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A Study on HER2NEU Overexpresson in Gastric Adenocarcinoma

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Abstract
Introduction: *Carcinoma stomach is the 5th most common cancer worldwide. In India though the incidence is 10.6 per 100,000 population, majority present in later stages and it is the second most common cause of cancer related deaths in Indian population. Surgery is the mainstay of treatment but the majority of patients present with advanced, inoperable disease and palliative radiotherapy & chemotherapy offer a little in improving survival of patients with advanced tumours (median survival 7-10 months)[6] and situation demands newer novel drugs or newer strategy to look for. Recent advances in molecular medicine have not only shed light on the carcinogenesis of gastric cancer but also offered novel approaches regarding prevention, diagnosis, and therapeutic intervention and HER-2 (human epidermal growth factor receptor 2) is currently in the spotlight. patients with HER2 overexpression receiving chemotherapy and trastuzumab had a significant longer median overall survival without any additional adverse side effects. HER2/neu testing and trastuzumab treatment also now becoming the standard of care for metastatic gastric cancer in few centres and HER2 has become a ''hot'' research topic.*
The aim of our study was to evaluate the frequency of HER2 expression in gastric adenocarcinoma and its association with various clinicopathological entities.
Material and Methods: *A consecutive prospective series of 150 patients of gastric carcinoma diagnosed on biopsy and resection specimens received in our department over a time span of 2 years (October 2016 to September 2018) were included in the study. Expression of HER2 in the histological specimen and correlation between the expression of HER2 & clinicopathological parameters (age, gender, tumour site, Lauren's type, histological differentiation and UICC staging) were evaluated in these patients.*
Result And Analysis: *Among the various clinicopathological entities HER2 positivity significantly associated in intestinal-type gastric cancers rather than diffuse/mixed-type cancers (29.6% vs 13.9%, p = 0.019), and moderate-differentiated cases 31(20.67%, p =0.01). Positive reactivity with anti cerbB-2 antibody was significantly more frequent in UICC stage-III (18%, p <0.001). But relationship of HER2 overexpression with sex, age, tumor site is not statistically significant (p > 0.05; Table 1). Within the subgroups, association of HER2 positivity was statistically more in M0 than M1(27.6% vs. 12.70%, p-value 0.028) but there was no assosciation with depth of invasion and lymph node metastasis.*
Conclusion: *So we may conclude that HER-2/neu expression in gastric carcinomas may be, related to their aggressive clinical behaviour but to stamp HER2 status as an independent prognostic factor and to further define the role of Her2 expression in gastric carcinoma in Indian patients further research is required.*

Introduction

One of the dreaded cancer because of its late presentation carcinoma stomach, in 2018, is the 5th most common cancer worldwide (~1milion cases, 5.7% of all cancers) and 3rd most common cause of cancer related death worldwide (~0.8 million deaths, 8.2%)[1] with acase fatality ratio of 70%[2]. Asia is the main geographical hub recording~75% of all gastric cancer cases[1]. In India though the incidence is 10.6 per 100,000 population, majority present in later stages[3] and it is the second most common cause of cancer related deaths in Indian population[4]. The incidence rate over the last few decades is in decreasing trend but the absolute incidence has risen due to the aging of the worldwide population[5]. Surgery is the mainstay of treatment but the majority of patients present with advanced, inoperable disease and palliative radiotherapy & chemotherapy offer a little in improving survival of patients with advanced tumours (median survival 7-10 months)[6] and situation demands newer novel drugs or newer strategy to look for.

Recent advances in molecular medicine have not only shed light on the carcinogenesis of gastric cancer but also offered novel approaches regarding prevention, diagnosis, and therapeutic intervention and HER-2 (human epidermal growth factor receptor 2) is currently in the spotlight. Her2/neu over-expression was first described in 1886[7] using immunohistochemistry. Her2/neu (c-erbB2), a proto-oncogene (chromosome 17q21)[8] encodes a 185-kDa trans membrane tyrosine kinase receptor, a member of the Epidermal Growth Factor Receptor Family (EGFRs). Comprising of four members: HER1 (EGFR), HER2, HER3 and HER4, they modulate various aspects of tumour cell biology like cell proliferation, adhesion, migration, differentiation and apoptosis[8]. Various malignancies shows HER-2 amplification and/or over expression like colon, bladder, ovarian, fallopian tube, endometrium, lung, uterine cervix, head and neck, prostate, pancreatic, salivary gland and oesophagealand gastric carcinoma[8].

