International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2019; 2(2): 85-89
Received: 09-05-2019
Accepted: 14-06-2019

Vicky S Budipramana
Digestive Surgery Division of Surgery Departement, Dr. Soetomo Hospital Surabaya, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia

Syahfreadi
Digestive Surgery Division of Surgery Departement, Dr. Soetomo Hospital Surabaya, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia

Dyah Fauziah
Departement of Patology Anatomi, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia

Correspondence
Vicky S Budipramana
Digestive Surgery Division of Surgery Departement, Dr. Soetomo Hospital Surabaya, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia

Correlation between GIST positive CD117 with EGFR expression based on tumor size and mitotic index

Vicky S Budipramana, Syahfreadi and Dyah Fauziah

DOI: https://doi.org/10.33545/pathol.2019.v2.i2b.83

Abstract
Background: Gastrointestinal Stromal Tumors (GIST) are mesenchymal malignancies in GI tract. GIST resistant to imatinib therapy are widely reported. Epidermal Growth Factor Receptor (EGFR) plays a role in progressivity of malignancy. This study aimed at analyzing the expression of EGFR on GIST that leads to the probability of using Anti-EGFR as a substitutive therapy for Imatinib-resistance GIST.
Methods: This study performed in Dr. Soetomo General Hospital in CD-117 positive GIST from 2009 to 2014. The number of the samples were 39, samples were measured using semi quantitative method, and analyzed using *Spearman formula.*
Results: CD-117 positive GIST showed EGFR expression in 74.4% samples, Correlation between EGFR expression with the tumor size (p=0.129) and mitotic index (p=0.626).
Conclusion: CD-117 positive GIST showed EGFR expression in 74.4% samples, though there was no significant correlation between EGFR expression with tumor size (p=0.129) and mitotic index (p=0.626).

Keywords: GIST, EGFR, tumor size, mitotic index

1. Introduction
Gastrointestinal stromal tumors (GIST) are one of the most common mesenchymal malignancies which occur along the muscle wall of the digestive tract with approximately 2% of prevalence, and the highest frequency in the gaster (60%) and small intestine (25-30%) [1-4]. These tumors usually occur to patients at the age, ranging from 60 to 70, without gender difference [4, 5].
Histologically, it is thought that GIST is originated from interstitial cells of Cajal, and it has various histological appearances which lead to the difficulty in determining the diagnosis and the prognosis [6]. NIH divides GIST into 4 categories: very low risk, low risk, intermediate risk, and high risk, according to tumor size and mitotic index [3, 7]. However, the distribution of these categories is still unable to predict the nature and malignant potential of GIST. "Modified grading system" includes the location of the tumor as a risk factor, where gastric-GIST aggressiveness tends to be lower than the intestinal-GIST. The difficulty in determining the grading of GIST, especially when the patient with a smaller size of tumor coming earlier for medical treatment, it requires an additional prognostic marker for predicting the grading [8].
Surgery is the main treatment of GIST for small size resectable tumors. However, 50% of post-operated patients were experiencing a recurrence or metastasis. Presumably, although the small size tumor is graded as low risk GIST, it is also important to know the mitotic index of the tumor as the prognostic factor [9]. One of the therapies is imatinib mesylate, which has become a major challenge in the treatment of GIST molecular targets. Acquired secondary mutation of KIT occurs in up to 90% of the cases and it is related to the mechanism of resistance to imatinib therapy. Primary resistance to imatinib therapy is 15% of the cases and 50% of the initially responders become resistance within 2 years after being treated with imatinib [11]. KIT mutations in other exons, the genes which encode other pathways such as RAF RTK/RAS, the MAPK-ERK, PI3K/AKT and mTOR is suspected as the cause of the resistance [12-15]. PDGFR-α amplification is the other mechanism of resistance to imatinib therapy, although the role of this mechanism is rare [15]. The high number of imatinib resistance causes an important consideration for alternative therapy other

than imatinib therapy, one of which is the use of broad-spectrum inhibitor, anti-EGFR therapy [16].

