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Original Article

Neuroendocrine Tumors, Possible Targets for Tyrosine Kinase Inhibitor Therapy

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ABSTRACT

Background: CD117 is a tyrosine kinase receptor encoded by c-kit proto-oncogene. It is expressed during normal development in some tissues and in a subset of neoplasia especially gastrointestinal stromal tumors (GISTs). It has been demonstrated that CD117 expression is necessary for the normal development of mast cells, melanocytes, some hematopoietic cells, and germ cells. The role of CD117 in normal hepatocyte maturation and regeneration of injured liver cells has also been shown. Overexpression of this receptor has been observed in some types of tumors, including lung, breast, skin, uterus, bladder and ovarian cancers, leukemias, germ cell tumors, Ewing sarcoma, and gastrointestinal stromal tumors (GISTs).

Objective: The objective of this study was to determine the frequency of CD-117 expression in neuroendocrine tumors of different grades

Methods: A Descriptive, Cross-sectional study was performed on 135 cases of neuroendocrine tumors of different grades from different sites at histopathology department, Chughtai Institute of Pathology, Lahore. The antibody used was polyclonal rabbit anti-CD-117 antibody from DAKO.

Results: In our study, out of 135 cases, 39.26%(n=53) were between 20-50 years of age whereas 60.74%(n=82) were between 51-80 years of age, mean$\pm$sd was calculated as 53.11$\pm$13.96 years, 53.33%(n=72) were male and 46.67%(n=63) were females, mean size of tumor was calculated as 2.82$\pm$1.70cm, frequency of CD-117 expression in neuroendocrine tumors was 22.96%(n=31).

Conclusion: We concluded that the frequency of CD-117 expression was higher in neuroendocrine tumors having a higher histologic grade, however, other local studies are required to validate our results.

Keywords: ***Neuroendocrine tumors, CD-117, Targeted therapy***

Introduction

Neuroendocrine tumors (NETs) are a diverse group of tumors arising from endocrine and pluripotent stem cells. They are rare, but recent data indicates a significant rise in both incidence and prevalence during the last few decades. NETs have a propensity to develop in a variety of tissues including the thyroid gland, lung, pancreas, gut, adrenal gland, kidney, uterine cervix, prostate, skin and others.[2,3]

Several biomarkers have been developed in the recent past for the prompt and accurate diagnosis of neuroendocrine tumors, amongst which the most applied general tumor marker is chromogranin A with a high sensitivity and specificity.[4]

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These tumors also express other neuroendocrine markers such as synaptophysin, NSE and CD-56.[5,6] NETs are graded as grade 1 (Ki-67 proliferation index <2%), grade 2 (Ki-67 proliferation index 2-20%), grade 3 neuroendocrine tumors (Ki-67 proliferation index >20% without architectural distortion and necrosis) and grade 3 neuroendocrine carcinomas (Ki-67 proliferation index >20% with architectural distortion and necrosis).[7] Recently, *c-kit* (CD-117) expression has been documented in many such tumors, especially in small cell carcinomas of the lung.[8,9]

The proto-oncogene *c-kit*, located on chromosome 4q12, encodes a trans-membrane tyrosine kinase receptor.[10] CD-117 is thought to play an important role in hematopoiesis, spermatogenesis, melanogenesis and, more recently, in carcinogenesis.[11] With the discovery of *c-kit* expression in neuroendocrine tumors, new hopes in molecular targeted tumor therapy have been raised.[12]

Usual treatment options for neuroendocrine tumors include surgery, biotherapy (somatostatin analogs),

chemotherapy and rarely molecular therapies.[13] However, somatostatin analogs commonly do not stop tumor growth; neither do chemotherapeutic regimens including 5-Fluorouracil and streptotozocin.[13] Positive KIT expression in NETs has encouraged investigators to initiate a therapeutic trial with the tyrosine kinase inhibitor imatinib mesylate (Gleevec), a drug that targets KIT and PDGF.[14] In vitro studies have suggested that imatinib can inhibit tumor growth in the most aggressive neuroblastic tumors and in neuroendocrine tumors.[15,16]

Many studies have been conducted and many more are currently in progress to analyze the importance of CD-117 in targeted therapy for neuroendocrine tumors. However, no such studies have been performed in our country.[9,17] Our aim is to determine the frequency of *c-kit* (CD-117) expression in neuroendocrine tumors of different grades, using Ki-67 proliferation index, arising in different tissue types that may benefit from possible targeted therapy.

Our aim is to determine the frequency of *c-kit* (CD-117) expression in neuroendocrine tumors of different grades, using Ki-67 proliferation index, arising in different tissue types that may benefit from possible targeted therapy.

