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Serum amphiregulin level and pancreatic adenocarcinoma relation in patients with chronic pancreatitis

Kronik pankreatit tanılı hastalarda serum amfiregülin düzeyi ve pankreatik adenokarsinoma ilişkisi

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ABSTRACT

Aim: We aimed in our study to determine serum amphiregulin level, which is an indicator of a malignancy, and to foresee pancreatic carcinoma in chronic pancreatitis.

Material and Method: Forty-two patients who applied to department of gastroenterology and were diagnosed with chronic pancreatitis and 43 healthy patients as the control group were enrolled in the study. Serum amphiregulin levels were studied with an Amphiregulin Human ELISA Kit ab99975 (Abcam, Cambridge, UK) by ELISA (Enzyme-Linked Immunosorbent Assay) method.

Results: Patients with chronic pancreatitis compared to healthy control group (p=0.007) and the group complicated with pseudocyst among chronic pancreatitis patient group, serum amphiregulin level (p=0.006) and CA 19-9 (p=0.03) level are high. We also found that the relationship between the long diameter of the pseudocyst and serum amphiregulin level is statistically significant (p=0.01) in the complicated chronic pancreatitis group.

Conclusion: Patients with chronic pancreatitis and the group complicated with pseudocyst among chronic pancreatitis patient group must be monitored more closely in terms of pancreatic cancer since serum amphiregulin level and CA 19-9 level are high and the relationship between the long diameter of the pseudocyst and serum amphiregulin level is statistically significant in such patient groups.

Keywords: Chronic pancreatitis, serum amphiregulin level, pancreatic carcinoma

ÖZ

Amaç: Çalışmamızda kronik pankreatit tanılı hastalarda malignitenin bir göstergesi olan serum amfiregülin düzeyi ve pankreas adenokarsinomu arasındaki ilişkiyi öngörmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya gastroenteroloji bölümüne başvuran kronik pankreatit tanısı alan 42 hasta ve 43 sağlıklı kontrol grubu dahil edildi. Serum amfiregülin düzeyleri Amphiregulin Human ELISA Kit ab99975 (Abcam, Cambridge, UK) ile çalışıldı. Serum amfiregülin düzeyi ELISA (Enzyme-Linked Immunosorbent Assay) yöntemi ile çalışıldı.

Bulgular: Sağlıklı kontrol grubu ile karşılaştırdığımızda, kronik pankreatit hastalarında (p=0,007), kronik pankreatit hasta grubunda da psödokist ile komplike olan grupta, serum amfiregülin düzeyinin (p=0,006) ve CA 19-9 (p=0,03) düzeyinin yüksek olduğunu tespit ettik. Ayrıca komplike olan kronik pankreatit grubunda psödokistin uzun çapı ile serum amfiregülin düzeyi (p=0,01) ilişkisinin istatiksel olarak anlamlı olduğu saptadık.

Sonuç: Kronik pankreatit hastalarıyla beraber, psödokist ile komplike olan kronik pankreatit grubunda, serum amfiregülin düzeyinin ve CA 19-9 düzeyinin yüksek bulunması ve psödokistin uzun çapı ile serum amfiregülin düzeyi ilişkisinin istatiksel olarak anlamlı olması nedeniyle bu hasta gruplarının pankreas kanseri açısından daha yakın takibi gerekmektedir.

Anahtar Kelimeler: Kronik pankreatit, serum amfiregülin düzeyi, pankreatik karsinoma

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INTRODUCTION

Pancreatic carcinoma is a fatal malignancy with very high mortality rates. In more than 80% of patients, the tumor has already exceeded surgical limits and caused distant metastasis at diagnosis. Treatment results are significantly better in small (< 20 mm) tumors diagnosed at an early stage (1).

Chronic pancreatitis is a progressive fibroinflammatory process in the pancreas, resulting in permanent functional failure is at the forefront (2). Chronic pancreatitis is a premalignant condition for pancreatic carcinoma and there is no effective scanning method (3,4).

