Journal of Interdisciplinary Histopathology
www.scopmed.org
DOI: 10.5455/jihp.20160328113716

Original Research

C-kit (CD117) expression is not valuable to predict prognosis in invasive ductal carcinoma of breast

Maha Shomaf[1], Al-Motassem Yousef[2], Jamal Masad[3], Murad Sahawneh[4], Ahmad Halawa[4]

[1]Department of Histopathology, Medical School, University of Jordan, Amman, Jordan, [2]Department of Biopharmaceutics and Clinical Pharmacy, College of Pharmacy, The University of Jordan, Amman, Jordan, [3]Department of Surgery, The University of Jordan, Amman, Jordan, [4]Department of Histopathology, University of Jordan, Medical School Amman, Jordan

Address for correspondence: Maha Shomaf, Department of Histopathology, Medical School, University of Jordan, Amman, Jordan. E-mail: mshomaf@ju.edu.jo

Received: February 04, 2016
Accepted: March 28, 2016
Published: April 03, 2016

ABSTRACT

Objective: C-kit functions as a tyrosine kinase receptor and represents a target for small molecule kinase inhibitors. The expression pattern for c-kit was studied in different human tumor types as gastrointestinal stromal tumor, malignant melanoma, breast and lung cancer, sarcoma and mastocytosis. C-kit expression in malignant tumors can be a factor that might affect the prognosis and its expression in different tumors was studied in respect of its correlation with the outcome. Imatinib or sunitinib therapy of KIT-positive tumors is an example of a targeted cancer therapy requiring immunohistochemical tumor analysis to identify patients that would benefit from this kind of therapy. This study is done on patients with breast cancer to find out the frequency of c-kit expression and to find out if it has any effect on the outcome of the patients and to compare the results with that of other ethnicities. **Materials and Methods:** Paraffin-embedded tumor tissues from 81 patients with breast invasive ductal carcinoma were analyzed immunohistochemically for c-kit expression and correlated to the survival of the patients after initial diagnosis using the follow-up data. **Results:** The mean age of the patients was 52.8 years. The median follow-up period was 22 months (range 1-37 months). C-kit immunopositivity was found in 37 cases (46%). Expression of c-kit was found in tumor samples with varying intensities and infrequently. **Conclusion:** There was no significant correlation between c-kit expression and prognosis.

KEY WORDS: Breast cancer, c-kit expression, Jordan

INTRODUCTION

Breast cancer (BC) is the most common cancer among women in Jordan and it is the leading cause of cancer death [1]. There are several clinical and histopathological prognostic parameters that have been studied [2]. C-kit expression has been detected in a variety of different tumor entities such as gastrointestinal stromal tumor (GIST), malignant melanoma, breast and lung cancer, sarcoma and mastocytosis [3-7]. In GIST, the frequency of KIT positivity is so high (90-95%) that immunohistochemistry (IHC) c-kit detection is considered a prerequisite for the histologic diagnosis of GISTs [3]. C-kit acts to regulate a variety of histological responses including cell proliferation, apoptosis, chemotaxis, and adhesion. C-kit alterations in malignant tumors are of high interest because KIT is one of the targets of tyrosine kinase inhibitors. Imatinib mesylate is a selective tyrosine kinase inhibitor has initially been shown to be effective in the treatment of chronic myeloid leukemia [8] where it targets the BCR-ABL fusion protein and the treatment of GIST targets the c-kit tyrosine kinase [9]. Different studies showed controversy results, some showed that tumors with either overexpression and/or mutations of c-kit have significantly poor prognosis [10-14], others found no prognostic significance of c-kit alterations [15-17].

Therefore, the aim of our study was to investigate the frequency of c-kit protein expression in our patients by standard IHC techniques and the relation to survival which would be a significant scientific advance in our understanding of expression pattern in BC.

MATERIALS AND METHODS

Patients and Tissues

A total of 81 patients with infiltrating ductal carcinoma of breast diagnosed in Jordan university hospital between January/2010 and May/2012 with a mean age of 52.8 years were included in this study.

Formalin-fixed, paraffin-embedded tissue sections were used for all cases. All cases were histologically classified according to the World Health Organization criteria.