Methods

After approval from institutional ethical committee, a descriptive, cross-sectional study was performed at Histopathology Department, Chughtai's Institute of Pathology, Lahore from: 01-01-2018 to 31-03-2019, using non-probability consecutive sampling technique. Total sample size was 135 (confidence interval: 95%, margin of error: 12%)**.** Neuroendocrine tumors of all sites and grades, in patients of both genders between the ages of 20 to 80 years were included. Blocks for review / immunohistochemistry from outside laboratories with clinical / pathological data available were also included. All autolysed and unfixed samples or tumors having neuroendocrine differentiation as an additional finding were not included in the study. The histological preparation was performed by classical method for inclusion in paraffin followed by hematoxylin–eosin staining. The immunohistochemical analysis was performed on serial sections using immune-enzymatic soluble complex method. The antibody used was polyclonal rabbitanti-CD-117 antibody from DAKO.

Data was entered and analysed using SPSS version 20. The mean and standard deviation was calculated for quantitative variables including age and size of tumor. Qualitative variables including gender, tumor grade, tumor site and immunohistochemical staining were presented in the form of frequencies and percentages. Effect modifiers like age, gender and size were controlled through stratification. Post stratification chi-square test was applied by taking P value of $\leq$0.05 as significant.

Results

135 cases fulfilling the selection criteria were enrolled to determine the frequency of CD-117 expression in neuroendocrine tumors of different grades. Age distribution of the patients is given in Table 1. Out of 135 patients, 72 were males.

The mean size of tumor was calculated as 2.82$\pm$1.70cm.

Frequency Of CD-117 Expression in Neuroendocrine Tumors

Frequency of CD-117 expression in neuroendocrine tumors in the complete sample size was 22.96% (n=31) whereas 77.04% (n=104) showed no expression (Table No. 2). Out of these positive cases, 67.7% (n=21) were of Grade III (Table 7) with a p-value of 0.04 (significant). Grade III neuroendocrine carcinomas expressed CD-117 in 36% of the cases (n=14).

Data stratification:

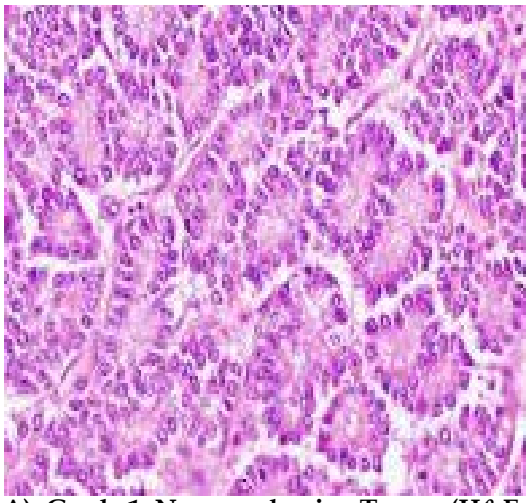
Effect modifiers like age, gender and mean tumor size was controlled through stratification. Post stratification chi-square test was applied by taking P value of 0.05 as significant. (Table No. 3-5)

Tumor site:

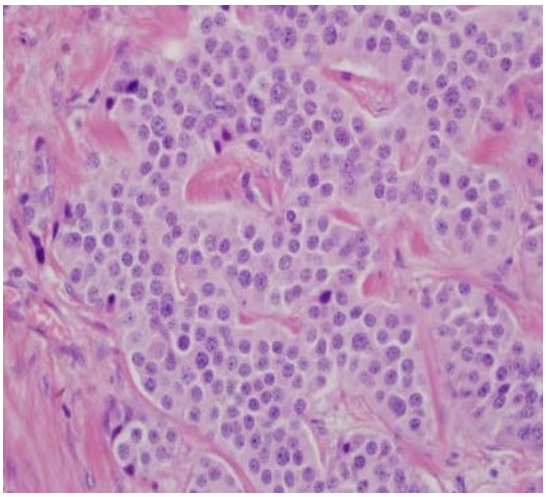
39.3% of the tumors were from gastrointestinal tract, 18.5% tumors were from lungs, 12.6% were from pancreas (Table No. 6). Other sites included the spine (4.44%), oral cavity, salivary gland (6.67%), breast (2.22%) and other miscellaneous sites (16.3%).