Human amphiregulin (AREG) is a glycoprotein consisting of 84 amino acids and was discovered and defined by Shoyab et al. in 1980 (5). Human AREG gene has a length of 10 kb and located at q13 - q21 of the 4th chromosome Multiple endogenous and exogenous stimuli may induce AREG synthesis. It was shown in the studies conducted that various cytokines and growth factors, prostaglandin - E2, interleukin 1β, TNF - α and EGF significantly induce AREG mRNA expression AREG is expressed in many tissues. It is especially expressed in reproductive tissues and in the urinary system (breast tissue, uterus/ovaries, placenta and prostate). Furthermore, it is also expressed in the pancreas, circulatory system, respiratory system and in the gastrointestinal system (5). Epidermal growth factor receptor (EGFR) serves in cell growth, differentiation and reproduction and in signaling pathways. Amphiregulin is a member of the epidermal growth factor family and shows mitogenic effect by binding to EFGR. It is known that EGFR expression increases also in colorectal carcinoma and liver metastases (6).

It is aimed in our study to determine serum amphiregulin level, which is an indicator of a malignancy, and to foresee pancreatic carcinoma in high-risk patients in chronic pancreatitis with premalignant lesions in pancreatic carcinoma.

MATERIAL AND METHOD

Patient and Control Groups

Forty-two patients who applied to department of gastroenterology and were diagnosed with chronic pancreatitis through clinical, laboratory and imaging methods i.e. magnetic resonance imaging, computed tomography and endoscopic ultrasound (according to Rosemont criteria) and 43 healthy patients as the control group were enrolled in the study. Informed consent form was given by the patients. After receiving local ethics committee approval, samples were taken from sera separated from bloods of patients taken during routine checks. In these sera, amylase, lipase, CA 19-9, CEA, sedimentation and CRP, complete blood count and biochemical parameters were studied. Demographic data of patients, their chronic pancreatitis etiologies, duration of chronic pancreatitis, whether they have diabetes mellitus in their histories, and if there is diabetes mellitus, their diabetic age were recorded. It was recorded whether there were any data (pseudocyst, etc.) indicative of any complications detected by imaging methods in the pancreas in patients with chronic pancreatitis.

Sera of patient and control groups separated by transferring into Eppendorf tubes were kept at -80 degrees until amphiregulin level analysis time. In our study, serum amphiregulin levels were studied with an Amphiregulin Human ELISA Kit ab99975 (Abcam, Cambridge, UK). Serum amphiregulin level was studied by ELISA (Enzyme-Linked Immunosorbent Assay) method. The lowest measurement limit of the kit was assumed as 10 pg/ml.

Ethics

After receiving local ethics committee approval, samples were taken.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) version 21.0 was used to evaluate the data in the study. In the presentations of continuous variables (qualitative variables), mean and standard deviation for variables consistent with normal distribution, and standard error or minimum-maximum values for variables not consistent with normal distribution were provided. T-test was used for comparison of parametric continuous variables, and Mann-Whitney U test was used for comparison of non-parametric variables. Frequency and percentage values have been used for presentation of categorical valuables (qualitative variables). Chi-square (X^2) test was used in the evaluation of categorical variables. Spearman's correlation test was used to investigate the statistical relationship between serum amphiregulin level, chronic pancreatitis age and the long diameter of the pseudocyst that is the complication of chronic pancreatitis.

Diagnostic decision-making characteristics of serum amphiregulin levels for forecasting chronic pancreatitis was investigated by Receiver Operating Characteristic (ROC) curve analysis. Sensitive, specificity values of these limits were calculated in the presence of significant limit values. Evaluation of the area under the curve was interpreted as diagnostic value of the test is statistically significant in cases where Type-I error level is below 5%. Level of significance was assumed as $p< 0.05$.

