# Clinicopathological study of gastric cancer in a tertiary care hospital .

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## ABSTRACT

**BACKGROUND**: Our aim is to study the  histological subtypes of gastric carcinoma and its association with H-Pylori.

**METHODS**: Over 120 endoscopic gastric biopsies and 80 gastrectomy specimens received in our department during the period  1st January 2015 to 30 Nov 2016 were  studied.

**RESULTS**: The total numbers of 200 cases of gastric carcinoma were evaluated out of which 80 were gastrectomy specimens and 120 were from gastric endoscopic biopsies. When Lauren classification was used to classify histological type of tumor ,out of 200 cases maximum were intestinal type 105 cases (52.5%) followed by diffuse type 81 cases (40.5%). There were 14 (7%) cases of unclassifiable/mixed type in this study. It was observed that Males were predominantly affected by intestinal type( 80 cases, 40%) of gastric adenocarcinoma, followed by diffuse type (53cases,26.5%) whereas the reverse was seen in females as they were affected by diffuse type in (28 cases,14%) followed by intestinal (25 cases,12.5%). It was also observed that diffuse type was  more frequent in women than non-diffuse .When WHO classification was used to classify histological pattern of gastric adenocarcinoma, the tubular type (42%) turned out to be most prevalent followed closely by( 40.5%)poorly cohesive (including signet ring cell carcinoma) & then by papillary adenocarcinoma (8.5%), mucinous adenocarcinoma 2% (MAC), mixed adeno-neuroendocrine (1%) and adenosquamous carcinoma (1%) in decreasing order. The SCC(Squamous cell carcinoma)& HEPATIOD variants were the least common variants (0.5%) each. The association of Helicobacter(H) pylori with histological  intestinal  type of adenocarcinoma was statistically insignificant (p >0.05).Majority of patients had score 0 for Human epidermal growth factor receptor2(HER2) (66.66%) while 16.66% of patients showed a score of 3+suggesting HER 2 positivity. A score of 2+ i.e., equivocal was seen in 13.33% of cases and 3.33% of cases were of score 1+

**CONCLUSION**: The present study provides a fair insight into the histological patterns of gastric cancer in our institution. Although it is a observational analysis yet several precursor lesions were found to have statistically significant correlation with intestinal type adenocarcinoma.

**Keywords**  :Gastric ,Cancer ,Subtypes, H-Pylori

**Introduction**: Although it is steadily declining in incidence, cancer of the stomach (also known as gastric cancer) remains one of the most common and deadly neoplasms in the world . Despite some advances in the prevention and treatment of gastric cancer, 5-year survival remains around 20% in most parts of the world and the prognosis of advanced gastric cancer remains poor.Among the malignant tumours of the stomach, carcinoma is the commonest accounting for about 90-95% cases.[2]Next in order of frequency being lymphomas (4%), carcinoids (3%) and malignant spindle cell tumours (2%) .[3]Gastric adenocarcinomas are primarily classified as cardia and non-cardia based on their anatomic site. Cancers of the gastric cardia arise in the region adjoining the esophageal-gastric junction and thus share epidemiological characteristics with esophageal adenocarcinoma (EAC). Non-cardia cancer, also known as distal stomach cancer, is more common and arises in the lower portion of the stomach.[4]Histologically the two main types of gastric cancer are the diffuse and intestinal type.[5]The identification of the association between gastric carcinoma and H. Pylori  infection has been the most important development in gastric cancer epidemiology, and H. pylori has been classified as a Group I carcinogen by the World Health Organisation (WHO).H. pylori infection results in chronic gastritis in the majority of infected personsand is strongly associated with gastric atrophy and intestinal metaplasia.[6]

**Methods**: The present study was observational in nature. This study was undertaken at the department of pathology in our tertiary care hospital. Over 120 endoscopic gastric biopsies and 80 gastrectomy specimens received in our department , during the period 1st January 2015 to 30 Nov 2016 were included in the study.

**Method of Study:**

**Inclusion & exclusion criteria**

All endoscopic gastric biopsies and gastrectomy specimens of histologically diagnosed gastric carcinoma were included in the study. Gastric biopsies with inadequate biopsy material and gastric lesions other than carcinoma are excluded. Additionally all endoscopic biopsies of histologically confirmed cases carcinoma of Gastroesophageal junction( GE Junction) were also excluded, while sharply observing the criteria laid down in 7th Edition of the TNM classification by American Joint Committee on Cancer (AJCC).

