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Research Article

# The Evaluation of S100A12 protein expression in patients with gastric cancer and its diagnostic significance

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**Abstract**

**Introduction**: The S100 protein family plays an important role in several types of tumors. One of the family members is the S100A12 protein, recently known as a new marker in tumorigenicity and gastric cancer research. Considering the diagnostic importance of S100A12 and the relatively high prevalence of gastric cancer in Iran, this study aimed at examining the expression of S100A12 in the tissues of such patients.

**Method**: The expression of S100A12 in 79 Gastric Cancer patients was examined using immunohistochemistry in tumor tissue cells, and 50 non-tumor samples were selected for comparison with tumor tissues. Data analysis was conducted using SPSS v16 and chi-square test with a confidence interval of 95 percent.

**Results**: The results showed that there was a significant difference between tumor tissues and non-tumor tissues with regard to the expression of S100A12 protein, with tumor cells showing significantly lower expression. Based on clinicopathologic results, S100A12 protein indicated a negative correlation with tumor dimensions, tumor invasion depth, metastasis stage of tumor, and no significant correlation with other clinicopathologic features, like age, sex and metastasis to lymph nodes.

**Conclusion**: The results showed that the low expression of S100A12 as a new marker was associated with tumor formation and Gastric Cancer progression.

**Keywords**: S100A12, Gastric Cancer, ImmunoHistoChemistry

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## Introduction

The S100 protein family is the largest subfamily of calcium-bound proteins [1] that execute key functions in the cell, including calcium homeostasis, cell proliferation, cell death, protein phosphorylation, transcription factor regulation, and inflammation [2, 3]. Most of the S100 protein genes are located on the human chromosome 1 q21, which is an area susceptible to genomic rearrangements, suggesting that S100 proteins may interfere with the progression of the tumor. Also, the modified expression of several members of the S100 family including S100A2 [2 , 3], S100A14 [4], and S100A4 [5] in various types of cancers have been shown. The high expression of several S100 proteins, such as S100A2, S100A3, S100A6, S100A8 / A9, and S100A11 has been demonstrated in a variety of cancers. By contrast, the low expression of these proteins has been exhibited in other types of cancer. The high expression of S100A2 is a poor prognostic marker for NSCLC and pancreatic cancer [6]. S100A4 is commonly expressed in a variety of cancers, and the high expression of S100A4 in the progression of tumor, invasion, metastasis, survival and poor prognosis have been demonstrated in different cancers [7]. S100 proteins exert their effects through specific target proteins. For example, intracellular S100 proteins affect the transcriptional activity of P53 protein, which ultimately affects the cell cycle regulation applied by P53. It has also been shown that some of these proteins impose varying effects on specific target proteins such as NF-κB and β-catenin, which are the pathways for inflammation and cell proliferation [2][8-10]. RAGE is a common receptor of S100 proteins. It is a cell-receptor that plays a major role in inflammation and cancer [11]. Studies have suggested that S100 proteins have an integral role in angiogenesis by affecting molecules such as MMP, TGF-β and FGF, which ultimately affect tumor metastasis [12].

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One member of the S100 protein family is a protein called S100A12, which has recently been introduced as a new marker of tumorigenicity and gastric cancer (GC) progression [13]. The S100A12 protein, also known as Calgranulin C, has high expressions in neutrophilic granulocytes and low expressions in lymphocytes and monocytes, while its expression in gastro-intestinal epithelial cells and fibroblasts is stimulated during inflammation [14]. Elevated serum level of S100A12 in acute and chronic inflammatory diseases is regarded as a biomarker [15, 16]. Recent studies have shown that S100A12 proteins play a vital role in detection of Intestinal inflammation caused by Crohn's disease and other inflammatory bowel diseases [16-18]. It also plays a protective role at various stages of atherogenesis development by inhibiting pro-inflammatory cytokines [19]. Recent studies have exhibited that increasing the expression of S100A12 protein can be a good diagnostic factor in the survival rate of patients with oropharyngeal squamous cell carcinomas (OPSCC) [20] In addition, the expression of this protein in suprabasal keratinocyte of normal mucosa cells is higher, while in 30.4% -51.9% of the primary cancer cells of OPSCCs, lower expression has been indicated. Moreover, this study suggested that the expression of this protein and the clinicopathological features of the tumor, such as tumor grade, metastasis to lymph nodes, and TNM [20].