Epidermal growth factor receptor (EGFR/Her1/erbB1) is one of tyrosine kinases receptor that plays a role in the proliferation, survival, adhesion, migration and cell differentiation. EGFR works by activating pathways such as the RAS/RAF/MAPK, JAK/STAT, PI3K/Akt, and PLC/Protein Kinase C. When these pathways are activated, they always force the cells to enter the cell cycle, which is characterized by high mitotic index [17]. Signalling of EGFR also causes tumor cells to undergo further development through several mechanisms such as increasing tumor cell proliferation, decreasing apoptosis, and enhancing tumor cell motility and neo-angiogenesis. EGFR is expressed in a variety of tumors including non-small cell lung cancer (NSCLC), head and neck, gastric, breast, colorectal, esophageal, etc. Therefore, EGFR could be a rational target for antitumor strategies. However, there are only a few studies that have examined EGFR expression on GIST [18].

Based on the reasoning above, the researchers want to analyze the expression of EGFR in patients with GIST who were hospitalized in Dr. Soetomo Hospital during 2009-2014. This study aimed at analyzing the expression of EGFR on GIST that leads to the probability of using anti-EGFR as an alternative therapy and to see the relationship between EGFR expression with tumor size and mitotic index, hopefully it can be used as a GIST's prognostic marker.

2. Methods

2.1 Study Design

It was a comparative cross-sectional retrospective study performed to evaluate not only the correlation between CD-117 positive GIST and Epidermal Growth Factor Receptor (EGFR), but also the correlation between tumor size and mitotic index with EGFR expression in patients with CD-117 positive GIST. Primary data was obtained from patients' paraffin block from January 2009 until August 2014.

2.2 Sample Population

The target population of this study was patients diagnosed with CD-117 positive GIST at Dr. Soetomo Hospital, Surabaya. The inclusion criteria were patients with complete medical records consisting size tumor, and positive CD117 in immunohistochemistry examination from paraffin block.

2.3 Pathological Evaluation

Blocks and slides were identified from each patient diagnosed with CD-117 positive GIST based on histopathology examination in Department of Pathological Anatomy, Dr. Soetomo Hospital Surabaya. Primary data including age, location, tumor size, and mitotic index were obtained from medical records of patients who met the criteria. Paraffin blocks were cut 4 μm and were stained with CD-117 antibody in immunohistochemistry examination to confirm diagnosis of GIST. Positive results were continued with EGFR antibody staining.

2.4 Diagnostic Standard

GIST was diagnosed based on histopathology appearance on paraffin block from intraoperative or biopsy specimens, with positive results on CD117 examination. Tumor size was determined from macroscopic examination on intraoperative specimens or CT-Scan on biopsy specimens. The size was classified as three categories: (a) small = <5 cm; (b) intermediate = 5-10 cm; and (c) large = >10 cm. Mitotic index was obtained from cells measurement on histopathology examination with *Hematoxylin-Eosin* (HE) staining. Index results were classified as: (a) low = mitosis <5/50 HPF; and (b) high = mitosis >5/50 HPF.

Epidermal growth factor receptor (EGFR) was reviewed from immunohistochemistry staining using monoclonal EGFR antibody. EGFR results were interpreted using scoring system (Table 1) and assumed to be positive if recorded on membrane cell or cytoplasm.

Table 1: Scoring System for EGFR Immunohistochemistry

Score	Results
0	Not Stained
+1	Stained>10% tumor cells, low intensity, *incomplete*
+2	Stained>10% tumor cells, intermediate intensity, *complete*
+3	Stained>10% tumor cells, high intensity, *complete*

2.5 Data Analysis

The distribution and characteristics of data from each variable were assessed using univariate analysis to evaluate descriptive statistics and presented in graphs and tables.

Bivariate analysis using 2x2 table was perfomed to evaluate the correlation between EGFR expression and tumor size and mitotic index. Analysis using Spearman method was performed to measure the correlation coefficient. This study was done using SPSS 20.0 program.

3. Results

Sample characteristics in this study were reviewed according to patients' gender, age, tumor size, and mitotic index. Age was classified into 4 categories: ≤40 years, 41-50 years, 51-60 years, and >60 years. Tumor size was interpreted into 3 groups: 0-5 cm, >5-10 cm, and >10 cm. Mitotic index was measured until 50 HPF, and were classified into 0/50 HPF, 1-5/50 HPF, 6-10/50 HPF, 11-15/50 HPF, 16-20/50 HPF, 21-25/50 HPF, and>25/50 HPF.