IHC

Sections from formalin-fixed and paraffin-embedded tumor tissue were processed for immunohistochemistry using a rabbit monoclonal anti-human c-kit antibody (Bio Genex YR 145) diluted in PBS. PH 7.6, containing 1% BSA and 0.09% sodium azide in ready to use vials. The sections were incubated with primary antibody for 1 h. For the detection of the primary antibody, a commercially available detection kit (liquid DAB, BioGenex) was used, and the procedure was conducted manually according to the manufacturer's instruction. Finally, the reaction is visualized by a chromogen. Appropriate positive controls were used throughout the testing process. Mast cells were used as internal positive control. Tumor cells that showed cytoplasmic and/or membrane immunoreactivity for c-kit were considered positive. Counterstaining was performed in hematoxylin solution. For each tissue sample, the percentage of positive cells was estimated and the staining intensity was recorded semi quantitatively from 0 (negative) [Figure 1], + (weak), ++ (medium), and +++ (strong) [Figure 2]. Staining results were interpreted by a single pathologist.

Statistical Analysis

Clinical data were obtained by reviewing the charts. Overall survival was defined as the period of time from initial diagnosis to death or last contact, that is, date of last follow-up visit. Survival analysis was determined according to the Kaplan–Meier method and statistical significance of the differences in survival distribution was evaluated by the log-rank test. The priori level of significance was set at $P < 0.05$. Hazard ratio was calculated utilizing cox regression. All statistical analyses were carried out using SPSS® software, version 15.0 (SPSS Inc. Chicago, IL, USA).

The research submitted for publication was approved by the ethics committee in Jordan university hospital (institutional review board) in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.

RESULTS

The patients' ages at diagnosis ranged from 30 to 74 years, with a median age of 51 years. Survival and follow-up data were available from hospital files of the patients. The median follow-up period is 22 months (range 1-37 months). 77 of the patients were alive and 4 died during the follow-up period. About 44 patients (54%) showed no c-kit expression, 14 patients (17%) showed low expression, 11 patients (14%) showed moderate expression, and 12 patients (15%) showed high expression. The mean survival time for the patients with no expression was 18.7 months while in low expression patients it was 20.1 months, in patients with moderate expression it was 26.41 months, and in patients with high c-kit overexpression, the mean survival was 19.33 months. The data is summarized in Table 1. None of the patients received kinase inhibitor therapy. No significant correlation was found between the survival of the patient after the first diagnosis of BC and c-kit expression.

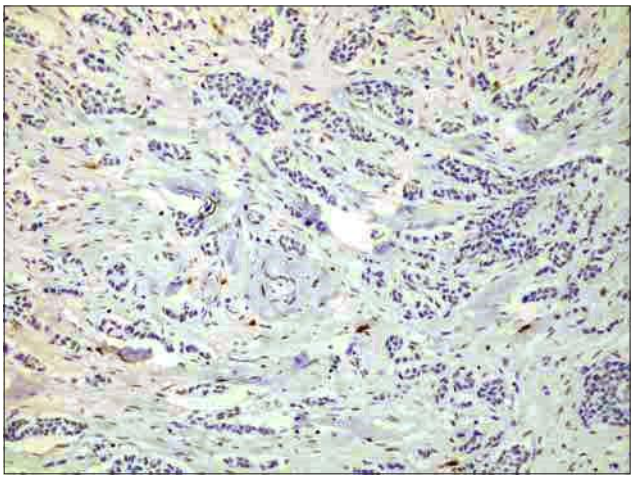

Figure 1: Negative immunohistochemical staining for c-kit in malignant cells of breast cancer (IHC, × 200)

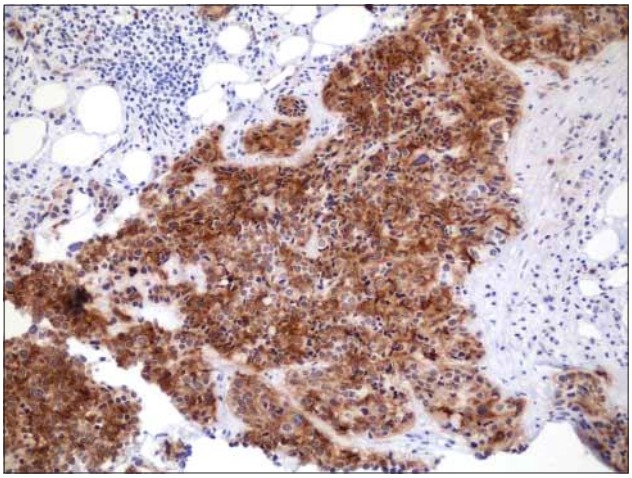

Figure 2: Intense immunohistochemical staining for C-kit in malignant cells of breast cancer (IHC, x200)