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In another study, however, proteomic studies showed that S100A12 shows a higher expression in colorectal cancer cells compared to normal tissues [21]. It has also been exhibited that in non-cancerous gastric mucosal tissues, S100A12 protein is present in not only gastric mucosal epithelial cells, but also in stem cells such as neutrophilic granulocytes, lymphocyte cells and monocytes. In addition, the association between the expression of this protein and the clinicopathologic features of patients with gastric cancer has been demonstrated [22]. Gastric cancer is recognized as the fourth most common types of cancer, and the second most fatal cancer worldwide [23]. Hence, in light of the importance of S100A12 diagnosis and the relatively high prevalence of gastric cancer in Iran, the goal of this study was to investigate the expression of S100A12 in tissues of patients with GC and to examine the relationship between the expression of S100A12 in glandular tissue cells and stromal of tumor specimens with clinicopathological features.

**Materials and methods:**

**- Sampling and data collection method**

The population of this study consisted of 79 patients with GC referred to Besat Hospital in Hamedan, who had undergone gastrocectomy in the period of 2012-17. Demographic and pathological data including age, sex, tumor size, invasion depth, metastasis, tumor differentiation and tumor/nodes/ metastasis (TNM staging system

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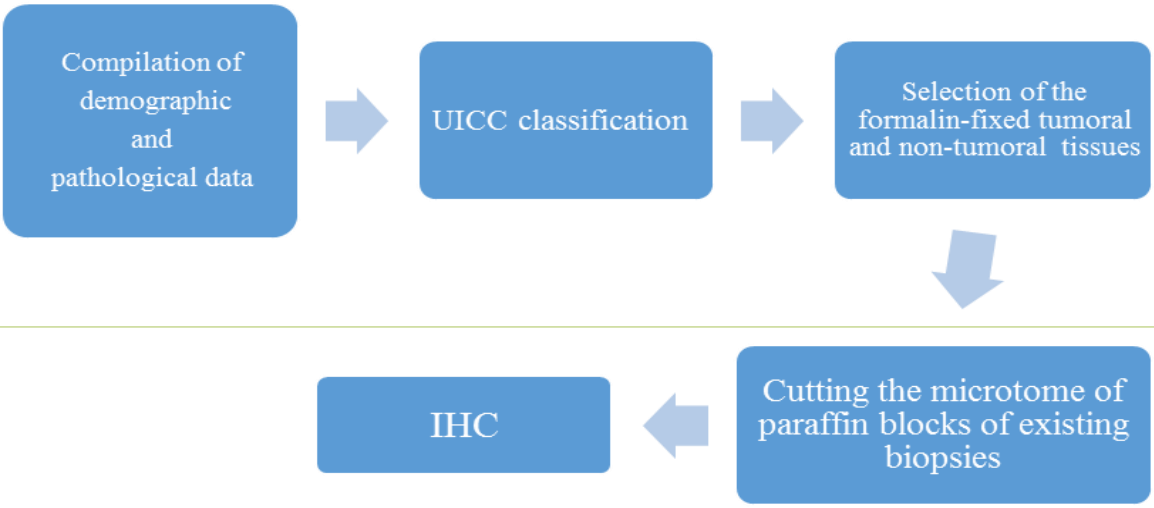
) based on UICC classification were collected. The objectives of the study were explained to the subjects and the informed consents were obtained. The formalin-fixed tumor and non-tumor tissues were selected. In the next step, paraffin blocks of existing biopsies, after cutting by microtome, were investigated by immunohistochemistry (IHC) in terms of the expression of S100A12 protein.-