In gastric cancer, the frequency of Her2/neu overexpression varies widely in the literature from about 4.4% to 53.4%[9].

Trastuzumab (Herceptin) is a monoclonal antibody which specifically targets HER2 protein by directly binding the extracellular domain of the receptor, enhances survival rates in both primary and metastatic HER2positive breast cancer patients inviting study regarding its antitumor activity in patients with HER2 positive cancers, including gastric adenocarcinomas. With a handful of literatures available on HER2/neu, it is turning out to be a useful molecule for which targeted therapy in the form of Trastuzumab is available. Combined with conventional chemotherapy (capecitabine or 5-fluorouracil and cisplatin), the humanized monoclonal antibody against HER-2, trastuzumab, is found to prolong overall survival and progression-free survival, and increase the response rate in HER-2-positive advanced gastric carcinoma [10]. According to the ToGA clinical trial reported in 2010[11], patients with HER2 overexpression receiving chemotherapy and trastuzumab had a significant longer median overall survival without any additional adverse side effects. HER2/neu testing and trastuzumab treatment also now becoming the standard of care for metastatic gastric cancer in few centres and HER2 has become a ‘‘hot’’ research topic.

The aim of our study was to evaluate the frequency of HER2 expression in gastric adenocarcinoma and its association with various clinicopathological entities.

Materials and Methods

A consecutive prospective series of 150 patients of gastric carcinoma diagnosed on biopsy and resection specimens received in our department over a time span of 2 years (October 2016 to September 2018) were included in the study. Expression of HER2 in the histological specimen and correlation between the expression of HER2 & clinicopathological parameters (age, gender, tumour site, Lauren’s type, histological

differentiation and UICC staging) were evaluated in these patients. Tissue samples retrieved either through endoscopy guided biopsy or from excised specimen from surgery were formalin fixed and paraffin embedded, and were subjected to histopathological examination followed by immunohistochemistry study with positive control(breast carcinoma tissue known case) and negative control.

For determination of Her2 expression, a semi quantitative scoring criteria proposed by Hofmann et al.[12] was applied in sections from resection specimens. Score of 3+ was taken as positive. Score values of 1+ and 0 were taken as negative. Confirmation of cases reported as equivocal (IHC Her2 score 2+) by fluorescence in situ hybridization (FISH) could not be done due to financial constraints. Therefore, score 2+ was also included as negative for all practical purposes. For biopsy specimens, the criteria of 10% cells showing reactivity were not applied, and scoring was done on the basis of intensity of staining as proposed by Hofmann et al. Strong membranous, complete, basolateral, or lateral Her2 expression in "any number of cells" were taken as positive in these cases.

Overall Her2 overexpression in gastric cancer was evaluated and was further correlated with various clinicopathological features. Survival analysis could not be done due to short follow up time during the study period.

Table no. 1: The Hoffmann scoring system

SPECIMEN	IHC SCORE	HER2 OVER EXPRESSION ASSESSMENT
No reactivity or membranous reactivity in <10% of cells (resection) : in biopsies only one cohesive cluster of <5cells is required	0	NEGATIVE
Faint membranous reactivity in >10% of tumor cells (resection): in biopsies only one cohesive cluster of >5cells is required.	1+	NEGATIVE
Weak to moderate incomplete (basolateral) membranous staining in >10% of tumor cells (resection): in biopsies only one cohesive cluster of >5 cells is required	2+	EQUIVOCAL
Moderate to strong incomplete (basolateral) membranous staining in > 10% of tumor cells (resection): in biopsies only one cohesive cluster of >5 cells is required	3+	POSITIVE

