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Original research

Plasma Kisspeptin-54 levels in gastric cancer patients

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ABSTRACT

Background: Kisspeptin (Kisspeptin-54; KP-54) is a 54-amino acid peptide was originally known as metastin that was implicated in suppression of tumor metastasis and circulating kisspeptin has been proposed as a tumor marker for numerous cancers in humans. However, the plasma level of KP-54 in gastric cancer (GC) remains undetermined.
Aim: We aimed to investigate the plasma levels of KP-54 in patients with GC.
Methods: Plasma KP-54 levels were quantified with enzyme-immunoassay from blood samples of 40 patients with GC at their initial staging and 59 age-matched controls.
Results: Plasma KP-54 levels were significantly higher in GC patients (63.3 ± 17.9) than in controls (49.0 ± 12.7) ($p = 0.000$). Cut-off value for KP-54 was determined as 44 ng/ml and sensitivity, specificity, positive predictive value and negative predictive value, were 60%, 78%, 63%, and 74% respectively. Plasma KP-54 levels were not correlated with any clinicopathological features of GC patients ($p > 0.05$).
Conclusions: Result of our preliminary study suggest that plasma KP-54 levels might be a useful parameter in diagnosis of GC.

1. Introduction

Gastric cancer (GC) is the fourth most common human malignant disease and second leading cause of cancer related death in both sexes worldwide.[1] Even though endoscopic examination is the most reliable method for the screening of GC, the feasibility and cost-effectiveness of this invasive approach in most countries except Japan remain questionable because of the lower incidence rates of disease.[2] A simple diagnostic test, such as a plasma biomarker assay might facilitate screening for GC.

Kisspeptin-54[3] encoded by KiSS-1 gene was identified as a human metastasis-suppressor gene in 1997 at breast cancer cells[4] and in 2001 at melanoma cells.[5] The endogenous ligand was initially called metastin, and now is referred to as Kisspeptin-54 (KP-54) after its role as a suppressor in metastasis was identified.[6] Circulating KP-54 has been proposed as a tumor marker for numerous cancers in humans. Prior to our study, elevated levels of plasma metastin-L1 (KP-54) have been reported in patients with pancreatic cancer[7] and colorectal cancer.[8] It has also been reported that high plasma and tissue levels of KP-54 were associated with good prognosis in patients with pancreatic cancer.[9] In this study, we aimed to determine the plasma KP-54 levels in patients with GC.

2. Materials and methods

2.1. Patients and controls

Forty caucasian GC patients at their initial staging admitted to Istanbul University of Turkey, Istanbul Medical Faculty, Gastrointestinal Surgery Services and 59 caucasian age-matched healthy controls were enrolled in the study between March 2009 and June 2010. All patients had histologically confirmed GC and their relevant clinicopathological details were obtained prospectively from our patients' records.

2.2. Preparation of plasma samples

All blood samples were collected in tubes containing 1 mg/ml EDTA-$2Na^+$, centrifuged at 1600 × *g* for 25 min at 4 °C, and then stored at −80 °C until assay.

2.3. Enzyme immunoassay (EIA) procedure for KP-54

Peptide levels in plasma were measured using highly sensitive enzyme immunoassay (EIA) for KP-54, that has been described previously.[10] Assays were performed using highly sensitive human Metastin- (1-54)-amide two sites EIA kit (EK-048-59) was purchased from the Phoenix Pharmaceuticals (Belmont, CA, USA), after extraction with Phoenix Peptide sep-columns (RK-Sepcol-2). The minimum detectable concentration was 5 ng/ml. The intra-assay variation and inter-assay variation were <4% and

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<10%. The linear range was 5–98 ng/ml. The immunoplate in this kit was pre-coated with a secondary antibody and the nonspecific binding sites were blocked. The secondary antibody bound to the Fc fragment of the primary antibody (peptide antibody), whose Fab fragment competitively bound by both biotinylated peptide and peptide standard or targeted peptide in samples. The biotinylated peptide interacted with streptavidin– horseradish peroxidase (SA–HRP) that catalyzes the substrate. The intensity of the yellow was directly proportional to the amount of biotinylated peptide–SA–HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. Absorbance was read at 420 nm.