**Immunohistochemistry technique:**

The immunohistochemistry was performed in conformity with previous standard methods [24]. In sum, the deparaffinized tissue sections were treated with 3% H2O2 and underwent antigen retrieval by citric acid (PH = 6). It was then treated overnight at a temperature of 4 °C with a primary antibody (anti-S100a12, produced by ABCAM Company and obtained from Arjans Co.). In the next step, tissue sections were treated at room temperature for 15 min by conjugated secondary antibody with horseradish peroxidase. Then they were treated with diaminobenzidine for 1 min. Finally, the sections were stained by hematoxylin and the tissue sections that had not treated with the primary antibody were considered as the control group. -

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**Immunohistochemical staining analysis**

The results of immunohistochemical staining for S100A12 protein were analyzed in all sections. In this study, S100A12 appeared in cytoplasm of tumor cells with varying color intensities. Therefore, the expression information of S100A12 in tumor cells, which was graded by color intensity, was analyzed according to the four-row grading system [0 = absent, 1 = poor, 2 = medium, and 3 = strong staining intensity]. Since S100A12 protein in stem cells is found in the nucleus and cytoplasm, the staining cells were brown and calculated independently. Hence, the number of cells stained in the stem cells was analyzed as immunohistochemistry score (IHC) and then divided into three groups based on the percentage of positive cells: group 0 (<10%), group +1 (10-25%); group +2 (> 50%). For further analysis, the expression levels of S100A12 were spilt into two groups: negative group (score <1 or +1) and positive group (score >1 or +1).

**Data analysis**

Following the experiments, all data were collected and entered into SPSS V16 software for analysis. Chi square tests with 95% confidence intervals were used to analyze the relationship between S100A12 protein and pathological features.

**Results:**

The study population comprised of GC patients referring to Besat Hospital in Hamadan, of whom 79 were included in this study. The formalin-fixed tumor tissues of these subjects were used for immunohistochemistry to determine the expression in in glandular and stromal cells separately

After performing immunohistochemical stages and observing the resultant samples, the outcomes of S100A12 protein were analyzed in all sections by an optical microscope with magnification ×200. In this study, S100A12 protein was observed in cytoplasm of tumor cells, which appeared with varying color intensities in different samples of patients, suggesting that its expression was different in individuals.

Further, to determine whether there is any significant difference between the expression of this protein in tumor and non-tumor tissues, 50 pairs of tumor and non-tumor tissue samples of mucosal epithelial of gastric tissue were randomly analyzed. According to the results, there was a significant difference in the expression of the protein between tumor and non-tumor tissues, in a way that tumor cells exhibited lower expression than non-tumor cells [48.5% in tumor cells compared to 87.7% in non-tumor cells at a significant level of ($P < 0.001$) (Fig. 1).

In this paper, the link between the expression of S100A12 cytoplasmic protein expression in glandular and stromal tumor cells and clinicopathological features of the samples was analyzed. The results are presented in Table 1 and 2. In the present study, out of 79 samples, 55 (69.6%) showed negative expression of S100A12 protein in glandular cells, whereas 24 (30.4%) exhibited the positive expression of this protein in the same cells The findings suggested a significant relationship between the expression of S100A12 in glandular cells and the degree of tumor differentiation, meaning that cells with a weaker degree of differentiation exhibited a lower protein expression ($P < 0.001$). It should be noted that cells with a weaker degree of differentiation are in more advanced stages of the disease. Moreover, there was a significant difference between the size of the tumor ($P < 0.02$) and TNM stage ($P < 0.004$), but S100A12 protein and other clinicopathological features such as age, sex and metastasis to lymph nodes were not significantly correlated ($P > 0.05$) (Table 1).

In this study, out of 79 samples, 16 (20.3%) displayed a negative expression of S100A12 protein in stromal cells of tumor samples while 63 (79.7%) showed a positive expression of this protein in the same cells

However, the results of statistical analysis revealed that the expression of S100A12 protein in stromal cells of tissue samples and clinicopathological features of participants such as age, sex, tumor size,

-depth of tumor invasion, metastasis to lymph nodes, TNM stage, and degree of cell differentiation were not significantly correlated (P> 0.05) (Table 2). Comparing the expression of S100A12 protein in glandular and stromal tissue cells in patients with gastric cancer did not suggest a significant relationship between the expression of such protein in these cells (p = 0.306) (Table 3).

