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Cross-sectional Study

Evaluation of serum levels of cathepsin S among colorectal cancer patients

Koroush Ghanadi [a], Saber Ashorzadeh [b], Asghar Aliyepoor [c], Khatereh Anbari [d,*]

[a] Hepatitis Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[b] Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
[c] Department of Pathology, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[d] Social Determinants of Health Research Center, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Objective: Colorectal cancer is the third most common cancer worldwide. Cathepsins are protease that are known to be involved in cancer progression and metastasis. The aim of this study is to evaluate the levels of serum cathepsin S in patients and control subjects and its effects on the prognosis of the cancer.

Methods: In this case-control study, colorectal cancer patients referred to our gastroenterology clinic were included. The control group consisted of healthy individuals. Cathepsin S levels were analyzed in these patients and the check list consisting of demographic data, cancer stage, colonoscopy findings, CEA marker and cathepsin S levels were recorded.

Results: Of 80 patients and healthy controls included in the study, age, gender and BMI were not significantly different among the two groups, $p = 0.265$, $p = 0.752$ and $p = 0.2$, respectively. Cathepsin S levels were significantly greater in-patient group $p < 0.001$ and was significantly correlated with the stage of the tumor. CEA marker was also linear related with the increased levels of cathepsin S, $p < 0.001$.

Conclusion: Our study concluded that cathepsin S is elevated in the cancer patients and can be a significant marker for the prognosis of colorectal cancer.

1. Introduction

Colorectal cancer is one of the leading causes of cancer death worldwide. It contributes to the 6% of all types of cancer and is the third most common cancer worldwide. Australia, New Zealand, Canada, the United States and parts of Europe are reported to have highest incidence of the cancer whereas countries with the lowest rates include China, India, parts of Africa and South America. Colorectal cancer accounts for 6.1% of all cancers in men and 13.1% in women [1]. According to the Health Ministry, colorectal cancer is the third leading cause of death in Iran after cardiovascular disease and accidents. Colorectal cancer is one of the nine most common cancers reported in Iran, and the prevalence is rising among young people [2,3]. The risk factors known to be associated with colorectal cancer include smoking, alcohol intake, obesity and intake of red meat, whereas, age, family history of the cancer and inflammatory bowel disease are non-modifiable risk factor [4–6].

Cathepsins are lysosomal peptidases belonging to the class of cysteine, serine, and aspartic protease. Cathepsins were initially described as intracellular peptide hydrolases, although several cathepsins also have extracellular function. Systemic B, C, F, H, L, K, O, S, V, W and X cathepsin belong to papain family and are the largest class of cathepsin. Cathepsins are produced in the form of passive enzymes and are converted into active and mature enzymes during a process [7].

Cathepsin S is distinguished from other cysteine proteins by its limited tissue distribution. While most members of the cathepsin family are expressed in a wide variety of tissues and organs, cathepsin S is found mainly in the spleen, lymph nodes, monocytes, macrophages, and several APC cells. This unique distribution pattern suggests that cathepsin S is highly involved in the immune response.

Cathepsins are known to play an important role in cancer metastasis and progression. Increased expression of cathepsin is associated with poor prognosis of tumor and is therefore suggested as the marker for the prognosis of cancer. Cathepsin S causes the degradation of extracellular matrix and promotes cell metastasis such as brain-to-breast metastasis [8]. A number studies have indicated the cathepsin contributes to tumor microenvironment and can be detected at high levels in colon, ovarian, lung, breast, liver, head and neck and brain cancer [9].

The aim of this study is to evaluate the serum levels of cathepsin S in colorectal patients in comparison to the healthy patients and its role in the prognosis of the cancer.

* Corresponding author. Lorestan University of Medical Sciences, Khorramabad, Iran.
E-mail address: md.anbari.kh@gmail.com (K. Anbari).

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2. Methods

In this case-control study, patients with colorectal cancer referred to gastroenterology clinic in 2019 for colonoscopy were included. The control group was composed of healthy individuals referred to the center for colonoscopy. Written content was obtained from all the patients included in the study. Exclusion criteria was patients presented with multiple primary malignancies, patients in whom colonoscopy could not be performed and those with hematological disorders.

9 cc of intravenous blood was obtained from the patients and sent to the laboratory. Blood samples were centrifuged and serum was frozen for the tests. The level of cathepsin S is measured by the Human Cathepsin S Elisa Kit.

Information on demographic characteristics, disease stage (based on additional tests performed on the patient in terms of disease stage determination), type of pathology and tumor anatomical location, as well as CEA level was obtained from patients' files and colonoscopy reports.

