**Aim of the study:** Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the gastrointestinal system. We aimed to determine whether nuclear transcription factor κB (NF-κB), CD9 and vascular endothelial growth factor (VEGF) have prognostic value in patients with GIST.

**Material and methods:** Thirty-five patients with GIST, who were diagnosed in the Pathology Department of Erciyes University, were included in the study. Cases were classified based on the 2002 NIH consensus. CD9, VEGF, and NF-κB immunohistochemistry were applied to GIST cases positive for CD117 and CD34, which are used to evaluate GISTs immunohistochemically.

**Results:** Although there are no statistically significant differences between NF-κB ($p = 0.329$), CD9 ($p = 0.269$), and VEGF ($p = 0.372$) and risk groups, 79.22% of cases that stained positive for NF-κB, 81% of cases that stained positive for CD9, and 80% of cases that stained positive of VEGF were in the high risk group.

**Conclusions:** It was found that NF-κB, CD9, and VEGF, which are important in predicting behaviors of other malign tumors, were expressed at high rates in high risk group GISTs. This can be used to determine prognosis with tumor diameter, mitosis rate under 50 BBS, Ki-67 proliferation index and other parameters.

**Key words:** CD9, gastrointestinal stromal tumor, NF-κB, VEGF.

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**Prognostic value of NF-κB, CD9, and VEGF in gastrointestinal stromal tumors**

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**Introduction**

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract (GIT) [1–5]. GISTs originate from interstitial cells of Cajal (ICH) that are normally located in the gastrointestinal tract and that act as an intestinal „pacemaker“ [1, 6–8]. Gastrointestinal stromal tumors are rare tumors, with an estimated incidence of 1.5/100 000/year [9]. They appear everywhere from the esophagus to the rectum, but most commonly they occur in the stomach and small intestine [10, 11]. 60–70% occur in the stomach, 25–35% in the small intestine, 5% in the colon, rectum or appendix, and 2–3% in the esophagus [1, 11, 12]. Gastrointestinal stromal tumors are typically seen in adults, especially over 40 years of age.

In terms of biological behavior, they exhibit a broad spectrum ranging from benign tumors to malignant tumors. When clinical behavior and prognostic parameters cannot be put as net, risk staging can be tried to prescribe by localization of the tumor, size, mitotic rate, and cellularity, showing infiltrative growth, necrosis, hemorrhage, and a lot of other determinants that increase in number every day in the literature [6].

The vast majority of GISTs have oncogenic proto-oncogenic tyrosine kinase receptor (KIT) mutation [1, 3]. KIT and platelet derived growth factor receptor α (PDGFR-α) genes are located on the 4th chromosome in people.

On Immunohistochemical studies that were performed on GISTs, CD117 is detected in 95–100%, CD34 is observed in 70–80%, smooth muscle actin is observed in 20–40%, desmin is observed in 1–2%, and S-100 is observed in 5% [4, 7, 13].

Nuclear transcription factor κB (NF-κB) is a dimeric transcription factor. REL includes family members such as REL-A (p65), c-REL, REL-B and p52. All NF-κB proteins include a very intense level of REL-homologous effect [DNA binding, dimerization, nuclear translocation and interaction with proteins 1kB] [14].

Angiogenesis is the process of formation of a new blood vessel from the existing vascular network. Angiogenesis plays an essential role in tumor growth and metastasis [15–18].

An increase in the density of vascular endothelial growth factor (VEGF) can be combined with many different types of carcinomas. It is found that if an increase in vascular density in the tumor is accompanied by immunohistochemical staining of VEGF, GIST will show poor prognosis [19].

CD9 is a member of the family of tetraspanin and shows a membrane-dependent effect. In the literature, it was found that CD9 is effective in cell motility and capacity of metastasis of tumor cells. For many cancer varieties such as breast, lung and colon, it was found that lower of expression of CD9
worsens the prognosis [20, 21], and with higher expression by increasing proliferation in gastric carcinoma, tumors behave more aggressively [22–24].