Results and Analysis

In our study there were 150 participants of whom 126 specimens were obtained from resected samples and rest 24 from endoscopy guided biopsy samples. Of these 82 (54.7%) are male and 68 (45.3%) are female (male: female ratio was 1.20:1) .The mean age was 63.68 ± 10.7 years ranging from 34 years to 82 years). Histologically as per Lauren's classification, 71 tumours (47.34%) were intestinal, 79 (52.66 %) were diffuse and mixed-type carcinomas. With respect to tumour differentiation 13 (8.67%) tumours were well differentiated, 31(20.7%) were moderately differentiated, 92(61.33%) were signet ring & poorly differentiated variety and 14 (9.33%) were mucinous variety. In terms of location, 47(31.8%) were located at the body, 49(32.2%) at the GEJ & Cardia and 54(36%) were at pylorus. According to UICC staging (TNM staging), 9(6%), 13(8.67%), 57(38%) and 71(47.33%) cases were staged as T1, T2, T3 and T4 respectively. Similarly 35(23.33%) has no nodal involvement and 57(38%), 34(22.67%) and 24(16%) has been grouped as N1, N2 and N3 respectively. While in 63(42%) cases there was metastasis (M1), in rest 87(58%) cases there was no metastasis (M0). So, 19(12.67%) of cases were stage I, 33(22%) stage II, 35(23.33%) stage III, and 63(42%) stage IV. Among our 150 cases 32 (21.3%) cases showed HER-2/neu protein overexpression (IHC score 3+).

Correlation of HER2 with clinicopathological characteristics:

Among the various clinicopathological entities HER2 positivity significantly associated in intestinal-type gastric cancers rather than diffuse/mixed-type cancers (29.6% *vs*13.9%, p= 0.019), and moderate-differentiated cases

31(20.67%, p=0.01). Positive reactivity with anti cerbB-2 antibody was significantly more frequent in UICC stage-III (18%, p <0.001). But relationship of HER2 overexpression with sex, age, tumor site is not statistically significant (p > 0.05; Table 1). Within the subgroups, association of HER2 positivity was statistically more in M0 than M1(27.6% vs. 12.70%, p-value 0.028) but there was no association with depth of invasion and lymph node metastasis (p>0.05; Table)

Table no. 2: Socio Demographic Character of Population

AGE DISTRIBUTIONS OF PATIENTS	
MEAN AGE± SD IN YEARS	63.68 ±10.71
MINIMUM AGE	34
MAXIMUM AGE	82
AGE GROUPS(YEARS)	**FREQUENCY (%)**
30-39	4(2.7%)
40-49	16(10.6%)
50-59	25(16.7%)
60-69	58(38.7%)
70-79	39(26%)
80-89	8(5.3%)
SEX	**FREQUENCY (%)**
FEMALE	68(45.3%)
MALE	82(54.7%)

Table no 3 Correlation of Human Epidermal Growth Factor Receptor-2 Expression with Clinicopathological Characteristics

CLINICOPATHOLOGICAL CHARACTERISTICS	**N**	**HER2 STATUS**		χ^2	**p-VALUE**
		POSITIVE	**NEGATIVE**		
SEX					
MALE	82(54.7%)	17(20.7%)	65(79.3%)		
FEMALE	68(45.3%)	15(22.1%)	53(77.9%)	0.039	0.843
AGE(IN YEARS)					
<60	45(30%)	14 (31.10%)	31(68.90%)		
≥60	105(70%)	18(17.10%)	87(82.90%)	3.662	0.056
TUMOUR SITE					
GEJ& CARDIA	49(32.2%)	9(18.40%)	40(81.60%)		
BODY	47(31.8%)	11(23.40%)	36(76.60%)		
PYLOROUS	54(36%)	12(22.20%)	42(77.80%)	0.402	0.818
LAUREN'S TYPE					
INTESTINAL	71(47.34%)	21(29.6%)	50(70.40%)		
DIFFUSE &MIXED	79(52.66%)	11(13.9%)	68(86.1%)	5.46	0.019
TUMOR DIFFERENTIATION					
WELL DIFFERENTIATED	13(8.67%)	2(15.40%)	11(84.60%)		
MODERATELY DIFFERENTIATED	31(20.67%)	14(45.20%)	17(54.80%)		
SIGNET RING TYPE & POORLY DIFFERENTIATED	92(61.33%)	15(16.30%)	77(83.70%)		
MUCINOUS	14(9.33%)	1(7.1%)	13(92.9%)	13.828	0.003
TUMOR INVASION					
T1-T2	22(14.67%)	3 (13.60%)	19(86.40%)		
T3-T4	128(85.33%)	29(22.70%)	99(77.30%)	0.91	0.34
LYMPH NODE STATUS					
N0	35(23.33%)	4(11.4%)	31(88.60%)		
N1	57(38%)	12(21.10%)	45(78.90%)		
N2	34(22.67%)	9(26.50%)	25(73.50%)		
N3	24(16%)	7(29.2%)	17(70.80%)	3.461	0.326
METASTATIS					
M0	87(58%)	24(27.6%)	63(72.40%)		
M1	63(42%)	8(12.70%)	55(87.30%)	4.826	0.028
UICC STAGING					
I	19(12.67%)	2(10.5%)	17(89.50%)		
II	33(22%)	4(12.10%)	29(87.90%)		
III	35(23.33%)	18(51.40%)	17(48.60%)		
IV	63(42%)	8(12.7%)	55(87.30%)	24.679	<0.001