The data was computerized and analyzed using SPSS v22. The mean and standard deviation was used to describe the variables. T test and Chi square tests were used to evaluate the relationship between the variables and hypothesis.

The study was approved by the ethical committee of (XXX).

Unique identifying number is: researchregistry7621.

The methods are stated in accordance with STROCSS 2021 [10].

3. Results

This study included 80 colorectal cancer patients and 80 healthy control. The mean age of the patients in the case and control group was 58.9 ± 11.7 and 56.9 ± 11.7 years, respectively. The highest prevalence of the colorectal cancer was in the age group 50–69 years (47.5%) and 49-30 years (27.5%) whereas the greater prevalence of control patients were aged 50–69 years. The difference between the mean age group of the patients and the control group based on the independent *t*-test was not statistically significant (P = 0.265). The difference in the frequency distribution of patients and control age groups based on chi-square was not statistically significant (P = 0.433). In patient group, 43 males (53.8%) and 37 females (46.2%) were included and in the control group there were 41 males (51.2%) and 39 females (48.8%). The gender difference among the two groups was not statistically significant, P = 0.752. In terms of education level, in case group, 63.7% had primary education and 23.8% did not finish their primary education. 8.8% had undergraduate certificate and 3.8% went to foreign universities. In patient and control group, majority of the participants were living in suburban areas, 71.3% and 80%, respectively. This was not statistically different in the two groups, p = 0.197. In terms of occupation, in the patient group, greatest number of participants were housewives (40%). 27.5% were jobless, 17.5% were self-employed, 5% were farmers and 6.3% were employees. In control group, majority of the individual were housewives 36.3% and self-employed 26.3%. In terms of employment status, the two groups had significant difference, p = 0.02.

In case and control group, 59% and 65.8% individual had normal BMI, respectively and 41% and 31.6% had BMI greater than normal, respectively. The two group was not significantly different in terms of BMI, p = 0.2 (Table 1).

7 patients (8.8%) were reported with a history of intestinal polyps and 6 patients (7.5%) reported a history of inflammatory bowel disease (IBD). Also, a history of colorectal cancer was seen in the first-degree relatives of 24 patients (30%).

In terms of staging, most tumors were in stage II (35%) and stage III (31.3%). The results of Post hoc test showed that the difference between serum cathepsin S level among patients with stage I and III tumor was significant, p < 0.001 and stage I and stage IV, p < 0.001. The cathepsin S level was not significantly different among stage I and stage II cancer patients, p = 0.348. Similarly, cathepsin S level were significantly different among stage II and III, p < 0.001 and stage II and IV, p = 0.001. However, this marker was not statistically significant among stage III and IV tumor stages, p = 0.408. It was seen that as the stage of the cancer increases, the serum cathepsin levels increases significantly too, p < 0.001(Table 2).

The mean cathepsin S level in patient and control group was 21.55 ± 6.3 and 12.35 ± 1.87 μg/L. The results of T-test showed that the difference in cathepsin S level among the two groups is statistically significant, p < 0.001(Table 3).Histopathological analysis of the tumor showed that the majority of tumors were in the descending colon (33.8%) and the ascending colon (25%) and the lowest frequency was in the rectosigmoid area of 6.3%. All tumors (100%) were adenocarcinoma. The mean levels of cathepsin S were not significantly different based on the location of the tumor, p = 0.984.

The distribution of cathepsin S level in different age groups. The serum cathepsin did not differ significantly among different age groups, p = 0.399 in patient group. Similarly, the gender, BMI and blood group type were also not associated with cathepsin levels, p = 0.342, p = 0.251 and p = 0.743, respectively, in this group.

In the above correlation matrix, no direct or inverse correlation was found between age and serum cathepsin S levels in the studied patients p = 0.164 however, there was a direct linear relationship between serum

Table 1
Comparison of frequency distribution of demographic characteristics of patients and control group.