Table 2: Subject's Characteristics

	Benign GIST	Malignant GIST	Total
Gender			
Male	7 (41.4%)	9 (40.9%)	16 (41%)
Female	10 (58.8%)	13 (59%)	23 (59%)
Age			
≤ 40 years	2 (1.2%)	2 (9.1%)	4 (10.3%)
41-50 years	4 (23.5%)	6 (27.3%)	10 25.6%)
51-60 years	7 (41.2%)	7 (31.8%)	14 (35.9%)
>60 years	4 (23.5%)	7 (31.8%)	11 (28.2%)

Tumor Size			
< 5 cm	7 (17.9%)	2 (5.1%)	9 (23.0%)
5-10 m	1 (2.6%)	5 (12.8%)	4 (15.4%)
>10 cm	9 (23.1%)	15 (38.5%)	24 (61.6%)
Mitotic Index			
0	1 (5.9%)	0 (0%)	1 (2.6%)
1-5	4 (23.5%)	1 (4.5%)	5 (12.8%)
6-10	4 (23.5%)	2 (9.1%)	4 (15.4%)
11-15	3 (17.6%)	3 (13.6%)	6 (15.4%)
16-20	1 (5.9%)	1 (4,5%)	1 (5.1%)
21-25	3 (17.8%)	10 (45.5%)	13 (33.3%)
>25	1 (5.9%)	5 (22.7%)	6 (15.4%)
EGFR Expression			
-	5 (29.4%)	5 (22.7%)	9 (2.,6%)
+1	8 (47.1%)	9 (40.9%)	17 (43.6%)
+2	4 (23.5%)	1 (4.5%)	5 (12.8%)
+3	0 (0.0%)	7 (31.8%)	7 (17.9%)

Based on these data, most of the GIST cases, either benign or malignant, were found in female 59% and in male 41%. From the total of 39 cases, it occurred to the patients ranging from 51 to 60 years old (35.9%) out of which only 31.8% of the patients were malignant. Based on mitotic index, the greatest number of mitosis is 21-25/50 HPF (33.3%), and based on the size of the tumor, the largest one is >10 cm (61.6%).

This study assessed the expression of EGFR protein in GIST tumors using semiqualitative assessment and the scores range from 0 to +3 (Figure 1).

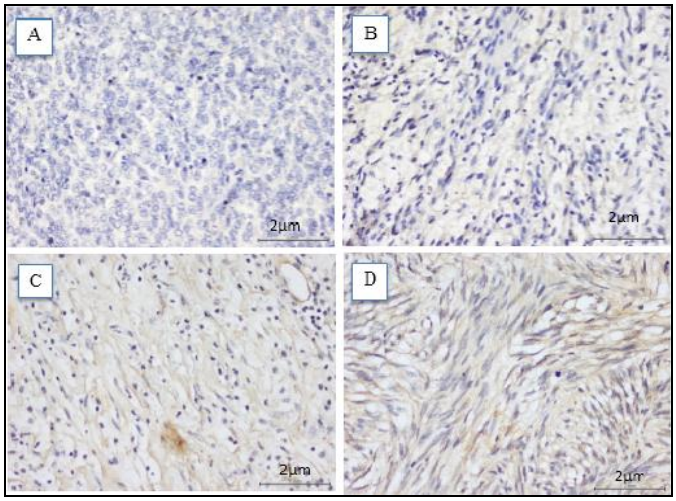

Fig 1: Expression of EGFR protein in GIST tumors. A: EGFR Expression with Negative Score. B: EGFR Expression with Score +1. C: EGFR Expression with Score +2. D: EGFR Expression with Score +3.

From EGFR antibodies staining, approximately 74.6% of total samples were expressed (stained) by EGFR antibodies. These samples were mostly stained with a score of +1 (43.6%), whereas the entire samples that are stained +3, of which a malignant GIST was found approximately 31.8%.

This study found the total samples in 5 years period (2009-2014) were 39 samples, which were divided into 17 samples that were diagnosed as benign (low risk) and 22 samples that were diagnosed as malignant (high risk) GIST (Figure 2).

