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Role and association of Hsp90, Her2, p53 and Ki-67 in Gastric and Colorectal Neopalasms in Indian context

Short Title: Expression of Hsp 90, Her2, p53 and Ki-67 in GIT Tumors

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Abstract: Introduction: Gastrointestinal malignancies are on raise and there is a need for predictive and prognostic markers to study the tumor behavior. In the present study we have evaluated four most important markers in these tumors in Indian population so that drugs targeting these proteins can be included in the therapeutic regimes. We also compared the expressions with the grade and stage of the tumors. Materials and methods: The present study is an observational cross-section study. We have included neoplasms of Stomach, Colon and Rectum, 30 benign and 30 malignant neoplasms from each organ. Results: HSP90 expression was associated with the grade and stage of all tumors except grade of colonic tumors. HER2 was associated with grade and stage of all tumors except stage of stomach & rectum and grade of carcinoma colon. Ki-67 expression was associated with grade and stage of all tumors except stage of rectal carcinoma. p53 is associated with grade and stage of all the tumors. Conclusion: Marked geographical variations have been identified in the phenotypic characters of the tumors. An understanding of the pattern of expression of prognostic and predictive markers shall be helpful in better analysis of the tumor behavior.

Keywords: Gastrointestinal neoplasms, HSP90 expression, Ki-67 expression, p53 expression, Her2 expression

1. INTRODUCTION

Gastrointestinal neoplasms are one of the leading causes of cancer deaths worldwide. In India, as per the incidence data derived from the recent report of ICMR/NCDIR-NCRP (2012-2016), cancer of gastrointestinal Tract accounts for 19.7% of total cancer incidences [1] next to Tobacco Related Cancers (Lung, mouth and esophagus). Recent statistics show that the overall incidence of cancer has increased in recent years. Several prognostic and predictive markers related to the gastrointestinal neoplasms are being identified and novel chemotherapeutic drugs are formulated targeting these markers. Human epidermal growth factor 2 (HER2), Heat shock protein 90(HSP 90), p 53and Ki-67 are few such important markers and drugs targeting these molecules are being included in the latest therapeutic regimens. Several studies have earlier looked to relate the expression of these markers to the staging, treatment response and survival of the patients with gastrointestinal malignancies in various population groups. All the studies have reported a wide range of inferences. Hence, there is a need to standardize the data by conducting studies in small population groups and deriving data pertaining to that geographical area. In the present study, we have analyzed the expression of all these four markers in carcinoma Stomach, Colon and Rectum in a tertiary care centre in south India.

2. MATERIALS AND METHODS

The present study is an observational cross-section study conducted in the department of pathology, Gandhi hospital, Hyderabad. Ethical clearance was obtained from Institutional Ethical Committee, Gandhi Medical College and informed consent was taken from the patients. We have included neoplasms of Stomach, Colon and Rectum in our study as these are the commonest tumors of gastrointestinal tract. Both endoscopic biopsies and resected samples were included in the study. Normal mucosa was used as control. Cases included 30 benign and 30 malignant neoplasms each from stomach, colon and rectum. The specimens were fixed in 10% buffered neutral formalin. The biopsies were processed in- toto. From the resected specimens, the tumor area was identified and tissue from that area was processed. The tissue selected was processed by the routine technique. The tissue blocks were cut for 4 micron thick sections and were stained with Hematoxylin and eosin stains. Later these slides were examined and sections for Immunohistochemistry were selected. The sections were later stained with antibodies to HSP90, HER2, Ki-67 and p53. Immunohistochemical staining was done using peroxidise-anti-peroxidase method according to the standard protocol and Primary antibody (HSP90, HER2, Ki67 and P53) was from Dako. Positive immunoreaction is characterized by dark brown color of the targeted protein. The scoring was done by the following standard protocols: HSP 90- score 0 < 5% of positivity(Negative),1+ 5% - 20%, 2+ 20% - 50% and 3+ >50% cells, HER 2 - Score 0 No reactivity or membranous reactivity in <10%, 1+ Faint/barely perceptible membranous reactivity in >10% of tumor cells; cells are reactive only in part of their membrane(Negative), 2+ Weak to moderate complete, basolateral, or lateral membranous reactivity in >10% of tumor cells(Equivocal), 3+ Strong complete, basolateral or lateral