The aim of this study was reconsideration of patients diagnosed with GIST, analysis of prognostic factors and determination of whether there is prognostic significance of NF-κB, CD9 and VEGF.

Material and methods

GIST originated mesenchymal tumor cases that were recognized in the Department of Pathology, Erciyes University Faculty of Medicine were analyzed retrospective-ly. Of these, 35 cases of GIST were included in the study. Fifteen cases (42.9%) were in the stomach, 12 (34.2%) in the small intestine, 7 (20%) in the large intestine, and 1 (2.9%) in the esophagus.

The best exemplifies the tumor slights were selected and re-evaluated under the light microscope. The cases were examined in terms of mitotic count in 40 BBS, 50 sites and tumor size. In order to determine the biological behavior of the tumor, risk was divided into 4 group as very low, low, intermediate and high on the basis of the 2002 National Institutes of Health (NIH) consensus risk categorization that is accepted all over the world and performed on the basis of tumor size and mitotic counts (Table 1) [13]. The number of patients according to risk categories was 2 (5.7%) in the very low risk group, 4 (11.4%) in the low risk group, 5 (8.6%) in the intermediate risk group, and 26 (74.3%) in the high risk group. The majority of the 35 cases were in the high risk group. Therefore, the cases with high risk were accepted as the high risk group where-as the other 3 groups (very low, low, and intermediate) were considered as the low risk group. So, there were 26 patients in the high risk group and 9 patients in the low risk group.

Immunohistochemical staining was examined by use of avidin-biotin-peroxidase. CD9, VEGF, and NF-κB immunohistochemistry were applied to GIST cases positive for CD117 and CD34, which are used to evaluate GISTs immunohistochemically.

For CD117, staining of ICHs was used as controls and diffuse cytoplasmic staining was accepted as positive for 50% and more for tumor cells for CD117 and CD34, which are used to evaluate GISTs immunohistochemically.

For CD117, staining of ICHs was used as controls and diffuse cytoplasmic staining and punctate staining accompanied by diffuse cytoplasmic staining were accepted as positive. For CD34, staining of the vessel was used as controls and diffuse cytoplasmic staining was accepted as positive.

Table 1. National Institute of Health (NIH, United States) consensus risk scheme for GISTs

| Risk     | Size      | Mitotic count (per 50 HPF) |
|----------|-----------|---------------------------|
| very low | < 2 cm    | < 5/50                    |
| low      | 2–5 cm    | < 5/50                    |
| intermediate | < 5 cm | 6–10/50                  |
|           | 5–10 cm   | < 5/50                    |
| high     | > 5 cm    | > 5/50                    |
|           | > 10 cm   | any                       |
|           | any       | > 10/50                   |

HPF – high power field

The cytoplasmic staining was accepted as positive for more than 15% of tumor cells in the area that the staining was most intense for VEGF, and staining was accepted as negative for 15% and under [25]. Cytoplasmic staining was accepted as positive for 50% and more for tumor cells for CD9, and others were accepted as negative [23]. For NF-κB, 5 different areas that have the cytoplasmic staining in the tumor tissue were examined and the areas with less than 10% for cytoplasmic staining were evaluated as negative, while the areas with more than 10% for cytoplasmic staining were evaluated as positive [14].

High risk groups in the risk groups were evaluated in a separate group and very low risk, low risk, and intermediate risk cases were evaluated as low risk cases (Fig. 1).

For evaluation of tumor characteristics and their relationship with the results of each antibody used, the chi-square test was used. Statistical significance was considered as p < 0.05.

Results

For this study 35 cases of GIST were taken. Twenty-one patients (60%) were male and 14 (40%) were female. The youngest patient included in the study was 31, and the oldest patient was 82 years old; mean age was 59.9.