The burden and dreadfulness of gastric cancer could not be overemphasized by the fact that it is the 5th most common cancer in the world and 3rd most common cause of cancer related death[1] with case fatality ratio of 70%.[2]. Although surgery is the main stay of treatment and for in advanced cases we have chemo and radio therapy for palliation, they improve the survivality a little (median survival 7-10 months)[6] and the control of advance stage of gastric cancer remains a challenge for the clinical surgeons. Recent advances in molecular medicine have not only shed light on the carcinogenesis of gastric cancer but also offered novel approaches regarding prevention, diagnosis, and therapeutic intervention .Currently, several molecular factors are under scanner as prognostic and predictive factors for gastric cancer like oncogenes and tumour suppressor genes, growth factors and receptors, cell adhesion molecules, proteolytic molecules and angiogenic factors (HER2, EGFR, p53, Cadherin,-catenin, cycloxygenase-2 (COX-2), matrix metalloproteinases, and vascular endothelial growth factor (VEGFR)[13]. These prognostic factors can also be considered as a marker of therapeutic response by molecular target to chemotherapeutics or a new class of antineoplastic molecules (HER2/neu targeted by Trastuzumab, COX-2 by nonsteroidal anti-inflammatory drugs, matrix metalloproteinases, EGFR and VEGFR by specific inhibitors).Over the last decade Her2/neu molecule has been a subject of interest with respect to association with gastric cancer and correlation with the outcome of the disease.

HER2 has been successfully targeted in breast cancer through Trastuzumab, a monoclonal antibody, that binds to domain IV of the extracellular segment of the HER2 receptor .The mechanism of action of trastuzumab is largely unknown but may act by (1) Prevention of dimerization of HER2 with other HER family members and stimulation of endocytosis, (2) Inhibition of HER2 shedding,(3) Inhibition of PI3K-AKT pathway,(4) Attenuation of cell signalling, (5) Antibody-dependent cellular cytotoxicity, (6) Activation of apoptotic signals of the tumour cells, and(7) Inhibition of tumour angiogenesis[14]. Though FDA originally approval fortrastuzumabin treatment of breast cancer came in September 1998 it was in October 2010, the US FDA approved trastuzumab (Herceptin, Genentech, Inc.) for gastric cancer in combination with cisplatin and a fluoropyrimidine (capecitabine or 5-fluorouracil) for the treatment of patients of metastatic gastric or GEJ carcinoma, who have Her2-overexpression or have not received any prior treatment for the same, as per the results of ToGA trial[15]. As clinical surgeons, we should accurate enough to identify patients which are suitable for Herceptin treatment. An accurate and reliable HER2 scoring system, together with clinical information, may help us to better determine whether a gastric cancer patient is a potential candidate for targeted therapy using Trastuzumab or not.