membranous reactivity in >10% of tumor cells (Positive)(Ruschoff et al), Ki 67- Score 0 <5% positive cells, 1+ 6% - 25% positive cells, 2+ 26% - 75% positive cells, 3+>75% positive cells, P53- Score 0 < 5% positive cells, 1+ 6% -25% positive cells, 2+26% - %75% positive cells, 3+>75% positive cells. The evaluation of the expression of the markers was done by two pathologists independently. The expression of these markers was correlated with the histologic grade and stage of the tumor. The tumors were categorized in to 4 stages, T1 to T4 stages (WHO classification of the Tumors, 5th Edition) .The results were compiled and statistically evaluated using Pearson chi-square test, SPSS 23rd version. P value was calculated with significance less than 0.05.

3. RESULTS:

A total of 210 samples were included in the study which included Controls as well as Test samples of three sites in the gastrointestinal system- Stomach, Colon and Rectum. For each site 10 controls (Normal Tissues), 30 Benign and 30 Malignant tumors were studied.

STOMACH:

In our study, we had three types of benign neoplasms in stomach. Majority of them were Gastric Hyperplastic polyps (24cases) comprising 80% of the benign tumors. The expression of all the four markers was studied in these neoplasms. HSP90 expression was negative in majority of the benign neoplasms (26 cases, 87%).4 cases (13%) showed +1 positivity. Her 2 was negative in 23 cases (77%) of the benign neoplasms. 1+ score positivity was seen in 7 benign hyperplastic polyps of the stomach. Ki-67 and P53 were negative in all the benign neoplasms of the stomach. The age of the patients with carcinoma stomach ranged from 38 years to 68 years with a mean of 51.96 years. Majority of the patients were males with male: female ratio being 6:1.Among 30 malignant cases, 29 cases were microscopically diagnosed as Adenocarcinoma NOS and 1 case was diagnosed as Neuroendocrine carcinoma. Only one case of Adenocarcinoma was poorly cohesive Signet Ring Cell carcinoma and rest of all the tumors were Tubular type. We have excluded neuroendocrine carcinoma and signet ring cell carcinoma from the grading. Out of the 28 cases, 16 were well differentiated (57%), 8 were moderately differentiated (28%) and 4 were poorly differentiated (15%).T1 stage had 7 cases (23.5%), T2 15 cases (50%), T3 had 8 cases (26.5% and there were no cases in T4 stage. HSP 90 expression was positive in 21 cases (70%) and negative in the remaining 9 cases (30%). Score 1 was seen in 11 cases, score 2 in 9 and score 3 in 1 case. Amongst well differentiated tumors, 5 showed score 0 ,7 cases 1+,4 cases 2+ .In moderately differentiated tumors, 3 showed score 0, 2 cases1+,3 cases2+ and none of them showed 3+ score. In poorly differentiated tumors, all the tumors showed positive staining with 1 case 1+ score, 2 cases 2+ and 1 case +3 score. These results were statistically correlated and P value was found to be Significant (P value 0.035).The expression of this marker was also correlated with the staging of these tumors. Stage T1 had 7 cases, T2 had 15cases, and T3 had 8 cases. In our study there was no case in T4 stage. The scoring of HSP 90 increased with the stage of the tumor. P value