Table no. 1: Age distribution for Neuroendocrine tumors (n=135)

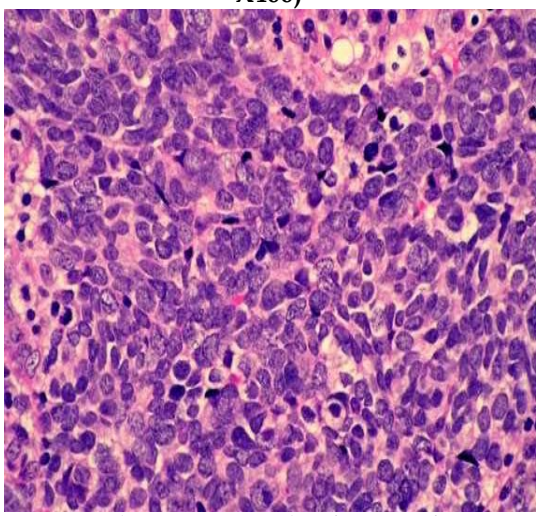
Age(in years)	No. of patients	%
20-50	53	39.26
51-80	82	60.74
Total	**135**	**100**
Mean$\pm$SD	**53.11$\pm$13.96**	



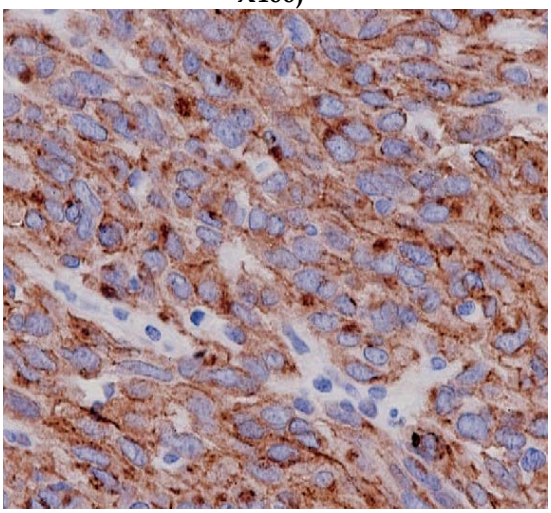
A): Grade 1, Neuroendocrine Tumor (H&E, X400)



B): Grade 2, Neuroendocrine Tumor (H&E, X400)



C: Grade 3, Neuroendocrine Carcinoma (H&E X400)



D): Positive CD-117 (IHC stain, X400)

Figure 1: Different grades of neuroendocrine tumors and CD 117 Positivity (D)

Table No. 2: Frequency of Cd-117 Expression in Neuroendocrine Tumors Of Different Grades (N=135)

Overexpression	No. of patients	%
Yes	31	22.96
No	104	77.04
Total	**135**	**100**

Table no. 3: Stratification for frequency of cd-117 expression in neuroendocrine tumors of different grades with regards to age89

Age (in years)	Overexpression		P value
	Yes	No	
20-50	9	44	0.18
51-80	22	60	

Table No. 4: Stratification for Frequency Of Cd-117 Expression In Neuroendocrine Tumors Of Different Grades With Regards To Gender

Gender	Overexpression		P value
	Yes	No	
Male	14	58	0.29
Female	17	46	

Table no. 5: Frequency of cd-117 expression in different grades of neuroendocrine tumors (n=135)

		Positive	Negative	Percentage of positive cases	P-value
Grade I (46)		7	39	15%	0.044
Grade II (24)		3	21	13%	
Grade III (65)	Grade III NET (26)	7	19	27%	
	Grade III NEC (39)	14	25	36%	

Table no. 6: Frequency of Neuroendocrine tumors at different sites (n=135)

Tumor site	Number of cases	Percentage
Gastro-intestinal tract	53	39.3%
Pancreas	17	12.6%
Lungs	25	18.5%
Upper-aerodigestive tract and salivary gland	9	6.67%
Breast	3	2.22%
Spine	6	4.44%
Others	22	16.3%

Discussion

CD117 is a tyrosine kinase receptor encoded by c-kit proto-oncogene. It is expressed during normal development in some tissues while also in a subset of neoplasia especially gastrointestinal stromal tumors (GISTs). C-KIT has been found to play a pivotal role in a variety of rapidly dividing normal body cells including mast cells, melanocytes, hematopoietic cells, and germ cells. Expression of this receptor has also been seen in tumors arising from the lung, breast, skin, urinary bladder and the female genital tract. Leukemias, germ cell neoplasia, soft tissue sarcomas including Ewing sarcoma, and gastrointestinal stromal tumors (GISTs) also demonstrate a significant percentage of positivity for CD-117.

This study was planned with the view to determine the frequency of *c-kit* (CD-117) expression in neuroendocrine tumors of different grades, using Ki-67 proliferation index, arising in different tissue types that may benefit from possible targeted therapy.

In our study, out of 135 cases, 39.26% (n=53) were between 20-50 years of age whereas 60.74% (n=82) were between 51-80 years of age, mean $\pm$SD was calculated as 53.11$\pm$13.96 years, 53.33% (n=72) were male and 46.67% (n=63) were females, mean size of tumor was calculated as 2.82$\pm$1.70cm, frequency of CD-117 expression in neuroendocrine tumors in the complete sample size was 22.96% (n=31). The study also demonstrated that CD-117 expression is a late event in the disease, explained by the findings that a higher percentage (32%) of Grade 3 tumors showed *c-kit* expression with a significant p value (0.044).