RESULTS

The mean age of patients enrolled in the study was 46.5 (21-83), and the mean age of the control group was 45 (23-70). Fifteen of 42 patients enrolled in the study were female and 27 were male. The control group consisting of 43 patients included 22 female and 21 male patients. No statistically significant difference was detected when the patient and control groups were analyzed in terms of age and gender distribution (p=0.19, p=0.15, respectively). The most common complaints for our patients' referral were abdominal pain (n: 39 patients, 92.86%) and jaundice (n: 3 patients, 7.14%). When we analyzed patients according to their pancreatitis etiologies, there was an etiology of alcohol in 9 patients (21.4%) and autoimmune pancreatitis chronic pancreatitis in 5 patients (11.9%) were detected. No etiologies could be detected in 28 patients (66.7%). In patients with chronic pancreatitis, sedimentation rate was found to be 33.26±2.94 mm/hour and CRP (C Reactive Protein) was found to be 18.36±3.97 mg/L. Patients were also evaluated in terms of CEA and CA 19-9, which are tumor indicators. Detected CEA level of patients was 2.03 (0.5-123) ng/ml on average, and CA 19-9 level was 10.04 (1.48-1200) IU/ml on average. The time to inclusion of patients in the study from the diagnosis of chronic pancreatitis (disease age) was 1 year on average (minimum: 6 months–maximum: 9 years). Fourteen of the chronic pancreatitis patients (33.33%) had a diagnosis of diabetes mellitus. Mean diabetes age was 5 years (minimum: new diagnosis–maximum: 15 years). Serum amylase level was found to be 162.04 (18-591) ng/ml, and serum lipase level was found to be 114.45 (4-369) IU/ml in the chronic pancreatitis patient group. Pseudocysts detected by an imaging method in our patients were recorded as a complication. Twenty-two (52.4%) of our patients had a complication and 20 (47.6%) of them did not have any complication. Long diameter of our patients' pseudocysts was 44.5 mm (13 mm-120 mm) on average.

Serum amphiregulin level was found to be 45.68±13.54 pg/ml in the chronic pancreatitis group, and 19.69±2.92 pg/ml in the control group. When chronic pancreatitis and control groups were compared in terms of serum amphiregulin level, a statistically significant difference was found (p=0.007). The ability of the serum amphiregulin level to foresee chronic pancreatitis was found to be high in values above 10 pg/ml with a rate of accuracy of 95% CI (0.53-0.77), p=0.017 (57.1% sensitivity and 76.7% specificity). In **Table 1,** the statistical relationship between serum amphiregulin level, age and gender is shown in the chronic pancreatitis group and control group. **Figure 1** shows serum amphiregulin levels in chronic pancreatitis and control groups.

Table 1. The statistical relationship of serum amphiregulin level, age and gender in chronic pancreatitis group and control group

	Chronic pancreatitis group	Control group	P value
Amphiregulin (pg/ml)	45.68±13.54	19.69±2.92	0.007
Age	46.5 (21-83)	45 (23-70)	0.19
Gender (F/M)	15/27	22/21	0.15

F: Female M: Male

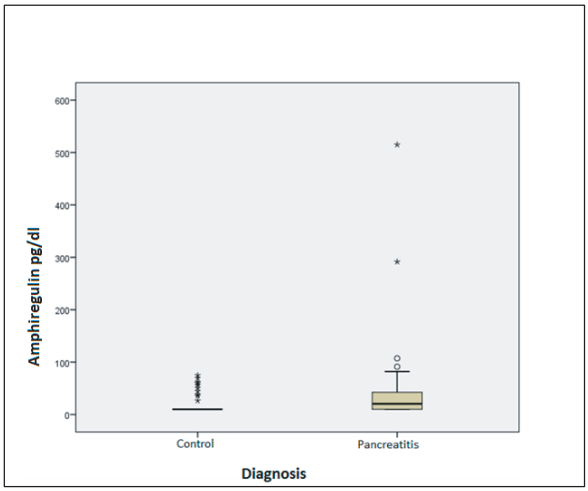

Figure1. Serum amphiregulin levels in chronic pancreatitis and control groups

The group which has complications and the group which does not have any complications were compared in terms of serum amphiregulin level. Serum amphiregulin level was found to be 46.76±12.57 pg/ml in the group which has complications, and the serum amphiregulin level was found to be 44.47±25.27 pg/ml in the group which does not have any complications. Serum amphiregulin level was found to be statistically higher than the group which has complications (p=0.006). **Figure 2** shows serum amphiregulin levels in patients with chronic pancreatitis in the group with complications and in the group without complications.