The largest tumor had a diameter of 43 cm and was located in the stomach, while the smallest tumor had a diameter of 0.7 cm and was located in the colon. Both the largest and the smallest diameter of tumors occurred in males. For 4 patients in the high risk group, liver metastases were found; also recurrence in the small intestine was found in a patient with liver metastases (Fig. 2), and another metastasis was found in the omental. For other patients, recurrence of small intestine and metastasis in omentum was detected. For male patients with localized high-risk rectum, recurrence was observed at the same locations. The highest rate of mitosis was 100/50 BBS, and it belonged to mixed cell type, small bowel tumors, 5.5 cm in size GIST cases. The lowest rate of mitosis was 0, and 3 of them were in low and lowest risk groups, 2 of them occurred in 5 cases in the intermediate group; and intermediate group cases were located as 6 cm in the small intestine and 7 cm in the stomach.

Positive stained number of cases was 24/35 for NF-κB, 21/35 for CD9, and 19/35 for VEGF.

Table 2 shows the comparison of NF-κB-positive cases (Fig. 3A) and NF-κB-negative cases in terms of risk category. Although there is no statistically significant difference, the percentage of high-risk group cases was higher (73.1%) in NF-κB-positive cases than in NF-κB-negative cases (63.6%).

Table 3 shows the comparison of CD9-positive cases (Fig. 3B) and CD9-negative cases in terms of risk category. Although there was no statistically significant difference, the percentage of high-risk group cases was higher (81.0%) in CD9-positive cases than in CD9-negative cases (64.3%).

Table 4 shows the comparison of VEGF-positive cases (Fig. 3C) and VEGF-negative cases in terms of risk category. Although there was no statistically significant difference, the percentage of high-risk group cases was higher.
Prognostic value of NF-κB, CD9, and VEGF in gastrointestinal stromal tumors

Discussion

GISTs can develop throughout the entire gastrointestinal tract from the esophagus to the anus and from the omentum to the mesentery and retroperitoneum.

GISTs are typically observed in adults aged 55–60 [7, 11]. Similarly, in this study mean age is 59.9 years. In the studies in general, while male and female gender have equal incidence [21], there are some series that show male dominance as 55% [13, 24, 26]. In this study, the male/female ratio is 21/14 (60% male, 40% female).

GISTs most commonly occur in the stomach and small intestine [10,11]. In this study, more cases were located in the stomach and small intestine in accordance with the literature (stomach localization is 43% (15/35), for small bowel it is 34% (12/35)). Occurrence in the esophagus has been reported in the literature as under 5% [1, 11, 12].

There is only one patient with esophagus localization in this series; this rate is consistent with the literature. There is no case located outside of the primary GIS area. There is omental metastasis in 2 small intestine GIST cases.

A preoperative biopsy is not generally recommended for operable tumors in which the radiologic studies have already diagnosed a GIST [27].

GISTs can be seen synchronously or metachronously with other epithelial cancers. Different genetic pathways in the tumorigenesis of two different neoplasms is suggested as a probable cause. However, the number of cases is limited [28].

C-kit expression studies were performed in 1998–1999 [29, 30]. GISTs form a specific tumor group because they include KIT tyrosine kinase receptors as different from other gastrointestinal tumors. These tumors express c-kit named a cell membrane receptor by result of exon mutation. Activation of this receptor causes uncontrolled cell proliferation and the development of resistance to apopto-

Table 2. Comparison of NF-κB-positive and NF-κB-negative cases

|          | NF-κB-positive, n (%) | NF-κB-negative, n (%) |
|----------|-----------------------|-----------------------|
| Low risk | 5 (20.8)              | 4 (36.4)              |
| High risk| 19 (73.1)             | 7 (63.6)              |
| Total    | 24 (100.0)            | 11 (100.0)            |

χ²: 0.952; p = 0.329

Table 3. Comparison of CD9-positive and CD9-negative cases

|          | CD9-positive, n (%) | CD9-negative, n (%) |
|----------|---------------------|---------------------|
| Low risk | 4 (19.0)            | 5 (35.7)            |
| High risk| 17 (81.0)           | 9 (64.3)            |
| Total    | 21 (100.0)          | 14 (100.0)          |

χ²: 1.222; p = 0.269
In recent years, for GISTs’ differential diagnosis, an immunohistochemical panel that also includes CD117 is applied, additionally to clinical and histological findings. This panel is useful to separate ICH originated tumors from right tissue originated tumors and from other pathologies in the differential diagnosis [13, 19].

