Research article

Utility of serum interleukin-18 (IL-18) as a tumour marker in gastric cancer

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ABSTRACT

Introduction: Gastric cancer (GC) is the fourth most prevailing cancer globally, attributing to more than 70% of cases in developing countries. Protein cancer biomarkers, such as CEA, CA- 125, AFP and PSA, are clinically helpful diagnostic tools, but they have low sensitivity and specificity for GC. Hence, it is essential to discover better markers for GC diagnosis. Interleukin-18 (IL-18) is the member of Interleukin-1 family. It is hypothesized to be a potent inhibitor of gastric acid secretion, leading to gastric atrophy and causing an increased risk of GC. This study was to evaluate the association between the serum IL-18 in GC.

Methodology: We included cases who underwent UGI Scopy and were proven to have GC histopathologically. The patients who presented to the out-patient who underwent UGI scopy and was found to have no growth were selected as controls. Twenty-eight cases and 84 control sample sizes were derived from nMaster V2. Blood samples from patients and controls were collected, and serum IL-18 levels were estimated using a solid-phase sandwich ELISA method.

Results: We found that the cut-off value of serum IL-18 was 85.59 pg/ml, had a sensitivity of 63.1% and specificity of 57.1%, with a positive predictive value of 81.5% and a negative predictive value of 34% in diagnosing GC. The study plotted the receiver operating characteristic curve against IL-18 for sensitivity and specificity. Statistically, we found through the Area Under the Curve (AUC) that the rise in serum IL-18 levels was a poor indicator of GC with a p-value of 0.078.

Conclusion: Statistically, a cut-off of 85.59pg/ml showed good sensitivity and specificity; however, the probability was insignificant, suggesting that IL-18 may not be of diagnostic importance. Studies with a larger sample size are required to further probe into the usefulness of estimating IL-18 in GC.

Keywords: Gastric cancer; serum Interleukin-18; Carcino Embryonic Antigen; Cancer Antigen-125; Alpha-Feto Protein; Prostate-Specific Antigen.

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer globally, with 70% of the cases reported in developing countries. Among these, 50% and more cases are recorded in Eastern Asia alone. In Asia, GC is the third most common type of malignancy after breast and lung cancer. GC is the second most common cause of cancer deaths after lung cancer (1). Many risk factors of gastric cancer have been identified. Most are related to lifestyle and dietary habits such as high salt intake (2), alcohol consumption (3) and tobacco smoking (4). It is more common in patients with pernicious anaemia, 'A' blood group, and patients with a family history of GC (5). Environmental factors are the significant cause of an intestinal type of gastric cancer than the more lethal diffuse type.

Physical examination is usually normal, with weight loss, reduced food intake due to anorexia and early satiety being the most common signs. Dysphagia is expected when the tumour is situated in or invades gastric cardia. Most of the time, abdominal pain is not severe & frequently overlooked. Other features include nausea, vomiting, and swelling. Acute GI bleeding seldom occurs, but chronic occult blood loss presenting as iron deficiency anaemia and heme-positive stool is usually observed. Based on clinical results and clinical history, it is difficult to differentiate between peptic ulcer and gastric cancer. Patients with dyspepsia and symptoms such as weight loss, frequent vomiting, dysphagia, signs of GI

bleeding or anaemia, and a family history of GC should undergo upper endoscopy and biopsy (1-5).

The lack of efficient diagnostic strategies is the main reason for high mortality in GC. However, protein cancer biomarkers are clinically helpful. The availability of diagnostic tools such as CEA, CA-125, AFP and PSA, commonly used for colon, ovarian, liver, and prostate cancer, have low sensitivity and specificity for diagnosing GC(6). Hence, it is essential to identify the new markers for diagnosing GC. The serum Interleukin-18 (IL-18) is a member of the Interleukin-1 family; IL-18 is synthesized as inactive from Pro-IL-18, converted into an active form by IL-1-Beta converting enzyme present in macrophages, Kupffer cells, dendritic cells & in few tumour cells. It strongly inhibits gastric acid secretion, which causes gastric atrophy, leading to increased GC risk. The serum IL-18 increases the metastasis and immune escape of stomach cancer via the downregulation of CD70 & maintenance of CD44 (7-9).