The age of patients with chronic pancreatitis was 51.5 (25-82) in the group with complications, and 44.5 (21-68) in the group without complications, and was not found to be statistically significant (p=0.084). When the group with complications and the group without complications were compared in terms of serum CEA level, gender, whether or not there is diabetes mellitus and its level and no statistically significant differences were found. In patients

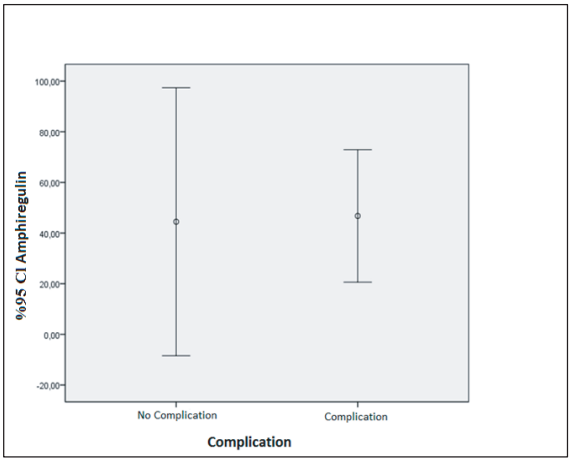

Figure 2. Serum amphiregulin levels in patients with chronic pancreatitis in the group with complications and in the group without complications.

with chronic pancreatitis, serum CA 19 -9 level was found to be 243.09±95.32 IU/ml in the group with complications, and 12.62±2.76 IU/ml in the group without complications. Serum CA 19-9 level was found to be statistically higher than the group which has not complications (p=0.03). **Table 2** shows the statistical analysis in terms of age, gender, serum amphiregulin level, diabetes mellitus, serum CA 19-9 and serum CEA in the groups with or without complications of patients with chronic pancreatitis.

When disease age and serum amphiregulin level were compared in patients with chronic pancreatitis, no statistically significant difference was found (p=0.37, r=0.14). When the long diameter of the pseudocyst and serum amphiregulin level were compared in patients with chronic pancreatitis who developed complications, a statistically significant difference was found (p=0.01, r=0.57).

Table 2. Statistical analysis in terms of age, gender, serum amphiregulin level, diabetes mellitus, serum Ca 19-9 and serum CEA in the groups with or without complications of patients with chronic pancreatitis

	With Complications	Without Complications	P value
Age	51.5 (25-83)	44.5 (21-68)	0.084
Gender (F/M)	6/14	9/13	0.46
Serum Amphiregulin	46.76 ± 12,57	44.47 ± 25.27	0.006
Diabetes mellitus (yes/no)	9/11	5/17	0.13
Serum Ca19-9	243.09 ± 95.32	12.62 ± 2.76	0.03
Serum CEA	10.18 ± 27.16	2.56 ± 2.11	0.74

F: Female M: Male

DISCUSSION

Chronic pancreatitis is an inflammatory syndrome of the pancreas characterized by progressive parenchymal fibrosis, maldigestion, diabetes mellitus and pain (7). In the epidemiological scan consisting of 6 national studies conducted by Hirota et al. (8) in Japan, male/female ratio of patients with chronic pancreatitis was found to be 4.5 and the average age was found to be 59.4. We found the male/female ratio of our patients to be 1.8 and the mean age of our patients was 46.5. These data suggest that chronic pancreatitis is seen at an earlier age and more commonly in women in Turkey compared to patients in Japan. In the study conducted by Hirato et al. the most common complaints of patients with chronic pancreatitis for applying to a hospital was reported to be abdominal pain (60.6%). We also found that the most common complaint of our patients to apply to the hospital was abdominal pain (92.86%).

Alcohol is the most common cause of chronic pancreatitis. As a result of clinical and experimental studies, it has been understood that alcoholic chronic pancreatitis starts as an acute process following chronic alcohol intake and progresses to chronic irreversible damage as a result of acute attacks. The studies show that alcohol is responsible in chronic pancreatitis etiology at a rate of 50-55% in the United States of America, 67-89% in Europe, and 56% in Japan. As can be seen in the study alcohol consumption varies according to countries in the etiology of chronic pancreatitis (9). Our study also found chronic pancreatitis etiology as 21.4% alcohol and 11.9% autoimmunity. No etiologies could be detected in 66.7% of our patients. Since alcohol consumption is less in our country compared to Western societies and Japan, its rate in chronic pancreatitis etiology has been found to be lower than in these countries. The reason could not be found for the high rate of patients whose chronic pancreatitis etiology could not be revealed and this requires re-examination of the patients in etiological terms.