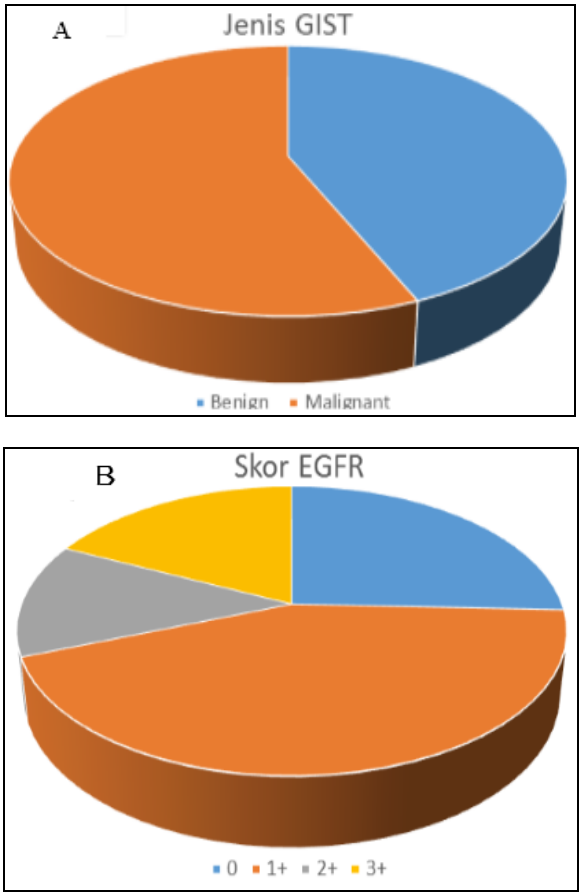

Fig 2: Distribution Based on Type of GIST (A) and Distribution Based on EGFR Score (B)

From overall sample positive stained with CD117 antibody, 10 samples (25.6%) were stained with score 0 (negative), 17 samples (43.6%) stained with score +1, 5 samples (12.8%) stained with score +2, and 7 (17.9%) samples were stained with score +3 (Figure 2).

As seen at Table 3, tumor size 5 cm mostly stained with score +1 (35.3%), tumor size 5-10 cm mostly unstained/ scored negative/0 (20%), while tumor size >10 cm mostly stained with score +1. However, we can also see that the samples which was stained with score +3 has a range of sizes >5-10 cm (57.1%) and >10 cm (42.9%).

Based on mitotic index in Table 3, most samples have mitotic index of 21-25/50 HPF (33.3%). At the mitotic index range can be seen that mostly have staining EGFR scores +3 (71.4%).

Table 3: Distribution of EGFR Score Compare to Tumor Size and Mitotic Index

	Score EGFR				Total
	(-)	(+)	(++)	(+++)	
Tumor size					
13 s/d 5 cm	0 (0%)	6 (35,3%)	3 (60%)	0 (0%)	9 (23,1%)
14 >5-10 cm	20 (20%)	0 (0%)	0 (0%)	4 (57,1%)	6 (15, 4%)
15 >10 cm	8 (80%)	11 (64,7%)	2 (40%)	3 (42,9%)	24 (61,5%)
Mitotic Index					
16 0	1 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)
17 1-5	1 (10%)	2 (11.8%)	2 (40%)	0 (0%)	5 (12.8%)
18 6-10	0 (0%)	5 (29.4%)	1 (20%)	0 (0%)	6 (15.4%)
19 11-15	1 (10%)	4 (23.5%)	0 (0%)	1 (14,3%)	6 (15.4%)
20 16-20	1 (10%)	1 (5.9%)	0 (0%)	0 (0%)	2 (5.1%)
21 21-25	4 (40%)	3 (17.6%)	1 (20%)	5 (71,4%)	13 (33.3%)
22 >25	2 (20%)	2 (11.8%)	1 (20%)	1 (14.3%)	6 (15.4%)

As seen at Table 4, there is no relationship between the expression of EGFR with mitotic index (p=0.626), between EGFR expression with tumor size (p= 0.129), and between mitotic index and tumor size (p=0.836).

Table 4: Correlation between EGFR, Tumor Size, and Mitotic Index in GIST

	EGFR Expression	Mitotic Index	Tumor Size
EGFR Expression	-	r_s=0.080 p=0.626	r_s=-0.247 p=0.129
Mitotic Index	r_s=0.080 p=0.626	-	r_s=-0.034 p=0.836
Tumor Size	r_s=-0.247 p=0.129	r_s=-0.034 p=0.836	-

4. Discussion

Over 5 years of study (2009 to 2014), total of CD117 positive GIST that was obtained are 39 samples, 17 samples of which are benign, while other 22 samples are malignant. As seen in Table 1, there are no difference between men and women who suffer GIST (men 41%, women 59%). It is appropriate to the prior studies which states that the incidence of GIST is not based on any particular gender [4, 5]. WHO also said the same thing, that the incidence of GIST almost similar among men and women (Male: Female, 1.1: 1) [6]. GIST mostly occurs in older people, the decade of the 6th to the 8th of life [6].