A previous study[3] recorded these findings in 6% of the cases, lower to our study. Another study conducted by Pelosi et al. [27] showed prevalence of CD-117 expression in around 70% of neuroendocrine carcinomas. But this study only included tumors from the lung. Furthermore, a similar study conducted by Araki et al [28] on neuroendocrine carcinomas of the lung demonstrated CD-117 expression in 45-55% of the lung neuroendocrine carcinomas. In our study, CD-117 expression was noted in 32% (21/65) of the Grade III NETs. In comparison to the reference studies quoted above, frequency of CD-117 in high grade NETs in our study is relatively lower. However, this percentage is for high grade NETs from all over the body and not only from the lungs. Grade III neuroendocrine carcinomas expressed CD-117 in 36% of the cases.

As far as lower grade NETs (Grade I and Grade II) are concerned, our study showed CD-117 expression in 15% (7/46) of grade I NETs and 13% of (3/24) grade II NETs. While Pelosi et al [27], in his study showed CD-117 expression in only 5% of carcinoids. But NETs from only lung were included in this study.

KIT expression has been detected in a variety of different tumor entities such as gastrointestinal stromal tumors (GIST) neuroendocrine Tumors (NET), malignant melanoma, breast and lung cancer, sarcoma and mastocytosis. In GIST, the frequency of KIT positivity is so high (90% to 95%) that immune histochemical KIT detection is considered a prerequisite for the histologic diagnosis.[18] New promising marker, DOG1 is highly specific for GIST.

The KIT protein is normally activated through binding to its ligand (stem cell factor). The neoplasms often harbor KIT activating mutations that result in constitutive ligand independent KIT phosphorylation and downstream activation.[21]

KIT mutations in malignant tumors are of great interest to pathologists and oncologists because KIT receptors are one of the targets for the class of tyrosine kinase inhibitors including imatinib mesylate, sunitinib, nilotinib and dasatinib. Imatinib mesylate is a selective inhibitor of certain tyrosine kinases, including ABL, BCR-ABL, ARG, KIT, and the platelet-derived growth factor receptors (PDGFRs).[22] Imatinib was initially shown to be effective in the treatment of chronic myeloid leukemia, where it targets the BCR-ABL fusion protein and is now being proposed for

usage in the treatment of NETs where it will target the c-kit tyrosine kinase receptor.[23] It is postulated that other KIT-positive tumors may also benefit from therapy using tyrosine kinase inhibitors directed against it and a particularly high response rate is expected in KIT-expressing tumors that also harbor activating KIT mutations.[24]

However, CD-117 expression by immunohistochemistry does not always correlate with underlying c-kit mutations [29]. Perkins et al reported a case of hepatic neuroendocrine carcinoma expressing CD-117 by immunohistochemistry with an underlying activating c-kit mutation. This patient showed a remarkable response to imatinib therapy [30].

The studies available until now about prognosis show controversial results regarding c-kit expression. While numerous clinical studies showed that cancer patients with either over-expression and/or mutations of c-Kit in their clinical samples have significantly poor prognosis, lower survival rates and show resistance to chemotherapy other studies found no prognostic significance of c-kit expression.[24-26] A study [31] revealed imatinib to inhibit neuroendocrine cell growth, irrespective of c-kit mutation. The results regarding efficacy of tyrosine kinase inhibitors in treatment of NETs are some-what conflicting. Some studies have shown good results [30, 31, 32], while other studies have revealed no efficacy of imatinib in treatment of NETs [33]. Further studies are required to determine c-kit mutation in NETs exhibiting CD-117 positivity on immunohistochemistry and clinical trials to determine effectiveness of imatinib therapy in NETs.

Conclusion

We conclude that a significant number of neuroendocrine tumors express CD-117 suggesting that these tumors are a potential target for imatinib mesylate therapy. Furthermore, our research points out that the expression of CD-117 was seen more frequently in tumors exhibiting a higher histologic grade. This stresses upon the notion that more aggressive tumors may respond well to such a therapy if our results are validated. However, whether this warrants effective treatment with imatinib mesylate therapy alone or requires additional treatment modalities remains unanswered and will require additional studies for confirmation.

Conflict of interest:

The authors declare no conflict of interest.

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- Sameen Afzal: Study design, Acquisition of data, Analysis of data and Drafting of manuscript
- Ghazi Zafar: Study design, Acquisition of data, Analysis of data and Drafting of manuscript
- Fatima Khalid: Acquisition of data, Analysis of data and Drafting of manuscript
- Beenish Fatima: Analysis of data and Drafting of manuscript
- Anila Chughtai: Acquisition of data
- Akhtar Sohail Chughtai: Critical revision and Financial support