Table 1: Relationship between expression of S100 A12 protein and glandular tissue cells in GC patients

| | | | S100A12 expression in Glandular tumor cells | | |
|---|---|---|---|---|---|
| Clinicopathological features | | All cases | Negative | Positive | p-Value |
| Gender | Male | 58 | 38[65.5%] | 20[34.5%] | 0.270 |
| | Female | 21 | 17[81.0%] | 4[19.0%] | |
| Age at diagnosis [years] | 57> | 57 | 49[85.9%] | 6[4.03%] | 0.777 |
| | 57≤ | 22 | 21[95.45%] | 1[4.5%] | |
| Size [cm] | 4.5> | 47 | 29[61.7%] | 50[38.29%] | 0.485 |
| | 4.5≤ | 32 | 23[71.87%] | 56[28.12%] | |
| Differentiation | Well | 32 | 13[40.7%] | 19[59.3%] | 0.001> |
| | Moderately | 23 | 17[73.9%] | 6[26.1%] | |
| | Poorly and undifferentiated | 22 | 22[100%] | 0[0.0%] | |
| Depth of invasion | T1 | 7 | 3[42.9%] | 4[57.1%] | |
| | T2 | 6 | 5[83.3%] | 1[16.7%] | |
| | T3/T4 | 66 | 47[74.2%] | 28[25.8%] | |
| Nodal metastasis | N0 | 25 | 15[60.0%] | 10[40.0%] | |
| | N1/N2/N3 | 54 | 38[70.3%] | 16[29.7%] | |

| | | | S100A12 expression in stromal tumor cells | | |
|---|---|---|---|---|---|
| Clinicopathological features | | All cases | Negative | Positive | p-Value |
| Gender | Male | 58 | 14[24.1%] | 44[75.9%] | 0.479 |
| | Female | 21 | 2[9.5%] | 19[90.5%] | |
| Age at diagnosis [years] | 57> | 57 | 15[26.31%] | 42[73.68%] | 0.742 |
| | 57≤ | 22 | 2[9.09%] | 20[90.90%] | |
| Size [cm] | 4.5> | 47 | 7[14.89%] | 40[85.10%] | 0.532 |
| | 4.5≤ | 32 | 9[28.12%] | 23[71.87%] | |
| Differentiation | Well | 32 | 5[18.5%] | 27[81.5%] | 0.095 |
| | Moderately | 23 | 5[21.7%] | 18[78.3%] | |
| | Poorly and undifferentiated | 22 | 4[18.2%] | 18[81.8%] | |
| Depth of invasion | T1 | 7 | 0[0.0%] | 7[100%] | |
| | T2 | 6 | 1[16.7%] | 5[83.3%] | |
| | T3/T4 | 66 | 11[16.6%] | 55[83.4%] | |
| Nodal metastasis | N0 | 25 | 4[16.0%] | 21[84.0%] | |
| | N1/N2/N3 | 54 | 10[18.5%] | 44[81.5%] | |

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Table 3: Relationship between expression of S100A12 protein in stromal and glandular tissue cells in subjects

| | | | glandular expression | | Total |
|---|---|---|---|---|---|
| | | | 0 | 1 | |
| stromal expres-sion | 0 | Count | 13 | 3 | 16 |
| | | % within stromal expression | 81.2% | 18.8% | 100.0% |
| | 1 | Count | 24 | 12 | 36 |
| | | % within stromal expression | 66.7% | 33.3% | 100.0% |
| | 2 | Count | 11 | 8 | 19 |
| | | % within stromal expression | 57.9% | 42.1% | 100.0% |
| | 3 | Count | 7 | 1 | 8 |
| | | % within stromal expression | 87.5% | 12.5% | 100.0% |
| Total | | Count | 55 | 24 | 79 |
| | | % within stromal expression | 69.6% | 30.4% | 100.0% |