was Significant, <0.05 (P value-0.017).HER 2 was positive in 18 cases(60%) and negative in 12 cases(40%).7 cases(23.3%) showed Score 1+,8 cases(27%) showed score +2 and 3 cases(10%) showed 3+ score. Out of 16 cases of well differentiated carcinoma,7 cases had score 0,5 cases +1,and 4 cases 2+.No cases showed +3.Out of 8 cases of moderately differentiated carcinoma ,4 cases score 0,2 cases 1+ and 2 cases showed +2 score. Majority of the poorly differentiated carcinoma showed score 3+. P value was found significant, <0.05 (P value 0.001). We also correlated the expression of the marker with stage of the tumor and P value was Not significant (P= 0.07).There was no association between the expression of the marker and stage of the tumor in Gastric Carcinoma.Ki-67 was positive in all the cases of carcinoma Stomach.8 cases (27%) showed Score 1+, 19 cases (63%) showed score 2+ and 3 cases (10%) showed score 3+.The expression of Ki-67 was studied in correlation with the grade and stage of the tumor. Out of 16 cases of Well differentiated carcinoma,6 showed 1+ and 10 score 3+.Out of 8 cases of moderately differentiated cases,1 showed 1+ and 7 showed 2+.Out of 4 cases of poorly differentiated ,1 case showed 2+ and 3 showed 3+ score. P value was calculated and was found significant (P value = <0.001). The association of Ki-67 with the stage of the tumor was statistically analyzed and P value was found to be significant,< 0.05(P value=0.003).P53 was positive in 26 cases out of 30 cases.4 cases (13%) were negative with score 0.16 cases(53%) showed score1+,8 cases(27%) showed score 2+ and 2 cases (7%) show 3+ score. The expression of this protein was studied in relation to the grade and stage of the tumor. In well differentiated tumors 3 cases were negative with score 0, 10 cases showed 1+ positivity and 3 cases showed 2+ score. Out of 8 cases of moderately differentiated cases,1 showed 1+ and 4 showed 2+and 1 showed score 3+.Out of 4 cases of poorly differentiated cases,2 showed 2+ and 2 showed 3+ score. P value was calculated and was found significant (P value = <0.001). The expression of P53 was correlated with the staging of the tumor and was found to be significant, P< 0.05.(Table 1)

Table 1: Expression of proteins in carcinoma stomach

Marker	Score 0	Score 1+	Score 2+	Score 3+
HSP 90	9(30%)	11(36.7%)	9(30%)	1(3.3%)
HER 2	12(40%)	7(23%)	8(27%)	3(10%)
Ki-67	0	8 (27%)	19(63%)	3(10%)
P53	4(13%)	16(53%)	8(27%)	2(7%)

COLON:

In our study out of 30 Benign tumors 12 cases were of tubular adenoma,.16 cases of Tubulovillous adenoma. We had 2 cases of Villous adenoma. The expression of the four markers was studied in these benign neoplasms. HSP 90 was negative in all the benign neoplasms. HER 2 was negative in 27 (90%) cases and was positive with score 1+ in 3 (10%) cases. Ki 67 and P 53 were negative in all the cases.

The mean age of the patients with adenocarcinoma ranged from 32 to 64 years with a mean age of 49.33 years. Males were predominant with a ratio of 1:6.Majority of the tumors (70%) were left sided.

Hsp90 was negative in 2 of the adenocarcinomas,11 cases showed score 1+,16 cases showed 2+ and only one case showed 3+.. HER 2 expression was positive in all the malignant cases.14 cases showed score 1+, 15 showed 2+ and only one case showed 3+.Ki 67was positive in all the cases.6 cases showed 1+, 22 showed 2+ and 2 showed 3+.P 53 was negative in 2 cases, score 1+ in 6 cases, score 2+ in 20 cases and 3+ in 2 cases. In our study, out of 30 cases, 18 were well differentiated, 9 were moderately differentiated and 3 cases were poorly differentiated. Out of 30 tumors, 4 cases were in T1 stage, 17 were in T2, 7 in T3 and only 2 cases in T4 stage.

In well differentiated tumors, negative expression was seen in 0ne case,1+ in 4 cases ,2+ in 13 cases and none of the tumors expressed score 3+.In moderately differentiated tumors 1 case showed no expression,6 showed score 1+,2 showed score 2+ and none of the cases showed 3+ score. Out of the 3 poorly differentiated cases, 1 showed 1+ score, 1 showed 2+ and one showed 3+ score expression. The P value was Not significant (P= 0.41) in correlation with the grade of the tumor. When the expression of this marker was correlated with the stage of the tumors, P value was found to be Significant (p=0.001).