The clinical information of the patients who underwent said procedures during this period was obtained from the histopathological requisition forms and any deficient relevant information wherever possible was procured from the clinical case sheets and the concerned clinician. The relevant investigations were obtained from the clinical case sheets and recorded.

The endoscopic gastric biopsies & gastrectomy  specimens received by the Department of Pathology were properly labelled, numbered and then subjected to gross and detailed histopathological examination. The patients details were carefully assessed to avoid double registration of any patient. Endoscopic Biopsies were taken and spread gently on a piece of filter paper 2x2cm. The raw surface adhered to the filter paper so curling of the tissue was prevented, then the tissue was put in 10% formalin along with the filter paper for 24hours. The gross serial sections & endoscopic biopsies after fixing in 10% buffered formalin, were

dehydrated with ascending grades of alcohol, cleared in xylene and embedded in paraffin. Multiple 4 micrometer thick paraffin sections were cut on a rotary microtome, dewaxed and stained routinely with haematoxylin and eosin.

### Immunohistochemistry (IHC) analysis

30 histologically confirmed tissues of gastric cancer were fixed in 10% formalin and embedded in paraffin. Immunohistochemical staining was carried out using anti-HER2/neu (Dako, Glostrup, Denmark) as the primary antibody for c-erbB-2.

### Statistical analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean + SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data were made,

Student t-test was used to find the significance of study parameters on continuous scale between two groups on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistics: Suggestive significance (p-value: $0.05 < p < 0.10$)

Moderately significant ( p-value: $0.01 < p < 0.05$)

Strongly significant (p-value: $p < 0.01$)

The Statistical software, SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables.

**Ethical Clearence:**Ethical clearance was taken by institutional ethical committee

## Results:

This study was undertaken from 1 Jan2014 to 30 Nov 2016 in the department of pathology, in our hospital . The total number of 200 cases of gastric carcinoma were evaluated out of which 80 were gastrectomy specimens and 120 were from gastric endoscopic biopsies.

Out of 200 cases 144 (72%) were male and 56(28%) were female, with male to female ratio of 2.6:1. The age range of the study patients was between 20 and 84 years and the mean age was 58.3±13.04 years. In our study all the 80 surgically resected stomach specimens were assessed for location of the tumor, out of which 42(53%) were located only in antro-pyloric region, followed by body 23(29%), antrum plus body 8(10%), Cardia &  fundus 6(7%),whole stomach1(1%) in decreasing order.Location of growth/ superficial ulcer [site] based on the gross examination of the resected specimens N=80.Five cases were of early gastric cancer type & only superficial ulceration could be identified .Antrum is the commonest location of growth of stomach carcinoma even on Endoscopic evaluation done. Out of 80(N=80) surgically resected specimens' of gastric carcinoma received in our department during the study period, 5 turned out to be cases of Early gastriccancers.

Bormann classification was used to classify the gross morphology of tumor in the  advanced gastric cancer cases.  Thus Gross type was evaluated for the 75 surgically resected stomach specimens (N=75).In advanced gastric carcinomas, infiltrative type of tumour was the commonest (47%), out of which 45.33% were localized type and & one case(1.33%)  was  of diffuse type (Linitisplastica). The other types in decreasing order were fungating (32%) ulcerative (17%),,and polypoidal (4%).

Lauren (1965) & WHO (2010) classifications was used to classify histological type of tumor.& comparison was done between the two classification systems.

(a) Lauren (1965)

Histopathological categorization based on Lauren classification in our study showed 52.5% cases of intestinal type of adenocarcinoma and 40.5% of diffuse type of adenocarcinoma.7% cases were unclassified.

(b)WHO CLASSIFICATION (2010): Histopathological categorization as per this classification showed 42% cases of tubular adenocarcinoma followed by 40.5% cases of signet ring cell carcinoma ,8.5% cases were of papillary adenocarcinoma,4% were of mixed type. Mucinous adenocarcinoma constituted 2% of cases. 2 caseseach of adenosquamous and mixed neuroendocrine carcinoma was also noted.One case of hepatoid carcinoma was also seen in our study.