MATERIALS AND METHODS

This case-control study was conducted after obtaining clearance from the Institutional ethical committee (IEC NUMBER INST.EC/EC/184/2018-19). We held this study between January 2019 and June 2020 at the Department of General Surgery of the tertiary hospital in Mangalore.

This study was based on Ye *et al.,* (7), wherein the positive expression of IL-18 was seen in 25% of the patients with GC, while 75% didn't express IL-18. Using the software nMaster v2, with the allocation ratio of 1:3(cases to controls) at 80% power alpha error of 5%, a sample size comprising 28 cases of GC and 84 controls was calculated.

The study participants were patients who underwent UGI scopy with a histopathologically proven diagnosis of GC constituted the cases; patients presenting to the out-patient with no growth in UGI scopy were taken as controls. The information sheet was given, and written consent was obtained from each study subject after explaining the study in a language they were well versed with. 2ml of peripheral blood sample was collected from the cases and the controls. The serum was separated from the blood sample and stored at -20°C. The serum IL-18 were estimated using the solid-phase sandwich ELISA method. The statistical analysis was performed using SPSS software v20. The ROC curve for serum IL-18 in gastric cancer was plotted, and the area under the curve was measured for the sensitivity and specificity of IL-18 for diagnosing the GC.

RESULTS

The study was cross-sectional, comprising a sample size of 28 cases of GC and 84 controls. The median age in cases is 58.64±12.81 years, and controls 48.92±11.67 years with a P-value of <0.001 with a male predominance.

The serum IL-18 was increased in GC compared to controls. However, the increased values were not statistically significant (Table 1).

The IL-18, with a cut-off value of 85.59 pg/ml, had a sensitivity of 63.1% and specificity of 57.1%. It showed a positive predictive value of 81.5% and a negative predictive value of 34%, but the Kappa value of 0.165, along with a p-value of 0.078, questioned its statistical significance (Fig. 1 and Table 2).

Among the 28 cases with GC, 4, 5, 13, and 6 patients were in stages I, II, III, and IV. IL-18 was the maximum in the cases of Stage III (Table 3).

Table 1: Comparison of IL-18 levels among cases and controls

Parameters	Cases (n=28) (Mean ±SD)	Control (n=84) (Mean ±SD)	T	p-value
Age	58.64±12.81	48.92±11.67	3.726	<0.001
IL-18 pg/mL	167.87±185.94	159.26±121.58	0.229	0.82

Table 2: Area under the curve

Area	Std. Error [a]	Asymptotic Sig. [b]	Asymptotic 95% Confidence Interval	
			Lower Limit	Upper Limit
.551	.069	.416	.416	.687
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

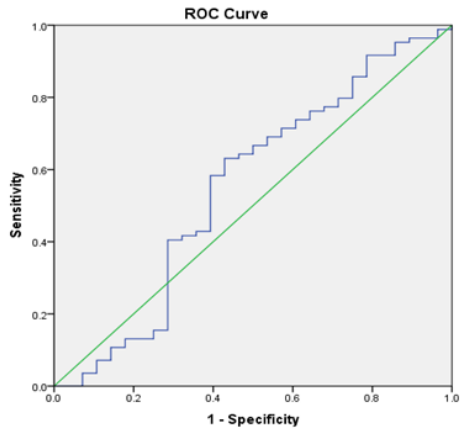

Fig. 1: ROC curve analysis for sensitivity, specificity and cut-off determination

Table 3: Comparison of IL-18 levels in different stages of GC

Parameters	**Staging**	**Valid Percent**
Control	84	75
I B	4	3.6
II A	4	3.6
II B	1	0.9
III A	7	6.3
III B	3	2.7
III C	3	2.7
IV	6	5.4
Total	112	100

DISCUSSION

The median age of patients in cases in our study was 58.64 years. We found this concordant with Sharma *et al.*, (11), who studied GC in India and found the median age to be 58 years. Our results were similar to the study by Kim *et al.*, (12), who investigated the clinicopathological characteristics and prognostic factors in 10783 patients with GC and observed a median age of 53.5 years.