Histopathologically, GIST is classified into benign and malignant, based on mitotic index and tumor size. Tumor size less than 5 cm is usually benign, whereas above 10 cm of size usually malignant. Tumor mitotic index >10/10 HPF categorized as malignant. Tumors with mitotic index <5/10 HPF categorized as benign [6]. Most common GIST of this study is malignant (22 versus 17); thus, tumor size of this study was mostly >10 cm, as well as the highest mitotic index is >21/50 HPF (Table 2).

As seen above, a few of malignant GISTs have low mitotic index (1-5/50 HPF), on the other hand a few of benign GIST also have high mitotic index (21-25/50 HPF). Such cases categorized as borderline or low-grade malignancies GIST (Table 2).

Based on the results, of 74.4% samples stained with EGFR positive, 43.6% samples score +1; 12.8% samples score +2; and 17.9% samples score +3. Unstained EGFR (negative score) was found in 25.6% samples. EGFR (Her-1) is a receptor tyrosine kinase that plays an important role in the regulation and normal cell proliferation. Activation of the EGFR pathway activates KRAS/BRAF which activates the transcription factor for cell proliferation. In cancer cells, the amplification of this gene may lead to excessive cell proliferation, angiogenesis, metastasis, and decreased apoptosis. The discovery of receptor tyrosine kinase inhibitors of EGFR such as geftinib, erlotinib breakthrough new area which could be used for malignancies. Nowadays, therapy using inhibitors of EGFR has been widely prescribed in lung adenocarcinoma.

The role of EGFR in several types of malignancies, particularly sarcoma, has been widely studied, one of which is a sarcoma sinovial. However, there was less research on the role of EGFR in GIST. Cai *et al*. in research on the expression of TGF and EGFR (Her 1 and Her 2) in GIST in the stomach and intestines found that mostly GIST are expressed with TGF, but only a few are expressed with EGFR [19]. Yoo *et al*. who analyzed the EGFR gene in 60 GIST using PCR method, suggests there is no mutation in the EGFR gene. It indicates the target in GIST therapy using EGFR inhibitors cannot be used [20].

Study by Lopez *et al*. in 82 GIST showed that EGFR is expressed on the mast case of GIST, but not found in genes that encode amplification of EGFR [16]. A similar trend was found by Tornillo *et al*., which EGFR amplification in GIST are found only 5.3% [12]. Several studies that found the presence of EGFR expression without being followed by the EGFR gene amplification was research of Bode *et al*. in synovial sarcoma. The study says that even only a few EGFR amplification are detectable, it can be used as the basis for therapeutic use of EGFR inhibitors [21]. Several studies of EGFR in glioma declared an EGFR overexpression is not only caused by the alteration in the gene EGFR. Some mechanisms that cause overexpression of EGFR are: (a) EGFR gene mutation inducer; (b) Increase of EGFR translation or transcription; (c) Decrease of protein destructive; (d) Overexpression of receptor-ligand binding. Therefore, EGFR overexpression mechanism is still not understood and the possibility of anti-EGFR therapy can still considered. According to that, this study, of which EGFR is expressed in 74.4% of GIST's samples, especially those scored +1 (43.6%), is appropriate.

Some studies also suggested that EGFR expression associated with bad progression, one of which is lung adenocarcinoma and glioma. GISTs are classified based on

its size and mitotic index. High grade GISTs, have size> 10 cm with a mitotic index >10/10 HPF, while benign GISTs have size <5 cm with a mitotic index <5/10 HPF. Thus, high grade GIST prognosis is worse than benign [6]. Objectives of this study to analyze the relationship between GIST with EGFR expression and between the expression of EGFR with mitotic index and tumor size, with hope can be used as a GIST's prognostic marker. Unfortunately, there is no significant correlation between the expression of EGFR and mitotic index, also between EGFR expression and tumor size.

5. Conclusion

CD117-positive GIST expresses EGFR in 74.4%, although there is no significant relationship between the expression of EGFR with tumor size ($p = 0.129$) and between the expression of EGFR with mitotic index ($p = 0.629$). This result leads to the probability of using Anti-EGFR as a substitutive therapy for Imatinib-resistence GIST.