**Table 1:** Showing the relative proportions of various Histopathological categorizations based WHO (2010)& LAUREN (1965) classification found in our study. A Comparative picture between various Histopathological categorizations of two classifying schemes is also presented.

| Lauren classification | % | WHO classification | % |
|---|---|---|---|
| Number Intestinal type Number=105 | 52.5% | tubular adenocarcinoma Number=84 | 42 |
| | | papillary adenocarcinomaNumber=17 | 8.5 |
| | | mucinous adenocarcinoma (MAC) Number=4 total=105 | 2 |
| Diffuse type Number =81 | 40.5% | Signet-ring cell carcinoma And other poorly cohesive carcinoma Number=81 | 40.5 |
| Unclassifiable /mixed Total=14 | 7% | Mixed Number =8 | 4 |
| | | Adenosquamous carcinoma Number=2 | 1 |
| | | Squamous cell carcinoma Number=1 | 0.5 |

| | | Mixed adeno-neuroendocrine carcinoma Number =2 | 1 |
|---|---|---|---|
| | | Hepatiod adenocarcinoma Number=1 | 0.5 |
| Total number=200(105+81+14) Table no 7 | 100% | Total number=200(84+17+4+81+8+2 +1+2+1) | 100 % |

The Japanese classification system categorizes gastric adenocarcinomas into two groups: differentiated and undifferentiated. The differentiated group consists of well-differentiated, moderately-differentiated and papillary adenocarcinoma(FIG 1A). The undifferentiated group consists of poorly differentiated adenocarcinoma (PDAC) and Signet ring cell carcinoma. Interestingly, Mucinous adenocarcinoma(MAC) can be regarded as either a differentiated or undifferentiated type depending on the predominant components.

In our study differentiated group comprised of following

Well differentiated tubular adenocarcinoma=15 cases

Moderately differentiated adenocarcinoma=60 cases

papillary adenocarcinoma=17 cases

**Total number of cases in differentiated group =92(46%)**

& the undifferentiated group comprised of

poorly differentiated adenocarcinoma (PDAC)=9 cases

poorly cohesive (including signet ring cell carcinoma=81 cases

Mucinous adenocarcinoma(MAC) =4 cases

**Total number of cases in undifferentiated group =94(47%)**

Five cases of early gastric cancers were identified in our study of 80 gastrectomy specimens carcinoma. These were categorized based on Japanese macroscopic classification for early gastric cancer².

**Table 2Showing the frequencies of all surgically resected gastric specimens with respect to early/advanced stage.**

| Stage | intestinal | Diffuse | Unclassifiable/mix ed |
|---|---|---|---|

|  | number | %=n/46 | number | %=n/28 | number | % |
|---|---|---|---|---|---|---|
| Early gastric cancer | 5 | 11 | 0 | 0 | 0 | 0 |
| Advanced gastric cancer | 41 | 89 | 28 | 100 | 6 | 100 |
| Total | 46 | 100% | 28 | 100% | 6 | 100% |

**Table 3:Showing frequencies of H pylori infection in different Histopathological types of adenocarcinoma based on Lauren classification. H pylori were detected only on microscopic examination of H&E, GIEMSA stained slides. N=110**

|  | Intestinal | Diffuse | Mixed |
|---|---|---|---|
| H pylori + | 29(42%) | 11(31%) | 2(33%) |
| H pylori - | 40(58%) | 24(69%) | 4(67%) |
| TOTAL | 69(100%) | 35(100%) | 6(100%) |

The association of H pylori with intestinal type adenocarcinoma was statistically insignificant (p >0.05.

IHC: Majority of patients was of score 0 (66.66%) while 16.66% of patients showed a score of 3+suggesting HER 2 positivity(Fig 1B). HER2 positivity was more in gastric biopsies (22.22%) as opposed to 8.3% in gastrostomies. A score of 2+ i.e., equivocal was seen in 13.33% of cases and 3.33% of cases were of score 1+.In our study the correlation of HER2 overexpression with gender was statistically weak (p>o.o5).