In 1986 HER2gene amplification and protein overexpression were first reported in gastric cancer[7]. Immunohistochemistry (IHC) or in situ hybridization (ISH) assays are main modalities of HER2 status assessment. Numerous studies since the discovery of HER2 shows HER2 gene amplification rates as 16%-27.1% by FISH analysis and HER2 protein overexpression as 4.4%-53.4% by IHC analysis[9]. Several factors including sample size, study design and differences in geographic location dictate this variability of the results of the two methods of HER2 assessment[16]. Also ASCO/CAP guidelines state that, HER2 genotype heterogeneity in gastric cancers can lead to discrepancies in the results from IHC and FISH[12]. However, it seems the most important variability factor is likely a consequence of having no standardized HER2 test and scoring criteria[17]. Fluorescent in situ hybridization (FISH) is considered to be the gold standard; however, because of its higher cost and time consumption, as well as the need for a fluorescence microscope, generally only equivocal cases are subjected to this technique. Furthermore,

the high concordance between FISH and IHC as reported in many literatures support the use of IHC, which turns out to be most familiar and readily accepted method in most laboratories[15]. The ToGA trial[12] reported aHER2 FISH and IHC concordance rate of 87.5%, and almost all HER2 IHC3+ cases were entirely HER2gene amplified (97.5% of cases). There is also ample evidence that the tumours that show IHC score 2+ few of them showHER2gene amplification by FISH, even there are a school of thought going on to eliminate the IHC score 2+ category as a criterion for Trastuzumab therapy[18]. Others have continued to advocate immunohistochemical analysis over FISH for its lower cost and greater availability, proposing manual or digital-assisted subtraction of background HER2 immunostaining using benign epithelium as a way of improving IHC Test specificity [19].

In the present study, Hofmann IHC scoring system were followed[12], which seems to be the most appropriate HER2 scoring system in human gastric cancer. We followed theguidelines on HER2 detection in breast cancer, recommended by the American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) [12] and theUnited States Food and Drug Administration certified test kit was used to ensure reliability of our results and all IHC 3+ tumours were accepted as HER2/neupositive cases. We found that out of 150 cases 32 cases (21.3%) show IHC score 3+ which is well within the range of results of studies published across the world till date (4.4%-53.4%)[9] and commensurate with results of ToGA trial[11]. According to literature analysis by Maresch et al.[20] median Her2 positivity of 20.2% was observed in studies involving >8000 patients which is close to the results of our study. As described above these variance could be explained by several factors including sample size, study design and differences in geographic location[16], geographic and ethnic heterogeneity of solid tumours[20], HER2 genotype heterogeneity in gastric cancers [12] and lack of standardization of HER2 tests and scoring criteria[17]. In the few Indian studies done till date, Her2 positivity in gastric carcinoma was found to be variable, ranging from 17% to 44.2% [21, 22]. Sekaran A et al in their study in 2012 in Indian population showed that results of HER2 overexpression in Indian population were quite high (44.2%)[21].The higher Her2 positivity by Sekaran et al was attributed to use of highly sensitive streptavidin-biotin elite kit monoclonal antibody and inclusion of both membrane and cytoplasmic staining of Her2. Piyali Ghosh et al. [23] in a study of 54 cases found HER2+ rates were 22.22% (12/54).Rajat Jagani et al.[24] and Gupta P et al.[15] reportedHER2/neu positivity in 22.6% and 24.5%of all tested gastric cancer samples.

In our study, there were no significant relationship between HER2 positivity and sex, age and tumour site ($p > 0.05$). Fassan et al.[25], Sekaran A et al[21] in 2012 and Federica Grillo et al[26] in their study in 2013 showed that there was no significant difference noted in HER2 overexpression or amplification when compared to age or sex of the patient or tumor site, butstudies by Yu G Zet al in 2009[27], Moelans CB et al in 2011[28] and Alina Bădescu et al in 2012[29]did found a positive correlation in older age and male sex. While Chua et al.[30] could not detect significant relation between Her2 expression and tumor location which is in agreement with our study.

A strong association of Her2 positivity with intestinal type (29.6%) of tumors as compared to diffuse type and mixed type together (13.9%) was observed ($P = 0.019$). The picture is also being reflected in other studies of previous years like study of M. Tanner et al in 2005[31] and Alina Bădescu et al in 2012[29]. This finding is in keeping withsimilar data from the ToGA trial[11] and Jan Trøst Jørgensen et al in their study in 2012[17] observed a higher level of HER2 overexpression or amplification in the intestinal phenotype compared to the diffuse or mixed types. In contrast Sekaran et al in 2012[21] found similar overexpression of HER2 in both intestinal and diffuse histological types. The preferential Her2 expression in intestinal type of tumors as opposed

to diffuse type can be explained on the basis of specific tumor characteristics, similar to breast carcinoma. In breast cancers, invasive ductal carcinoma expresses Her2 in much higher percentage as compared to lobular carcinoma which shows loss of E-cadherin gene function. Diffuse carcinomas of the stomach also show loss of E-cadherin gene. An inverse relation between Her2 expression and E-cadherin loss has also been proven in gastric tumours[32].