Table 1: Medians for survival time of the patients in correlation with c-kit expression

c-Kit expression	Survival				
	Median (months)	Standard error	95% CI	Hazards ratio*	*P* value
No expression (*n*=44)	18.7	0.8	19.4-22.6	Reference	
Low expression (*n*=14)	20.1	3.3	11.6-24.4	1.1	0.71
Moderate expression (*n*=11)	26.4	2.5	22.1-31.9	0.53	0.067
High expression (*n*=12)	19.3	6.9	4.4-31.6	0.89	0.72
Overall (*n*=81)	22.0	1.3	19.5-24.5		0.21

*Cox regression

DISCUSSION

C-kit expression plays a pathogenic role in different types of tumors besides GIST. Inhibition of the c-kit and the PDGFR pathway with low-molecular-weight kinase inhibitors showed clinical benefits in patients with GIST [18] and dermatofibrosarcoma protuberans [19]. Imatinib or sunitinib therapy of KIT-positive GISTs represents an example of a targeted cancer therapy requiring immunohistochemical tumor analysis to identify patients that would benefit from this kind of therapy. The response to these kinase inhibitors (e.g., imatinib) may not correlate with the c-kit expression level. It has been shown by different studies that the benefit from imatinib is related to location and type of mutation of c-kit as well as the interaction with other signal pathways [20,21]. Tumors with c-kit expression are potential targets for imatinib and other selective tyrosine kinase inhibitors therapy. However, it was found that patients with advanced GIST harboring a KIT exon 11 mutation have the best response rate to imatinib and long-term survival as well as overall survival than either exon 9 KIT mutations or wild type [22]. Another study done in Korean patients with advanced GIST was unable to find an association between KIT mutational status and clinical outcome of imatinib [23]. There was a trend toward better outcomes for patients with wild-type KIT or exon 11 mutations compared with exon 9 mutations, although this was not statistically significant compared with previous studies in western populations, these results suggest that ethnic differences may influence the relationship between KIT genotype and clinical outcome to imatinib.

C-kit expression was found in 21% of BC [24]. In our study, c-kit expression was found in 46% of our cases which is high in comparison with other studies, and there was no significant correlation with the survival.

Concerning c-kit expression and prognosis the available data are controversial. In a recent study by Charpin *et al*., it was found that c-kit expression in patients with breast carcinomas correlated with a poor patient outcome while other studies found that a loss of the c-kit expression is associated with an advanced stage of BC [14].

In a study done in Egypt, it was found that the prevalence of CD117 immunoreactivity in invasive breast carcinoma was 28.6% which is lower than our finding (46%) and the lack of CD117 immunoreactivity in invasive breast carcinoma was associated with features of more aggressive tumor behavior as higher microvessel density, larger size, higher tumor grade, more lymph node metastasis, and negative estrogen and progesterone receptors which significantly related to lowering overall survival and prognosis [25].

Other studies found no statistically significant relationship between the expression of c-kit proto-oncogene product in BC tissue and histological type, tumor size, lymph node metastasis, distant metastasis, stage, and age of the patients [24,26].

Similar findings also reported in a study done in Indian patients with BC which showed that c-kit gene plays a poor role in the prognosis of ductal and lobular carcinoma and the point mutations in c-kit gene did not significantly correlate with tumor size, estrogen receptor status and lymph node metastasis [27].

Our results possibly influenced by the number of patients in each category since the majority of patients showed no expression of c-kit.

CONCLUSION

This study supports previous studies analyzing c-kit expression in solid tumors showing that a strong c-kit expression is rarely observed in solid tumors, still, screening for the expression of specific growth factor receptors in tumor cells is very relevant since it offers a significant knowledge about the pathogenesis of tumors and to understand the complex interactions of cellular signal transduction pathways which will help and guide us in the development of target therapies which can be more efficient in cancer treatment.

Limitations

The follow-up period of the patients was not long and the survival at the time of the study ranged between 1 and 37 months. In addition, our patients do not receive kinase inhibitors as imatinib routinely and as such the effect of treatment could not be evaluated.

ACKNOWLEDGMENTS

This study was supported by a grant from the Deanship of Scientific Research/The University of Jordan/Jordan.

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Source of Support: Nil, Conflict of Interest: None declared.