**Discussion:**

Globally, variations observed in gastric cancer incidence and histological appearance in populations show geographical distribution. In India, South Indians are found to be more prone to gastric carcinoma and it comprises about 22.5%-32% of all gastrointestinal tract carcinomas in South India. Kashmir, one of the three provinces of Jammu & Kashmir state is in the northern most part of the country too has a high incidence of gastric cancer possibly due to their, special personal habits.[7] This study was undertaken from 1 Jan 2015 to 30 Nov 2016 in the department of pathology of our college. A total number of 200 cases of gastric carcinoma were evaluated out of which 80 were gastrectomy specimens and 120 were from gastric endoscopic biopsies.

Of the 200 patients of gastric carcinoma, 72% were males & 28% were females. Thus males showed a higher incidence of gastric carcinoma as compared to females with male to female ratio being 2.6:1. There is wide variation in the gross appearance of advanced carcinoma of the stomach. Bormann (1926) based his time-honoured gross classification of advanced gastric carcinoma into four types on the relative proportions of exophytic and endophytic components. Many intermediate stages exist between the two extremes, represented by the

fungating tumor growing mainly into the lumen and the flat, ulcerated, and deeply invasive tumor growing through the wall of the stomach.[8]Though being the commonest, relatively lower proportion of infiltrative lesions in our study than the aforementioned studies could be explained by the fact that intestinal type which contributed 105(52.5%) of adenocarcinoma usually form exophytic lesion. Majority (60.29%) of patients presented with vague symptoms of dyspepsia, abdominal pain. This finding is supported by Sigonet aland Chattopadhyay et al., who also found that pain abdomen was commonest symptom (84%) in gastric cancer patients rather than obvious mass. [9,10]Present study showed positive family history in a single case of diffuse gastric carcinoma (0.8%).

When Using Lauren's classification 81(40.5%) each of diffuse 105(52.5%) intestinal & 14(7%) unclassifiable /mixed gastric adenocarcinomas were diagnosed. In the present study, ratio of diffuse and intestinal adenocarcinoma is 0.8:1 .Several studies found wide variation in relative incidence of these two subtypes throughout the world. Where some authors like Mihailovic et al., and Biggar et al., found that diffuse variety occurred more frequently than intestinal type, (55% and 62%, respectively)[.11],[12] have found the reverse (diffuse 36%). Due to endemicity of *H. pylori* in our country relative incidence of intestinal adenocarcinoma is higher in India equating to diffuse variety. Sakitaniet al., showed this hypothesis that intestinal type is prevalent with people carrying high risk factors like intestinal metaplasia andGonzαlez et al., showed intestinal metaplasia is one of the significant risk factor, also identified several risk factors like cigarette smoking.[13,14]Though H pylori endemicity might explain the relative increased incidence of intestinal type adenocarcinoma ,but this appears to be less contributory as previous studies (Malik G.M et al. 1999)& ours study failed to show any significant association between H pylori infection & intestinal adenocarcinoma.[15] So the most appropriate answer would lie in peculiar dietary habits of our region. Kashmir, one of the three provinces of Jammu & Kashmir state is in the northern most part of the countryand the beautiful hilly terrain, gifted natural settings and the peculiar life style of the people are significantly different from the rest of country. The various factors can be linked to the high risk of gastric cancer in Kashmir. First, the peculiar Geography of the valley (situated at an altitude of 1800-2400 m above the sea level) with severe cold winter may have a bearing on the etiology.Further, the intake of a large quantity of rice by a Kashmiri native may lead to delayed gastric emptying, which can also be accounted for a possiblepredisposition.[15] But, the most important of the predisposing factors may be the special & peculiar dietary habits of the Kashmiris.[15]. The dietary items containing substantial amounts of N-nitroso compounds include: salt tea, dried fish, vegetables especially Brassica olerecea (haak), red chillies, and spice cake (wur).[15]Thus due to our special dietary habits relative incidence of intestinal type adenocarcinoma is higher in the present study than to diffuse type adenocarcinoma. WHO classification was also utilized for Histopathological categorization. Following observations were made tubular adenocarcinoma (42%) was most frequent followed by poorly cohesive & signet ring cell carcinoma (40.5%), papillary adenocarcinoma (8.5%), mixed (4%) & other uncommon variants i,eAdenosquamous carcinoma (1%), Squamous cell carcinoma (0.5%). Mixed adeno-neuroendocrine carcinoma (1%), Hepatiod adenocarcinoma (0.5%)(Table 4)