2.4. Statistical analysis

All data obtained from patients and controls were evaluated by using SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA) and MedCalc-version 11.5.1 (MedCalc Software free trial, Mariakerke, Belgium). Values are presented as the mean ± standard deviation or median ± SEM. In comparison of groups, Student *t*-test, one-way variance analysis (ANOVA) followed by Tukey HSD Post Hoc tests, Spearman's correlation analysis, χ^2 analysis or, when appropriate, the Fisher exact test, Mann Whitney *U*-test, and the Kruskal Wallis tests were used. Repeated measures analysis of variance was used to analyze changes in variables over time. Receiver operator characteristic (ROC) analyses were used to determine the cut-off value, specificity and sensitivity of KP-54.[11] Non-parametric Spearman's R correlation coefficients were computed to analyze the relationship between plasma KP-54 levels and different clinical parameters (e.g. tumor stage and grade). A *p*-value of <0.05 was considered significant.

3. Results

Patients and primary tumor characteristics are shown in Table 1. Total plasma KP-54 levels that were below the level of detection were assumed to be 5 ng/ml for statistical analysis. The median plasma KP-54 was 63.3 ± 17.9 ng/ml for GC patients ($n = 40$) and 49 ± 12.7 ng/ml for controls ($n = 59$) (Mann Whitney U, $p = 0.000$). Data of plasma KP-54 levels were analyzed by ROC curve (Fig. 1) and area under the curve (AUC) was determined to be 0.725 ± 0.054 (95% confidence interval (CI):0.627–0.810; $p < 0.0001$) (24). The best cut-off value of KP-54 levels according to our study group was selected as 44 ng/ml according to ROC curve analysis (Fig. 2). When we take the cut-off value as 44 ng/ml, 24 of 40 (60%) GC patients had their plasma KP-54 levels equal to or above that concentration; whereas 46 of 59 (78%) controls had KP-54 levels below 44 ng/ml. Sensitivity, specificity, PPV and NPV for the cut-off value of 44 ng/ml were 60%, 78%, 63.1%, and 73.7%, respectively. When we calculated likelihood ratios (LR), positive LR was 2.53 and negative LR was 0.52 for our plasma cut-off value of KP-54 level.

Table 1
Characteristics of GC patients and controls. Data are presented as mean ± SD. $p < 0.05$; was considered significantly difference between patients and control group.

Parameters	Patients ($n = 40$) (%)	Controls ($n = 59$) (%)	*P* value
Mean age, years±SD	58.05 ± 13.4	57.1 ± 7.44	0.670
Gender			
Male	26 (65%)	30 (50.8%)	
Female	14 (35%)	29 (49.2%)	0.117
Differantiation			
Well	8 (20%)		
Moderately	23 (57.5%)		
Poor	9 (22.5%)		
Tumor size			
T1	2 (5%)		
T2	7 (17.5%)		
T3	25 (62.5%)		
T4	6 (15%)		
Nodal involvement			
N0	7 (17.5%)		
N1	7 (17.5%)		
N2	26 (65%)		
Stages			
Stage 1	4 (10%)		
Stage 2	11 (27.5%)		
Stage 3	11 (27.5%)		
Stage 4	14 (35%)		
Metastases+ direct invasion			
No	14 (35%)		
Yes	26 (65%)		

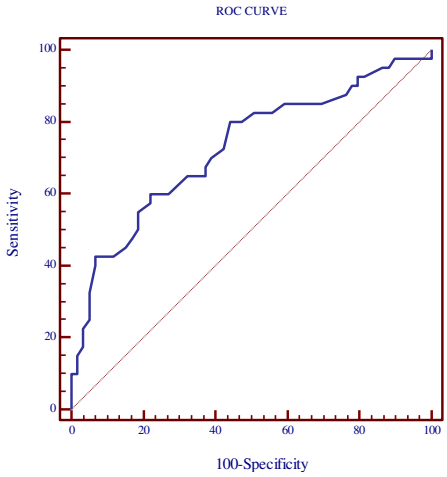

Fig. 1. Receiver operating characteristic (ROC) curve for KP-54. (The best cut-off value is 44 ng/ml). Area under the ROC curve (AUC) is 0.725; Standard Error[a] ([a] DeLong et al., 1988(18)): 0.054; 95% CI[b] ([b] Binomial exact): 0.627–0.810; Z statistic: 4.188, significance level $P = 0.000$.