Pancreatic diabetes is characterized by insulinopenia. Evidence from the studies point out to the fact that glucose intolerance and insulin resistance also accompany insulinopenia (10-13). In a single-centered study including 445 patients with chronic pancreatitis conducted by Wang et al. (14) the prevalence of diabetes mellitus was found to be 52%. Diabetes mellitus accompanied chronic pancreatitis in 33.33% of our patients in our study.

Pancreatic cancer has a poor prognosis and 5-year-survival is below 5%. Most patients die within 6 months after diagnosing with pancreatic cancer. The

etiology of this malignancy is still not clear. Factors that bear potential risk associated with pancreatic cancer may be classified as smoking, men with gastric ulcer, diets containing high fat and chronic pancreatitis (14). In the studies conducted, the risk of developing pancreatic cancer for patients with sporadic chronic pancreatitis was reported to be 1.8% in 10 years, and 4% in 20 years. Risk of pancreatic cancer has increased by 50-70 times in patients with hereditary pancreatitis, a rare form of chronic pancreatitis (4). All of our patients were sporadic chronic pancreatitis patients. Mean disease periods of our patients was 1 year (minimum 6 months–maximum 9 years) and they were much below the periods reported in the literature.

Signal transmission scenario associated with EGFR (epidermal growth factor receptor) family and their ligands are quite complex. Members of EGFR may be listed as ErbB or HER, ErbB-2 (HER-2, NEU), ErbB-3 (HER-3), ErbB-4 (HER-4). There are many ligands for EGFR. These are; EGF (epidermal growth factor), TGF-α (transforming growth factor-α), amphiregulin, heparin binding EGF (HB-EGF), crypto, epiregulin and betacellulin (16). Amphiregulin is associated with cell growth and apoptosis. Amphiregulin mRNA is expressed in many normal tissues such as placenta, testicles, pancreas, spleen, lungs, breasts, ovaries and the intestines (17). Serum amphiregulin level was also found to be 19.69±2.92 pg/ml in our study. In the studies conducted, amphiregulin was found to have a significant role in the development of breast tissue, breast cancer and colorectal cancer (17). Moreover, with the in vitro studies conducted, amphiregulin was shown to induce proliferation of malignant intestinal, breast, cervical, prostatic and pancreatic cells through autocrine effect. In addition, it has been reported that it is often excessively expressed in intestinal, stomach, breast and pancreatic cancers and that amphiregulin level is associated with tumor progression and short patient survival (18). Based on prior gene expression studies, AREG has been found to increase anterior gradient 2 (AGR2) expression in all pancreatic adenocarcinomas. Adenocarcinoma cells stimulated by AGR 2 proliferate and AGR 2 expression causes development of many characteristics associated with malignant transformation (19).

In current literature scan, it was found that no studies were reported concerning serum amphiregulin level and other factors affecting that in chronic pancreatitis patients; which is a risk factor for pancreatic cancer. In the study consisting of 33 patients conducted by Tun et al. (19) during which AREG level was measured in pancreatic cyst fluid, it was found that AREG values over 300pg/ml has an accuracy rate of 78% in cancer or high grade dysplasia (sensitivity 83%, specificity 73%). As a result of the study, it was found that AREG levels in pancreatic cyst fluid were significantly high in malignant and high-grade dysplastic cysts when compared with benign mucinous cysts. We found in our study that serum AREG levels of patients with chronic pancreatitis were significantly higher than those of healthy individuals (p=0.007).

We divided patients with chronic pancreatitis into groups of complicated chronic pancreatitis or uncomplicated chronic pancreatitis according to whether a pseudocyst is developed. We also found that in the complicated group, serum amphiregulin level and serum CA 19-9 level were significantly higher than the uncomplicated group (p=0.006 and p=0.03, respectively). Besides, we found that a statistically significant relationship exists between long diameter of the pseudocyst and serum amphiregulin level (p=0.01). However we found that diabetes mellitus is seen at a similar rate in complicated chronic pancreatitis group and in uncomplicated pancreatitis group.