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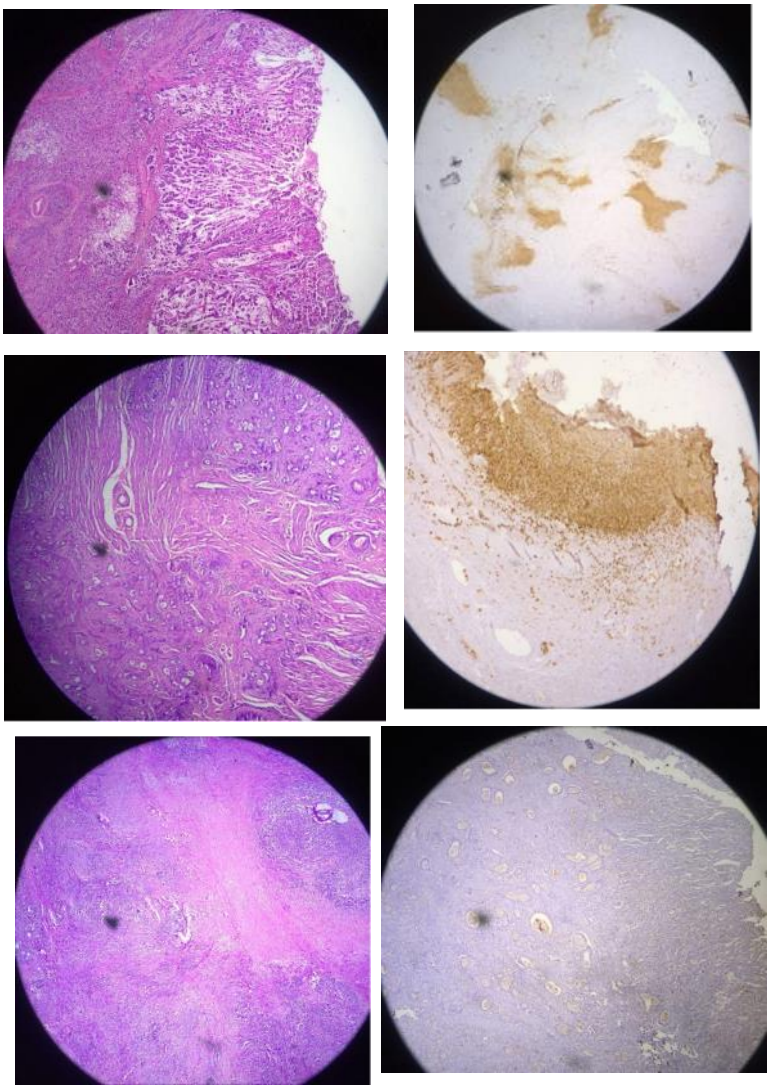
**Figure**: Immunohistochemical staining of S100A12 protein in cancer tissue samples in patients with GC



*brown: [IHC staining]

*Purple: [H & E staining] [×200]

**Discussion:**

Several proteins belonging to the group of calcium -binding proteins (i.e. S100) have been identified so far. The expression level of these proteins in relation to tumor progression has received growing scholarly attention. In this regard, the relationship between various S100 proteins and gastric cancer (GC) has been investigated in various research. Among these proteins, some subunits have been used as biomarkers in GC. Zhang reported that serum levels of S100A6 in GC patients were remarkably higher than that of healthy subjects (P <0.001), which could be used as a diagnostic biomarker in gastric cancer. S100A6 inhibition restricts metastatic ability of cells containing S100A6 [25, 22]. Conversely, a meta-analysis has shown that an increase in the expression of S100A4 may lead to poor prognosis in Asian patients with GC, but this protein could be a useful marker for assessing the progression and diagnosis of patients with GC [4].The Kaplan-Meier analysis showed a lower survival rate for patients with moderate or poor S100A4 expression than those without S100A4 expression (P <0.001). There was also a correlation between depth of tumor invasion, metastasis to lymph nodes, TNM, and S100A4 expression, with these factors acting as a criterion for poor prognosis of patients (P <0.05). Increased expression of S100A4 is directly related to pathogenesis, growth, invasion, metastasis and differentiation of gastric carcinoma. Therefore, S100A4 can be an indication of aggressive behavior and progression of gastric carcinoma. Recent studies have shown that lower expression of S100A12 is associated with the deterioration of gastric cancer. In addition, S100A12 has lower expression in GC tissue samples compared to non-cancerous gastric tissues [26].