All the malignant tumors expressed HER2 .None of them had score 0.14 out of 30 cases expressed score1+,15 cases 2+ and 1 case showed score 3+ . The expression was correlated with the grade and stage of the tumor.18 cases were well differentiated ,out of which 11 showed score 1+,7 showed 2+ .Amongst 9 cases of moderately differentiate tumors,2 showed 1+,6 showed 2+ and 1 showed 3+ scoring. In poorly differentiated group, 2 cases showed 2+ and 1 showed 3+ scoring. P value in association with the grade of the tumor was found to be Insignificant (P=0.149).When correlated with the stage of the tumor P value was found Significant (P=0.009). Ki 67, was found to be positive in all the malignant tumors .6 cases showed 1+,22 showed 2+ and 2 showed 3+ score. The expression was also correlated with the stage and grade of the tumor. Out of 18 well differentiated tumors,4 had score1+,13 had 2+ .In moderately differentiate tumors,2 showed score 1+,6 showed 2+ and 1 showed 3+ score. Amongst 3 cases of poorly differentiated carcinoma, 2 showed 2+ and 1 showed 3+ scoring. The P value was found to be Significant (P=0.001).When the marker's expression was compared with the stage of the tumor, P value was Significant (P=0.007). P53 showed positivity in most of the tumors. Only 2 cases out of 30 showed negative staining.6 showed score 1+, 20 cases showed score 2+ and 2 showed score 3+.The expression was compared with the stage and grade of the tumor. Out of 18 cases of well differentiated carcinoma only one case was negative.5 cases showed 1+, 12 showed 2+ and none of them showed 3+ score. In moderately differentiated tumors,1 was negative,1 case showed 1+ and 7 cases showed 2+.In poorly differentiated carcinoma 1 case was 2+ and 2 cases showed 3+ scores. P value was found to be Significant (P=<0.001).When the marker expression was correlated with the staging of the tumors, P value was found to be Significant (P=0.001).(Table 2)

Table 2: Expression of the markers in malignant neoplasms of colon

MARKER	SCORE 0	+ 1	+2	+3
HSP90	02	11	16	01
HER 2	00	14	15	01
KI 67	00	06	22	02
P53	2	6	20	2

RECTUM:

Out of 30 benign neoplasms, 16 were Tubular Adenoma, 4 Villous Adenoma, 10 were Tubulo villous adenoma. The expression of all the four markers was negative in all the neoplasms except one cases of Tubulovillous adenoma which showed positivity for P53. Age of the patients with carcinoma rectum ranged from 38 years to 61 years with a mean age of 50.36 years. The tumor was common in males with the Male: female ratio being 10:1.The expression of these markers was studied in these tumors .HSP 90 was positive in 20 cases and remaining 10 cases showed score 0.14 cases showed score 1+, 4 score 2+ and 2 showed score 3+.HER 2 was negative in 4 cases, 1+ in 21 cases, 2+ in 4 cases and 3+ in 1 case. Ki 67 was positive in all cases, 20 showed 2+ and 8 showed 3+.P53 was negative in 2 cases, 1+ in 22 cases, 2+ in 6 cases and 3+ in 2 cases.

HSP 90 expression was studied in correlation with the grade and stage of the tumor. In well differentiated tumors, 10 tumors were negative, 14 cases 1+and 2 cases 2+. In moderately differentiated tumors, 2 cases showed score 2+ and 1 case showed 3+ score. One case of poorly differentiated carcinoma showed score 3+. P value was found to be significant (P = 0.001) in correlation to the tumor grade. The expression of HSP 90 was statistically analysed with the stage of the tumor and P value was found to be Significant (P=<0.001). HER2 expression was correlated with the grade and stage of the tumor. Out of 26 cases of well differentiated tumors,3 showed score 0,20 showed 1+,3 showed 2+.In moderately differentiated tumors,1 case showed score 0,1 case showed 1+ and only one case showed 3+.One case of poorly differentiated tumor showed 2+ score. The association of the marker with the tumor grade was found to be Significant (P=0.007).When expression of the marker was correlated with the stage of the tumor P value was Not significant (P=0.45). Ki67 expression was correlated with the grade and stage of the tumor. Out of 26 cases of well differentiated tumors, 22 showed 2+ score, 4 showed 3+ score. In moderately differentiated tumors, all the 3 cases expressed score 3+.One case of poorly differentiated carcinoma expressed score 3+. P value was found to be Significant (P=0.002).The expression of this marker when correlated with the stage of the tumors was found to be Not Significant (P=0.167). P 53 was scored as per the criteria and the results were correlated with the grade and stage of the tumor. In well differentiated tumors,1 case showed score 0, 20 cases showed score 1+ and 5 cases showed 2+.None of the cases showed 3+.In moderately differentiated tumors,1 cases showed score 0, 1 showed 2+ and one showed 3+ score. One case of poorly differentiated tumor showed score 3+.These results were statistically analysed and P value