Regarding the grade of tumour our study showed it had a significant relation with HER2 overexpression with higher frequency of expression in moderately differentiated type (45.20%, p-value 0.003). The Ghosh et al.[23] study favours HER2/neu over expression poorly differentiated gastric carcinomas with a statistical significance difference between the two histological grade (poorly differentiated versus well and moderately differentiated, p=0.0159). Some other studies like the Alina Bădescu et al in 2012[29] have shown that it is more common in cases of well-differentiated gastric carcinomas, but they added that positive staining was also found in poorly differentiated carcinomas indicating that HER2 is not uniquely linked to a specific differentiated type.

According to UICC staging of gastric tumour we found that it has a statistically significant relation with HER2/ neu overexpression and more frequently associated with UICC stage III (51.40%, p-value <0.001). Within sub groups HER2 positivity is not associated with depth of tumour invasion and lymph node status, but there is a statistical significant correlation with M0 stage (27.6%, p-value 0.028). Yan et al in the year 2011[33] reported that HER-2 status was correlated with the depth of invasion, TNM stage, lymph node and distant metastasis of gastric cancer (P < 0.05) and well supported by El-Gendi et al. [34]and other studies. In contrast Abdel- Salam et al. in 2018[35] could not found the relation and that was in agreement with Chua et al.[30].

HER-2 amplification and over-expression has a definite role in initiation, progression, and metastasis of some common cancers, including breast and gastric cancer. HER-2 has been implicated as an important prognostic factor for these cancers. The patients with HER-2 positive breast cancer have significantly shorter survival time than those with HER-2 negative tumours. The prognostic value of HER2 gene amplification and protein overexpression in gastric adenocarcinoma is not well established as it is in breast cancer. A retrospective study in Singapore showed that patients with intestinal-type gastric cancer who underwent curative surgery the HER2overexpression was 9.4% but had poor survival[26]. On the contrary, in a large retrospective study of over 900 cases though there was similar rates of HER2 positive cases (<10%), but a correlation with survival was not concluded. In A review of 35 published studies for the evaluation the prognostic value of HER2, majority of studies found no differences in the overall survival (OS), while two of them found a longer OS, 13 reported a significantly poorer OS[30].

Our study, as regards to the prognosis of gastric carcinoma, revealed a significant correlation between HER-2/neu and tumour stage (stage III), and 29 HER-2/neu-positive cases were T3/T4, representing 22.7% of T3/T4 cases, though T stage or N stage by themselves have no significant correlation with HER2 expression and M0 stage is significantly related to HER2 in our study. Supporting this few of previous studies concluded that though there were HER2/neu overexpression in advanced cases, there was a non-significant correlation between positivity and different poor prognostic factors [23, 27, and 8]. On the other hand few studies also reported that there was a significant correlation and close association between HER-2 expression and aggressiveness of the tumour[36]. In our study survival analysis could not be done due to short follow up time during the study period.

Conclusion

HER2 has been decorated as a prognostic and predictive biomarker in breast and gastric cancers

and the recent introduction of trastuzumab for the treatment of advanced gastric cancer has now made the assessment of HER2 status mandatory for selecting patients eligible for this treatment. Assessment of Her2 expression in gastric tumours should be made routine to provide a therapeutic advantage in Indian patients, and there should be standardized criteria for Her2 detection in order to bring uniformity and accuracy of results. In our study we found a significant association of HER2/ neu with intestinal and moderately differentiated subtype and UICC stage III gastric cancer without any association with age, sex and tumour location. So we may conclude that HER-2/neu expression in gastric carcinomas may be, related to their aggressive clinical behaviour but to stampHER2 status as an independent prognostic factor and to further define the role of Her2 expression in gastric carcinoma in Indian patients further research is required.

Limitations

- The gold standard test (FISH) could not be done, as was not available in our setup, for confirmation of HER2/neu overexpression.
- Follow-up of the cases was not feasible due to short study period wide residential spread of patients and hence was not included study design.

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