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In our study, the analysis of S100A12 protein expression in the cytoplasm of tissue samples in patients was performed by immunohistochemistry (IHC) technique. This technique has already been used for S100A14 and S100A16 proteins in breast cancer as a way of detecting protein expression [19]. The relationship between S100A12 expression in glandular and stromal tumor cell samples of 79 patients with GC and their clinicopathological features were analyzed. 69.6% of patients had negative expression of S100A12 protein in glandular cells, while 30.4% had positive expression of this protein in the same cells. In addition, the results showed there was a significant difference between tumor and non-tumor tissues in the expression of the target protein, so that tumor cells exhibited lower expression than non-tumor cells. The results also suggested a significant relationship between expression of S100A12 protein in glandular cells and degree of tumor differentiation. Moreover, there was a significant relationship between tumor size ($P < 0.02$) and TNM stage ($P < 0.004$), but S100A12 protein and other clinicopathological features such as age, sex and metastasis to lymph nodes ($P > 0.05$) were not significantly correlated. Table [1-3], in agreement with the results of our study, shows that S100A12 protein can be helpful to identify GC patients with lower invasive severity and to evaluate the progression of GC tumors. Also in this study, a significant positive accumulation of S100A12 in stromal cells was observed under tumor cells. Stromal cells, which have positive S100A12 expression, often exhibit higher levels of immune activity. In these cells, immune cells with a myelogenous source have a more active presence, which indicate cancer-induced inflammationIn our research, of 79 samples, 20.3% had negative expression of S100A12 protein in stromal cells of tumor samples, while 79.7% had positive expression of this protein in the same cells. The results of statistical analysis did not show any significant correlation between the expression of S100A12 protein in stromal cells of tissue samples and the clinicopathological features of patients such as age,

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sex, tumor size, tumor invasion depth and metastasis to lymph nodes, TNM stage and degree of cell differentiation (P> 0.05). The results of our study are consistent with those reported by Dan Li et al., 2016. In this study, which explored the expression of S100A12 protein in 207 patients with gastric cancer in China using immunohistochemistry, it was found that 64.25% of glandular tumor cells had positive protein expression, and in the case of stromal cells, the expression level of this protein was 48.86%. Therefore, in GC, the protein expression of S100A12 increased compared to non-cancerous tissues (p <0.05). The expression of S100A12 was inversely correlated with tumor size (p = 0.004), invasion depth (p = 0.022), TNM stage (p = 0.018) and cell differentiation (p <0.000). Survival analysis indicated that S100A12 protein was a factor affecting the prognosis of GC [22]. The researchers exhibited that serum levels of S100A6, a member of the S100 family, were significantly associated with the TNM stage, metastases of lymph nodes and depth of invasion, and it may be considered as a diagnostic biomarker in GC [3].In the study of Ji Liu in 2008 [5], the modified expression of the S100 family genes in normal and malignant stomach tissues was compared. Among genes studied, S100A2, S100A4, S100A7 and S100A3 genes had higher expression in gastric cancer. There was a 2.5-fold increase in the expression of S100A3 compared to non-tumor tissues and its expression was correlated with tumor differentiation and TNM stage, which is not in line with the results of the present research. The findings reported by Fan in 2012 [27] are also contradictory with our results.

**Conclusion**: Our results suggested that low expression of S100A12 protein could be used as a biomarker for diagnosis of gastric cancer and its progression. However, further research is needed to shed more light on its mechanism.

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