was Significant (P=<0.001).On correlation with the stage of the tumor, Significant P value (P=0.001) was derived.(Table 3)(Table 4).

Table 3: Malignant Tumors of the Rectum

MARKER	SCORE 0	+ 1	+2	+3
HSP90	10	14	04	02
HER 2	04	21	04	01
KI 67	00	00	22	08
P53	2	20	6	2

Table 4: Significance of expression of Markers

	Hsp90 stomach grade	*HER 2 stomach grade*	*Ki 67 Stomach grade*	*P53 Stomach Grade*	*HSP 90 stomach stage*	*HER 2 stomach stage*	*Ki67 stomach stage*	*P53 Stomach Stage*
Chi-square, df	13.53	23.866	23.857	27.32	15.516	11.667	16.327	29.72
P value	0.035	0.001	<0.0001	<0.0001	0.017	0.07	0.003	0.003
Statistically significant? (alpha<0.05)	Yes	Yes	yes	yes	Yes	No	Yes	Yes

	HSP 90 colon grade	*HER 2 colon grade*	*Ki67 colon grade*	*P53 Colon Grade*	*HSP 90 colon stage*	*HER 2 colon stage*	*Ki 67 Colon Stage*	*P 53 Colon Stage*
Chi-square, df	13.158	9.34	14.673	14.673	22,331	13.44	17.86	45.3
P value	0.041	0.149	0.001	0.001	0.001	0.009	0.007	<0.0001
Statistically significant? (alpha<0.05)	No	No	yes	Yes	Yes	Yes	Yes	Yes

	HSP 90 rectum grade	*HER 2 rectum grade*	*Ki 67 rectum grade*	*P53 Rectum Stage*	*HSP 90 rectum stage*	*HER 2 rectum stage*	*Ki 67 rectum stage*	*P53 rectum Stage*
Chi-square, df	28.846	17.647	12.692	13.723	40.6	5.8	3.6	47.32
P value	P<0.0001	0.007	0.002	0.002	P<0.0001	0.45	0.167	<0.0001
Statistically significant? (alpha<0.05)	Yes	Yes	Yes	Yes	Yes	No	No	Yes