The clinical, histopathological and immunohistochemical panel are used for diagnosis, classification, and prognosis of GISTs. They are evaluated immunohistochemically by vimentin, smooth muscle actin, desmin, S-100, CD34, CD117, and Ki-67. CD117 positivity is observed in GISTs in 95–100%, CD34 in 70–80%, SMA in 20–40%, desmin in 1–2%, and S-100 in 5% [13, 19]. Ki-67 that was added to the immune panel is a predictor of proliferation and used for determining prognosis. Wong et al. and Carrillo et al. showed that Ki-67 is an independent prognostic factor, and high Ki-67 staining is associated with poor prognosis, but the mitotic index does not give a better result from the indicated prognosis [31,32].

Suster et al. [33] studied determination of prognosis by using cellularity and a type parameters. In 2002, Fletcher et al.’s GIST study group [34], who contributed to the National Institutes of Health (NIH), determined diagnostic criteria for malignant behavior for GIST by using tumor size and mitotic activity. As to the NIH consensus approach, GISTs were separated into 4 groups as very low risk, low risk, intermediate risk and high risk [24]. 74.3% (26/35) of the cases in this study are in the high risk group.

An increase in the density of VEGF can occur in a wide variety of malignancies. The expression can be seen in lung, thyroid, breast, stomach, small intestine, colon, bladder and ovarian tumors. The tumors that show VEGF expression in breast cancer have a poor prognosis and are associated with early relapse. Likewise, for patients with gastric cancer, VEGF positivity is associated with vascular invasion, lymph node and liver metastases; it is an indicator of poor prognosis [35].

In a study in the literature, it was found that VEGF immunohistochemistry was applied for 53 GIST cases that were located in the stomach; in 26.4% (14/53) of cases VEGF expression was found. In these cases, tumor expression of VEGF was found to be correlated with liver metastasis, Ki-67 index and microvessel density.

For the tumors that show VEGF expression, prognosis was found to be worse than the cases that do not. In GISTs, an increase in vascular density in the tumor is associated with poor prognosis when it accompanied by immunohistochemical staining of VEGF [19]. In the literature, when VEGF expression in the microvessel density, and increasing is associated with poor prognosis and aggressive behavior [25]; in another study, it was found that the positive staining rate in GISTs is 78.8% and for the high risk group and high mitosis GIST, the VEGF cytoplasmic dying is increased [36]. In this study, 80% (16/20) of the cases that were dyed with VEGF positively are in the high risk group, and this is

![Fig. 3. Positive immunohistochemical staining with NF-κB (A), CD9 (B), and VEGF (C) (magnification 20×)](image)

Table 4. Comparison of VEGF-positive and VEGF-negative cases

|            | VEGF-positive, n (%) | VEGF-negative, n (%) |
|------------|----------------------|----------------------|
| Low risk   | 4 (20.0)             | 5 (33.3)             |
| High risk  | 16 (80.0)            | 10 (66.7)            |
| Total      | 20 (100.0)           | 15 (100.0)           |

$\chi^2$: 0.798; $p = 0.372$
consistent with the literature. But, because 66.7% (10/15) of VEGF negative cases are in the high risk group, it was not found statistically significant.