CONCLUSION

Consequently, our study is a first-time as there is no study conducted to determine the serum amphiregulin level in chronic pancreatitis patient group and to analyze the factors affecting serum amphiregulin level in chronic pancreatitis patients. Our study has determined that patients with chronic pancreatitis compared to healthy control group and the group complicated with pseudocyst among chronic pancreatitis patient group must be monitored more closely in terms of pancreatic cancer since serum amphiregulin level and CA 19-9 level were found to be high and the relationship between the long diameter of the pseudocyst and serum amphiregulin level is statistically significant in such patients. However, studies conducted with a higher number of cases are needed.

DECLARATION OF CONFLICTING INTERESTS

The author declared no conflicts of interest with respect to the authorship and/or publication of this article

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REFERENCES

1. Kaur S, Baine MJ, Jain M, et al. Early diagnosis of pancreatic cancer: challenges and new developments. Biomark Med 2012; 6: 597-612.
2. Steer ML, Waxman I, Freedman S. Chronic pancreatitis. N Engl J Med 1995; 332: 1482-90.

3. Yeo TP, Lowenfels AB. Demographics and epidemiology of pancreatic cancer. Cancer J 2012; 18: 477-84.
4. Dítě P, Hermanová M, Trna J, et al. The role of chronic inflammation: chronic pancreatitis as a risk factor of pancreatic cancer. Dig Dis 2012; 30: 277-83.
5. Busser B, Sancey L, Brambilla E, et al. The multiple roles of amphiregulin in human cancer. Biochi Biophys Acta 2011; 1816: 119-31
6. Kuramochi H, Nakajima G, Kaneko Y, et al. Amphiregulin and epiregulin mRNA expression in primary colorectal cancer and corresponding liver metastases. BMC Cancer 2012; 12: 88.
7. Yadav D, Hawes RH, Brand RE, et al. North American Pancreatic Study Group. Alcohol cosumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. Arch Intern Med 2009; 169: 1035-45.
8. Hirota M, Shimosegawa T, Masamune A, et al. The sixth nationwide epidemiological survey of chronic pancreatitis in Japan. Pancreatology 2012; 12: 79-84.
9. Herreros-Villanueva M, Hijona E, et al. Alcohol consumption on pancreatic diseases. Worl J Gastroenterol 2012; 19: 638- 47.
10. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012; 35: 64–71.
11. Bank S, Marks IN, Vinik AI. Clinical and hormonal aspects of pancreatic diabetes. Am J Gastroenterol 1975; 64: 13–22.
12. Joffe BI, Bank S, Jackson WP, et al. Insulin reserve in patients with chronic pancreatitis. Lancet 1968; 2: 890-2.
13. DiMango MJ, DiMango EP. Chronic pancreatitis. Curr Opin Gastroenterol 2012; 28: 523-31.
14. Wang W, Guo Y, Liao Z. Occurrence of an risk factors for diabetes mellitus in Chinese patients with chronic pancreatitis. Pancreas 2011; 40: 206-12.
15. Lai HC, Tsai IJ, Chen PC, et al. Gallstones, a cholecystectomy, chronic pancreatitis, and the risk of subsequent pancreatic cancer in diabetic patients: a population-based cohort study. J Gastroenterol 2013; 48: 721-7.
16. Michalopoulos G, Khan Z. Liver regeneretion, growth factors, and amphiregulin. Gastroenterology 2005; 128: 503-6.
17. Pei R, Chen H, Lu L, et al. Hepatitis C virus infection induces the expression of amphiregulin, a factor related to the activation of cellular survival pathway and requared for efficient viral assembly. J Gen Virol 2011; 92: 2237–48.
18. Johansson CC, Yndestad A, Enserink JM, et al. The epidermal growth factor-like growth factor amphiregulin is strongly induced by the adenosine 3'5'-monophosphate pathway in various cell types. Endocrinology 2004; 145: 5177-84.
19. Tun MT, Pai RK, Kwok S, et al. Diagnostic accuracy of cyst fluid amphiregulin in pancreatic cysts. BMC Gastroenterol 2012; 12: 15.