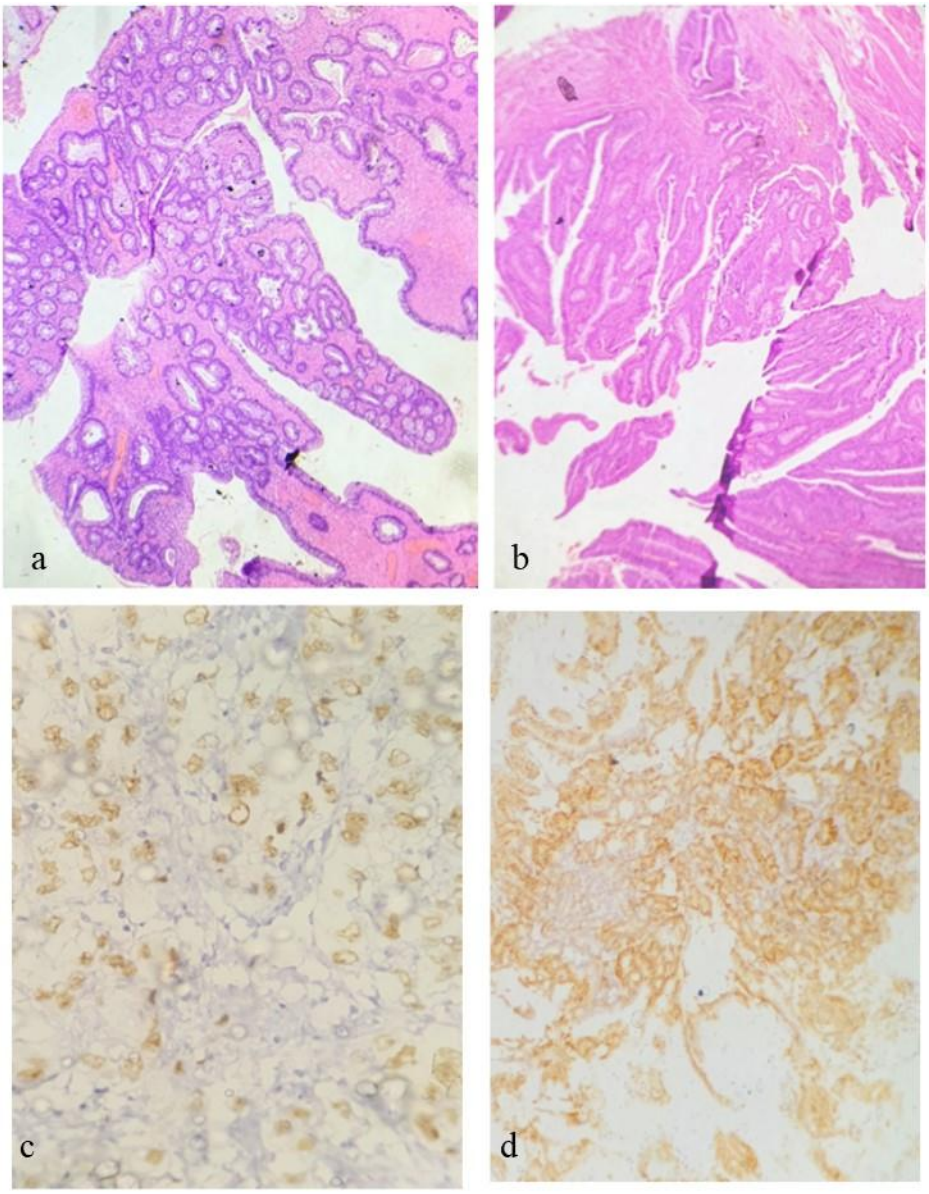

Figure 1. a. H and E staining. Tubular Adenoma, b. H and E staining. Villous Adenoma, c. IHC: HSP 90 expression , d. IHC: HER2 expression

4. DISCUSSION:

The overall cancer burden by gastrointestinal tumors is 273982 cases by 2020 march i,e 19.7% of the total cancers. It is expected to raise to 19.8% by 2025(ICMR/NCDIR-NCRP (2012-2016). An improvised diagnostic and therapeutic modalities are warranted to curtail the increase in the incidence of these tumor. In addition, gastrointestinal tumors show a varied behaviour in different patients with the same histologic type and same stage. Most of the patients present at a late stage. Hence it is necessary to include other predictive and prognostic markers in addition to the basic parameters like histologic typing and staging.

In our study, carcinoma stomach was most common in males and the mean age was 51.96

years. Arun Kumar Barad et al., has reported increased incidence of carcinoma stomach in males and the mean age in their study was 60 years [2]. They also observed that the most common histologic type as Adenocarcinoma NOS (95.6%).Similar findings were reported by OT Muslim et al., where in the tumors were common in males and the commonest age group was 40-60 years [3]. In our study, carcinoma colon was common in males and mean age was 49.33 years. Huan-Cheng Changet al., in their study reported that majority of cases of carcinoma colon were under the age of 50 years [4]. RudreshaA Haleshappa et al., also had male preponderance in carcinoma colon [5]. In our study carcinoma rectum was common in men and the mean age was 50.36 years.