Properties		Patients	Control	p-value
		Number (percent)	Number (percent)	
Age	30–49	22(27.5)	21(26.3)	0.433
	50–69	38(47.5)	45(56.3)	
	70 ≤	20(25)	14(17.5)	
Sex	Male	43(53.8)	41(51.2)	0.752
	Female	37(46.3)	39(48.8)	
Level of education	Illiterate and elementary	51(63.7)	45(56.3	0.714
	Middle school	19(23.8	21(26.3)	
	High school and diploma	7(8.8)	11(13.8)	
	University course	3(3.8)	3(3.8)	
Residency	City	57(71.3)	64(80)	0.02
	Rural	23(28.7)	16(20)	
Job	Workless	2(27.5)	7(8.8)	0.02
	Free	14(17.5)	21(26.3)	
	Employee	5(6.3)	7(8.3)	
	Labor	3(3.8)	5(6.3)	
	Farmer or rancher	4(5)	11(13.8)	
	housewife	32(40)	29(36.3)	
BMI	<18	-(–)	-(–)	0.2
	18–24.9	46(59)	52(65.8)	
	25–29.9	32(41)	25(31.6)	
	≥30	–	2(1.3)	

Table 2
Comparison and standard deviation of serum cathepsin S levels in patients with colorectal cancer by anatomical location and tumor stage.

Properties	Serum level of cathepsin S	Type of statistical test	p-value
	Standard Deviation ±mean		
Anatomical location of the tumor	Ascending colon Colon transverse Descending colon Rectum Rectosmoid	ANOVA (F = 0/094	0.984
Tumor stage	STAGE 1 STAGE2 STAGE3 STAGE4	ANOVA (F = 20.73)	<0.001

Table 3
Comparison of mean serum cathepsin S levels in colorectal cancer patients and control group.

Groups	cathepsin level	*t*-test	p-value
	Standard Deviation mean		
Patients	21.55 ± 6.3	12.519	<0.0001
Control	12.35 ± 1.87		

CEA levels and cathepsin S, $p < 0.001$. Increase in CEA levels was associated with increase in serum cathepsin S levels(Table 4).

Table 4
Matrix correlation of linear relationship between age and serum CEA levels with serum cathepsin S levels in patients with colorectal cancer.

Properties	
Age	R = 0.157 PV = 0.164
CEA Serum	R = 0.799 PV<0.001

4. Discussion

In current study, we evaluated the serum levels of cathepsin S in colorectal cancer patients in comparison with healthy control. The findings of our study demonstrated that cathepsin S levels are significantly elevated in colorectal cancer patients irrespective of their age, gender, BMI and blood group. Furthermore, the increase in the cathepsin S was directly correlated with the advancement of the cancer. It is also significantly correlated with carcinoembryonic antigen, colon cancer marker.

Cathepsin S, is a secretory protein and its expression has been reported in a number of cancerous studies such as hepatocellular carcinoma [11], lung and prostate cancer [12,13]. Antibodies against cathepsin and RNA silencing have been reported effective to inhibit tumor inhibition and progression and induce apoptosis [14–16]. In a retrospective study conducted by Gormley, Hegarty [17] on 560 colorectal cancer patients, reported that the expression of cathepsin S is 1.3 folds greater in patients, compared to the control. The study also showed that more than 95% of the patients were presented with an increased expression of cathepsin S.

In a cross-sectional study, Liu, Liu [18] evaluated the levels of cathepsin S in gastric cancer along with esophageal, nasopharyngeal, liver, colorectal cancer patients in comparison with healthy controls (n = 496) and reported that the cathepsin S levels are significantly greater in these patients. The study also reported that these levels were lower in stage I and II patients as compared to stage III and IV. The findings of our study also indicated that the progression of the cancer to later stages is significantly associated with greater levels of serum cathepsin, as compared to the early stages. CEA marker was also seen to be significantly associated with cathepsin levels. The study found that cathepsin S is not associated with gender, smoking and alcohol status, grade of the tumor and age.

In an in-vivo study by Burden, Gormley [13], Fsn0503, cathepsin S antibody inhibit proteolysis and has anti-angiogenic properties. The study also showed from the biopsy samples of colorectal cancer tissue that the expression of cathepsin was significantly enhanced, as compared to the healthy tissue. Huang, Chen [19] reported that targeting cathepsin S can induce autophagy in colorectal adenocarcinoma cells.

Our study doesn't report the outcomes of chemotherapy or cancer surgery on cathepsin levels. Clinical trials and studies on therapies targeting cathepsin S can give better conclusion.

5. Conclusion

In line with previous publications, we also report that cathepsin S is significantly elevated in colorectal cancer patients and is associated with poor prognosis.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the.

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No funding was secured for this study.

Author contribution

Dr. Koroush Ghanadi: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. Dr. Saber Ashorzadeh and Dr.Asghar Aliyepoor: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.Dr. Khatereh Anbari: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

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Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declaration of competing interest

The authors deny any conflict of interest in any terms or by any means during the study.

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