In our study, among 28 cases of GC, 18 were male, and 10 were female, with a highly significant p-value of <0.001. A similar study was done by Forman D *et al.*, (13) on GC: global pattern and environmental risk factors have identified the incidence rate of 22.0 and 10.3 per 100,000 per annum in males and females, respectively. Kim *et al.*, (12) also found male dominance with a male-to-female ratio of 2:1.

The study by Kim *et al.* (12) found that 16.6%, 20.0%, 36.5% and 13.2% of the patients belonging to TNM stages I, II, III, IV. The findings of our study were identical, with the majority of the 13 patients belonging to stage III.

Dimarello (14) studied the relationship between pro-inflammatory cytokines in cancer. The study showed that patients with carcinoma will have raised levels of IL-18, leading to tumour progression and poor prognosis. A study done by Tas *et al.* (9) on the significance of IL-18 levels in patients with GC found that the levels in GC are significantly higher than the control group, with a P-value of 0.001.

A study by Kang *et al.*, (8) showed that the serum IL-18 was significantly higher in patients with GC compared to normal individuals. In our study also, the cases of GC had higher mean IL-18 levels compared to controls. However, the increase was statistically insignificant. The possible reason could be the low sample size in our study.

A study was done by Kawabata *et al.*, (15), whose objective was to investigate preoperative IL-18 level as a prognostic marker in GC patients. The IL-18 levels in 94 patients of GC and 54 controls were analysed. The serum IL-18 levels in patients with stages II and III were significantly higher than the healthy controls (P <0.01), and the IL-18 levels in patients with stages I and IV did not show significant alterations compared to healthy controls. A similar study by Lissoni *et al.*, (16) showed serum IL-18 levels are elevated in patients with metastases from lung and gastrointestinal tumours compared to healthy individuals.

A study done by Kim *et al.* (17) on IL-18 as a critical factor for vascular endothelial growth factor enhanced migration in human GC cell lines. It has concluded that the concentration of IL-18 would be higher in advanced stage than in early-stage cancer patients. It has been suggested that raised IL-18 are seen in the metastatic process. IL-18 induces angiogenesis with a potent mediator of VEGF, leading to the migration of cancer cells SNU-601. In our study, a total of 28 patients had GC, out of which stages I, II, III, and IV are 4, 5, 13, and 6, respectively, and it was found that the mean Serum IL-18 was maximum in the case of Stage II and III.

A study was done by Thong-Ngam D *et al.*, (18) whose objective was the diagnostic role of IL-18 in 51 histologically proven GC. He has stated that inappropriate production of IL-18 would cause the pathogenesis of cancers which will influence the clinical outcome of patients. He also showed that IL-18 levels in GC patients (58.54+43.96 pg/mL) are significantly higher than the control group (30.84+11.18 pg/mL) with a sensitivity of 52.17% and a positive predictive value of 92.31%. In our study, the cases were 28, the sensitivity was 63.10%, and a positive predictive value of 81.50%, but the p-value was insignificant.

A study by Vidal-Vanaclocha *et al.*, (19) showed IL-18 in the pathophysiology of cancer progression. Its levels are increased in most patients associated with

disease recurrence, poor prognosis and long-term survival.

A study by Majima *et al.,* (20) on IL-10 and IL-18 on interferon-gamma production by peritoneal exudate cells in patients with GC found that IL-18 levels are significantly raised in serum and peritoneal lavage fluids in patients with GC and it is related to poor prognosis. Yao *et al*., (21) did a meta-analysis of the prognostic role of IL-18 in various human cancers and radiation injuries. It was concluded that high IL-18 levels are related to a poor prognosis in cancers. The statistical analysis of our study also correlates with a cut-off of 85.59, proving its good sensitivity and specificity. However, plotting the data in the ROC curve, the value indicated that IL-18 is a poor marker of GC.

Limitations of the study

This was a single-center study, and the sample size was limited. Hence, the results may not be accurately extrapolated to the general population. A standard cut-off value for IL-18 as a marker for GC is not established; therefore, there was difficulty in interpreting the tabulated values following estimation.

CONCLUSION

Our study aimed to estimate the association of IL-18 as a tumour marker in GC. Although the 85.59 pg/ml cut-off showed considerable sensitivity and specificity, it was not statistically significant. The present study indicates serum IL-18 cannot be used as a novel biomarker in GC diagnosis, and studies with a larger population are needed to confirm our findings.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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