6. References

1. Agaimy A. Gastrointestinal stromal tumors (GIST) from risk stratification systems to the new TNM proposal: more questions than answers ? A review emphasizing the need for a standardized GIST reporting. 2010; 3(5):461-71.
2. Arne G. Expression Profiling of Gastrointestinal Stromal Tumors Biomarkers for Prognosis and Therapy. Sahlgrenska Cancer Center. Departement of Pathology. Sahlgrenska Academy at the University of Gothemburg. Sweden, 2012.
3. Fletcher CDM, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ *et al*. Diagnosis of gastrointestinal stromal tumors: A consensus approach. Int. J Surg Pathol. 2002; 10(2):81-9.
4. Rossi S, Gasparotto D, Toffolatti L, Pastrello C, Gallina G, Marzotto A *et al.* Molecular and clinicopathologic characterization of gastrointestinal stromal tumor (GISTs) of small size. Am J Surg. Pathol. 2010; 34:1480-1491
5. Nielsen TO, Hsu FD, O'Connell JX, Gilks CB, Sorensen PH, Linn S *et al*. Tissue microarray validation of epidermal growth factor receptor and SALL2 in synovial sarcoma with comparison to tumors of similar histology. Am J Pathol. 2003; 163:1449-1456
6. Miettinen M, Lasota J. Gasstrointestinal stromal tumors. Review on Morphology, Molecular Pathology, Prognosis, and Differential Diagnosis. Arch Pathol Lab Med. 2006; 130:1466-1478.
7. Yamamoto H, Kojima A, Miyasaka Y, Imamura M, Nakamura N, Yao T *et al*. Prognostic Impact of blood vessel invasion in gastrointestinal stromal tumor of the stomach.Human Pathology. 2010; 41:1422-1430.
8. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol. 2005; 29:52-68.
9. Rammohan A, Sathyanesan J, Rajendran K, Pitchaimuthu A, Perumal SK, Srinivasan UP, Ramasmy R, Palaniappan R *et al*. A GIST of Gastrointestinal stromal tumors: A review. World J Gastrointest Oncol. 2013; 6:102-112.
10. Ksienski D. Imatinib Mesylate: Past Successes and Future Challenges in the Treatment of Gastrointestinal stromal tumors. Clinical Medical Insight: Oncology. 2010; 5:365-379.
11. Serrano C, George S, Valverde C, Olivares D, García-Valverde A, Suárez C *et al*. Novel Insights into the Treatment of Imatinib-Resistant Gastrointestinal Stromal Tumors. Target Oncol. 2017; 12(3):277-88.
12. Tornillo L. Biology of Gastrointestinal stromal tumour and mechanism of imatinib resistance. Mini Symposium: Pathology of gastrointestinal stromal tumours. Diagnostic Histopathology. 2013; 19:6.203-210.
13. Miranda C, Nucifora M, Molinari F *et al*. KRAS and BRAF mutations predict primary resistance to imatinib in gastrointestinal stromal tumors. Clin Cancer Res. 2012; 18: 1769-76.
14. Bauer S, Duensing A, Demetri GD, Fletcher JA. KIT oncogenic signaling mechanism in imatinib resistant gastrointestinal stromal tumor: PI3-kinase/AKT is crucial survival pathway. Oncogene. 2007; 26:7560-8.
15. Jamali FR, Darwiche SS, El-Kinge N, Tawil A, Soweid AM. Disease Progression Following Imatinib Failure in Gastrointestinal Stromal Tumors: Role of Surgical Therapy. Oncologist. 2007; 12(4):438-42.
16. Lopes L, Bacchi C. EGFR and gastrointestinal stromal tumor: an immunohistochemical and FISH study of 82 cases. Modern Pathology. 2007; 20:990-994.
17. Kumar V *et al*. Robbins and Cotran: Pathologic Basic of Disease 8th Ed. Philadelphia. Saunders, 2010.
18. Growth E, Receptor F. O ncologist in Human Tumors: More Than Just Expression? Cancer. 2002; 7(4):31-9.
19. Cai YC, Jiang Z, Vittimberga F *et al*. Expression of transforming growth factor-alpha and epidermal growth factor receptor in gastrointestinal stromal tumours. Virchows Arch. 1999; 435:112-115.
20. Yoo NJ, Lee JW, Soung YH *et al*. Mutational analysis of the epidermal growth factor receptor gene in gastrointestinal stromal tumors. J Korean Gastric Cancer Assoc. 2004; 4:268-271.
21. Bode B, Frigerio S, Behnke S *et al*. Mutations in the tyrosine kinase domain of the EGFR gene are rare in synovial sarcoma. Mod Pathol. 2006; 19:541-547.