Table : 4 ShowingSchematic comparison between Lauren & who classifications with respect to the data of present study

| Histological variant | Intestinal | Diffuse | Mixed | Mixed Adeno-neuroend | Hep-adenocarcinoma |
|---|---|---|---|---|---|
| | | | | | |

| Microscopic & Macroscopic (size) properties | | | 46 | n/46% | 28 | %n/28 | 4 | 1 | 1 |
|---|---|---|---|---|---|---|---|---|---|
| Size (greatest tumor dimensions) | ≤5cm | | 29 | 63.04 % | 6 | 21.42 % | 1 | | |
| | >5cm | | 17 | 36.95 % | 22 | 78.57 % | 3 | 1 | |
| Level of infiltration | LP | T1 | 5 | 11% | 0 | 0% | | | |
| | M M | T2 | 9 | 20% | 3 | 11% | | | |
| | SS | T3 | 14 | 30% | 5 | 18% | 1 | 1 | 1 |
| | I S | T4a | 18 | 39% | 19 | 68% | 3 | | |
| | NO | T4b | 0 | 0% | 1 | 3% | | | |
| Lymph node involvement | Nil | N0 | 26 | 57% | 5 | 18% | 1 | | |
| | 1-2 | N1 | 8 | 17% | 4 | 14% | 1 | | 1 |
| | 3-6 | N2 | 7 | 15% | 6 | 22% | | 1 | |
| | 7-15 | N3a | 5 | 11% | 11 | 39% | 2 | | |
| | >15 | N3b | 0 | 0% | 2 | 7% | | | |
| Lymphovascular invasion | | | 19 | 41% | 15 | 54% | 3 | 1 | 1 |
| Perineural invasion | | | 17 | 40% | 18 | 64% | 3 | 1 | 1 |

H.pylori is classified as a group I carcinogen by WHO & is strongly associated with gastric carcinoma. H.pylori infection is found in both intestinal and diffuse type of gastric carcinoma.[16] The association of H pylori with histological type of adenocarcinoma based on Lauren classification was found to be statistically insignificant (p >0.05).

However to know the exact correlation between H pylori & gastric adenocarcinoma Histopathological observations have to be substantiated with culture, serology and other immunological techniques.

We did find statistically significant correlation of HER 2 positivity with level of differentiation & with respect to lauren classification.(p<0.5) The reasons for the selective overexpression of HER2 in intestinal type of gastric carcinomas are complex. The association of HER2 with a specific histologic Tumour type suggests that intestinal and diffuse types develop along different molecular pathways. None of the diffuse cases were positive for HER2. Though review of literature shows decreased percentage of diffuse type of gastric cancers with HER2 overexpression, none of the studies have recorded a total absence of HER2 expression HER2 positivity.

One of the drawbacks of our study was that we were not able to confirm IHC 1+ and 2+ cases by FISH. Studies have shown excellent IHC-FISH concordance (>95%) in IHC 0 and IHC 3+ cases, suggesting that these IHC scores may not require routine FISH confirmation. Reliable separation of IHC 1+ and IHC 2+ patterns is challenging in small biopsy specimens, due to crush and edge artifacts.

**Conclusion**: The newest WHO classification of gastric carcinoma is by far the most comprehensive classification , describing the morphologic characteristics of each subtype in detail. Hopefully, it will help understand the clinicopathologic entity of each subtype by correlating its histologic feature with molecular profiling and clinical behaviour.

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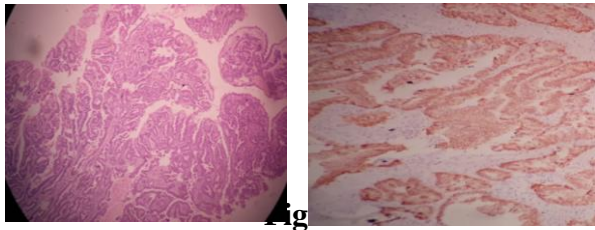
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FIGURES:



Figure 1 A/B:  HPE (H&E STAIN) Papillary adenocarcinoma stomach, IHC showingstrong  membranous reactivity  for HER2 .