In cell motility, CD9 is effective in migration-adhesion function of cells and metastatic capacity of tumor cells; and it is a member of the tetraspanin family. It shows a membrane-dependent effect. It is expressed on a variety of hematopoietic and nonhematopoietic cells such as CD9 B-cell precursors and platelets. It has been reported in the literature publications that the loss of expression of CD9 in breast, lung, and colon cancer worsens the prognosis [20, 21]. And it was reported that it increases proliferation in gastric carcinoma, and that tumors behave more aggressively and exhibit lymph node metastasis, peritoneal dissemination, vascular invasion, and advanced stage, when it is more expressed [22, 23]. In this study, while 81% (17/21) of the tumors that show CD9 expression were in the high risk group, 19% (4/21) of them were in the low risk group. CD9 expression was seen in 3 of 4 cases that show liver metastasis (75%). In one of these cases, there was also omentum metastasis.

NF-κB is a dimeric transcription factor. It supplies DNA binding, nuclear translocation and interaction with signal-dependent phosphorylation target κB proteins. It includes REL family members such as REL-A (p65), c-REL, REL-B, p50, and p52 [14, 37]. Agents that damage DNA such as NF-κB topoisomerase inhibitors are activated in cancer cells by gamma radiotherapy [34]. It also plays an important role in inflammatory and immune responses [36]. In this study, while 79.2% (19/24) of the cases that show NF-κB expression are in the high risk group, 20.8% (5/24) of them are in the low risk group. In 2 of 4 cases with liver metastases and in a case that showed recurrence in the small intestine with omental metastasis, strong NF-κB expression was seen.

After radical surgical operations the recurrence rate is 60–70%. Most of the recurrences for GISTs are in the intra-abdominal region. Before the operation or after the operation, rupture of the abdominal cavity is associated with a high risk of recurrence. After complete resection of the localized primary tumor, 5-year life expectancy is in the range 50–65% [38]. However, 40–90% of surgical patients experience postoperative recurrence or metastasis. In patients with metastatic or locally recurrent GIST, the average life expectancy was about 10–20 months before treatment with imatinib [39].

For GISTs that do not have metastases, surgery is the standard treatment. It must be removed en bloc or with an adequate margin of resection with the tumor pseudo-capsule. For GISTs, because lymph node metastasis is very rare, radical lymphadenectomy is not recommended. Peritoneal tumor rupture is associated with an increased risk for the development of the implant [13]. Macroscopic extra-abdominal metastases are rare even in advanced disease. The most common metastases are seen in the mesentery and liver [19]. Standard therapy for metastatic GIST is imatinib. For 65–70% of patients, a partial response is obtained after an average of 12–15 weeks. For imatinib-resistant patients, sunitinib is used as a multiple tyrosine kinase inhibitor. In this study that examined 35 GIST cases, the multiple GIST case that 19 items nodule structure were in the high risk group had applied with the omental metastasis 6 months after the diagnosis, and with the liver metastasis 5 months after this. Inoperable liver metastasis was seen in the high-risk group, and bone marrow metastasis was seen in another female patient with high plasma. A female patient had a 10 cm diameter small intestine located liver metastasis in the diagnosis applied after 9 months with omental metastasis and recurrence in the small intestine. Patients who the imatinib therapy was initiated applied with recurrence in small intestine and liver metastases after break of 3 months because of adverse events of the treatment. Two cases with diagnosis of GIST were lost due to illness. Low and very low risk patients are on follow-up without treatment.

As the result, the correct diagnosis in GISTs, classification, the morphological and immunohistochemical studies for prognosis and treatment are important. To determine the clinical behavior and prognostic parameters, it is tried to prescribe by many other determinants that have a growing number of literature every day. In this study NF-κB, CD9 and VEGF that were found important for predicting the behavior of many other malignant lesion were evaluated as to whether they are prognostic indicators also in GIST.

It was observed that all three markers are expressed in high rates in the high risk group of GIST. For determining the prognosis of NFκB, CD9 and VEGF it can be used with tumor size, mitotic rate in 50BBS, Ki-67 proliferation index and other parameters.

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