Correlation analysis was performed using Spearman correlation test in order to determine whether plasma KP-54 levels changed in terms of clinicopathological findings such as tumor size, lymph node involvement, tumor grades, distant metastases and stages. Plasma KP-54 levels greater than 44 ng/ml were found to be not correlated with any of the clinicopathological parameters of patients with GC ($p > 0.05$, Table 2).

4. Discussion

GC has a complex network of molecular alterations along its carcinogenesis pathway and is diagnosed at advanced stages in the majority of cases. Despite numerous studies focused on these issues, many crucial questions and early diagnosis with convenient diagnostic tools of GC still remain to be clarified. Therefore, one of the most important requirements for GC is that the necessity of

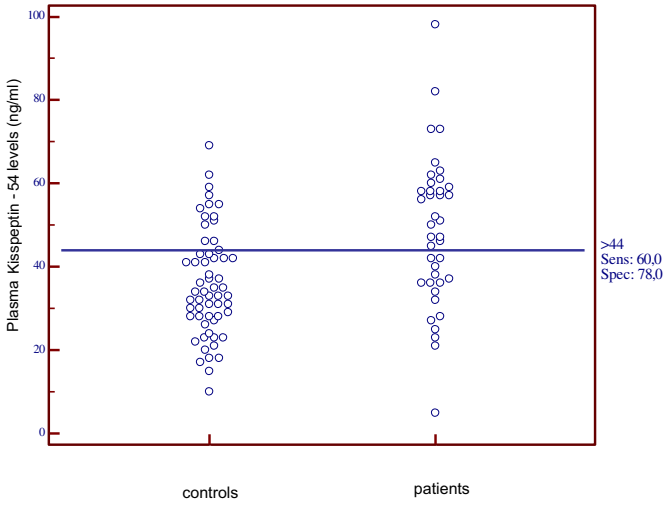

Fig. 2. Cut-off value of plasma Kisspeptin-54 (KP-54) levels. When 44 ng/ml was selected as cut-off value, 78% of controls had plasma KP-54 levels below 44 ng/ml, and 60% of GC had plasma KP-54 levels above 44 ng/ml.

Table 2
No correlation was detected between plasma KP-54 levels and patients' cahar-acteristics. $P < 0.05$ was considered to be significant.

Clinicopathologic characteristics of GC patients	KP-54 (ng/ml)		
	>44 ($n = 24$)	<44 ($n = 16$)	*P*
Age			
<60	14	6	0.206
≥60	10	10	
Sex			
Male	17	9	0.356
Female	7	7	
Differentiation			
Poor	3	6	0.441
Moderately	17	6	
Well	4	4	
Primary tumor size			
T1	1	1	0.938
T2	5	2	
T3	14	11	
T4	2	4	
Lymph node metastasis			
N0	12	6	0.863
N1	6	1	
N2	8	9	
Distant metastasis			
Present	7	7	0.356
Absent	17	9	
Stages			
Stage I	1	3	0.410
Stage II	10	1	
Stage III	6	5	
Stage IV	7	7	

emerging new non-invasive, simple diagnosing and screening markers for detection of GC at earlier stages.

Our study is the first report on investigations of plasma KP-54 levels in GC that demonstrates the plasma KP-54 levels are significantly increased in patients with GC at their initial diagnosis compared with control group supporting diagnostic value of plasma KP-54 in GC. Even though significantly elevated plasma KP-54 levels were detected in our GC patients, we have not observed that KP-54 was associated with any clinicopathological parameters of these patients. For GC, Dhar et al.[12] showed that GC with low *KiSS-1* had frequent venous invasion, distant metastasis and tumor recurrence. Guan-Zhen et al.[13] detected that reduced *KiSS-1* expression in lymph node and liver metastases compared with primary tumours of GC.

