal fibroblasts have an important role in the formation process of strictures in CD. Transforming growth factor (TGF)-b1 cytokine has a central place in this process [16]. Both TGF-b1, and its receptors are expressed in abundant amounts in the bowels of the patients diagnosed as CD [16]. When we considered that TGF-β can transform mesenchymal epithelium in a way to produce tenascin-C [16], one can assume that increased serum tenascin-C levels play a role in the development of CD-related strictures.

In summary, stricture formation in Crohn's disease is regulated by intestinal fibroblasts. Transforming growth factor-β1 (TGF-β1) stimulates synthesis of tenascin-C, and it is very important in the activation of fibroblasts. Starting from this point, serum tenascin-C levels can be said to be helpful in the determination of the type of Crohn's disease without resorting to invasive methods Studies performed with large patient series should aim to substantiate the use of tenascin-C levels in the prediction of stricturing type Crohn's disease.

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