There are several limitations that need to be addressed regarding the present study. The first limitation concerns the validation set of ELISA with immunohistochemistry or RT-PCR has not included in the study. A prospective clinical trial study including comparison of plasma expression with using other techniques will better assess the performance of our assay. The second limitation is that size of the study groups who needs to be enlarged with long-term follow up. Longer follow-up in big study size group will give more information regarding the prognostic value of plasma KP-54 in GC patients. The population of normal volunteers included blood donors who are selected for not presenting any symptom or acute disease that they may have an occult or undiagnosed disease during the sample collections. Further validation will need to select negative controls after screening for GC in future studies. Another limitation of the study is that the applicability of our study findings to other ethnic groups. We investigated the plasma KP-54 levels in Turkish caucasian population. However comparison of other ethnic populations in terms of importance of plasma KP-54 in GC patients need to be clarified. The gender sizes were also different between patient and control groups in our study. Pita J et al.[14] pointed out adult and obese females showed higher KP-54 levels in plasma. Therefore, equalization of the gender and BMI are also needed in future studies. Finally, the sensitivity of the test needs improvements. Additional proteins will be included in the bioassay. These proteins will be selected to be encoded by genes overexpressed in GC. And, tumor and adjacent normal tissue expressions of *KiSS-1* gene, protein and its receptor need to be determined in order to conclude reflection KP-54 tissue levels to the plasma.

In conclusion of our preliminary results, preoperative plasma KP-54 levels in GCwere increased with respect to plasma levels of healthy controls. Further prospective evaluation of plasma KP-54 levels, in GC with larger study groups using other validation tests and comparison with tissue expressions of *KiSS-1* and its receptor are needed, to emerge its clinical value in GC.

Ethical approval

Ethical approval was given by Local Ethical Committee of Istanbul University.

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Author contribution

Arzu Ergen, performed the experiments.

Emel Canbay ,wrote up the project and made data interpretation and wrote up the MS.

Dursun Bugra, read the article, made data interpretation.

Umit Zeybek, performed the experiments.

Sumer Yamaner, made data interpretation.

Turker Bulut, made data interpretation.

Conflicts of interest

Authors declare no conflict of interest.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;**127**:2893–917.
2. Gent R. Screening for gastric cancer: does it make sense. *Aliment Pharmacol Ther* 2004;**20**:42–7.
3. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 2001;**411**:613–7.
4. Lee JH, Welch DR. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene KiSS-1. *Cancer Res* 1997;**57**:2384–7.
5. Shirasaki F, Takata M, Hatta N, Takehara K. Loss of expression of the metastasis suppressor gene KiSS1 during melanoma progression and its association with LOH of chromosome 6q16.3-q23. *Cancer Res* 2001;**61**:7422–5.
6. Kirby HR, Maguire JJ, Colledge WH, Davenport AP. International Union of Basic and Clinical Pharmacology. LXXVII.Kisspeptin receptor nomenclature, distribution, and function. *Pharmacol Rev* 2010;**62**:565–78.
7. Katagiri F, Nagai K, Kida A, Tomita K, Oishi S, Takeyama M, et al. Clinical significance of plasma metastin level in pancreatic cancer patients. *Oncol Rep* 2009;**221**:815–9.
8. Canbay E, Ergen A, Bugra D, Yamaner S, Eraltan IY, Buyukuncu Y, et al. Kisspeptin-54 levels are increased in patients with colorectal cancer. *World J Surg* 2012 May 3 [Epub ahead of print] PubMed PMID: 22552499.
9. Nagai K, Doi R, Katagiri F, Ito T, Kida A, Koizumi M, et al. Prognostic value of metastin expression in human pancreatic cancer. *J Exp Clin Cancer Res* 2009;**28**:9.
10. Chen X, Mo Y, Li L, Chen Y, Li Y, Yang D. Increased plasma metastin levels in adolescent women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2010;**149**:72–6.
11. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;**44**:837–45.

12. Dhar DK, Naora H, Kubota H, Maruyama R, Yoshimura H, Tonomoto Y, et al. Downregulation of KiSS-1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma. *Int J Cancer* 2004;**111**: 868–72.
13. Guan-Zhen Y, Ying C, Can-Rong N, Guo-Dong W, Jian-Xin Q, Jie-Jun W. Reduced protein expression of metastasis-related genes (nm23, KiSS1, KAI1, and P53) in lymph node and liver metastases of gastric cancer. *Int J Exp Pathol* 2007;**88**: 175–83.
14. Pita J, Barrios V, Gavela-Pérez T, Martos-Moreno GA, Munoz-Calvo T, Pozo J, et al. Circulating kisspeptin levels exhibit sexual dimorphism in adults, are increased in obese prepubertal girls and do not suffer modifications in girls with idiopathic central precocious puberty. *Peptides* 2011;**32**:1781–6.