HSP 90:

In our study the expression of HSP 90 was associated with the grade and stage of carcinoma stomach. The expression of the marker increased with the grade of the tumor. Similar findings were reported by Jiahong Wang et al., where in the over expression of HSP90 was observed in majority (69.6%) of gastric cancers [6]. According to them HSP 90expression was more in tumor with poor prognosis. Sabina berezowska et al., had reported contradictory results in their study where in HSP 90 expression was found to be associated with lower local tumor burden, absence of lymph node metastasis and better tumor differentiation [7]. Several researchers have proposed that inclusion of inhibitors of HSP90 would be beneficial to patients with advanced gastric cancer. Inhibition of HSP 90 expression could reduce angiogenesis and gastric cancer cell proliferation and overcome the resistance to chemotherapy [8, 9]. In our study, the expression of HSP 90 was negative in all of the benign neoplasms of colon but 96.6% of the malignant tumors showed positivity .In addition, the expression of the marker was not associated with the grade of the tumor in our study. On the contrary, in the study done by Qiu-Ran Xu1 et al., there was association between the expression of HSP 90 and grade of the tumor[9]. Similar findings were reported by Dong X,Lang L et al., ChenY, Ran ZH et al., and Z Milicevic et al., where in ,the expression of HSP 90 was associated with the grade of the tumor [11,12,13].In our study, expression of HSP 90 was found to be associated with the stage of the tumor. These findings were similar to the studies done by Qiu-Ran et al., where in, the expression of the marker increased with Dukes staging and lymphnode metastasis [9]. Z Milicevic et al., in their study, has reported that tumor progression of colonic carcinoma is associated with the expression of HSP 90(13).Christian Moser et al has proved that use of HSP 90 inhibitors inhibited the invasive properties of colonic cancer cells [14].

In our study, the expression of HSP 90 was seen in 66.6% of the Rectal adenocarcinomas .The expression of the marker was associated with the grade and stage of the tumor. Poorly differentiated tumors expressed more of the protein. The expression also increased with tumor invasiveness .These findings were similar to that of the study done by Qiu-Ran wi et al. Z Milicevic also reported similar results wherein the expression increased with tumor Stage(13).

HER2:

The staining pattern of HER 2 in gastric cancers has always been a debatable issue. Heterogenesity is the hall mark of these tumors. Several studies have been done in terms of Her2 testing by taking in to account inter-observer and inter-laborotary observer consensus for scoring the expression of the marker on tissue microarray. Josef Ruschoff, mafred Dietel et al., has standardized reporting of HER 2 expression in endoscopic biopsies as well as resected specimens [15]. Similar scoring system was also proposed by Hofmann et al.[16]. The positivity of this marker in gastric cancer showed an extensive range from 2%, in the study done by Grabsch H et al., to 90%, in a study conducted by Allgaver H et al.[17,18]. In our study HER 2 was expressed in 60% of the malignant tumors. Laxmi V et al., has shown 35.9% HER 2 positivity in their study [19]. Raj Aditi, Rau Aarathi et al., reported HER 2 expression in 27.6% of the carcinomas where as Sekaran et al., had reported a very high positivity of 44.2 % in their study [20,21]. In our study, the expression of the protein was not associated with the grade of the tumor .The findings were similar to that of study conducted by Raj Aditi et al., where in, there was no positivity in well differentiated tumors.Studies done by Kim et al., and Marx et al., showed that majority of HER 2 positivity was seen in moderately differentiated tumors,51.8% and 60% respectively [22,23]. On the contrary, in the studies done by Tateshi et al., had shown that the expression was more in well differentiated tumors compared to poorly differentiated tumors [24]. Similar findings were reported by Phillips et al., and Fisher SB et al.[25,26]. In our study, the expression of the marker increased with the stage of the tumor and there by tumor progression. Yan et al., and Chua et al., also stated that the protein expression is associated with only stage of the tumor rather than site and type of the tumor [27,28].

We have adopted same scoring system as that of gastric cancer for scoring the expression of HER 2 in colonic carcinoma.In our study, the expression of HER2 in colonic adenocarcinoma was 100% and majority of the showed score1+.According to our knowledge no study has reported such high percentage of positivity. Ingold Heppner et al., have reported a very less positivity,1.6% [29]. Various studies have shown a varied positive percentage of HER 2 expression in colonic carcinoma. A maximum of 80% was reported by Kruszewski WJ et al., and a minimum of 0% was reported by Blok EJ et al [30,31] . In our study, the expression of HSP 90 was not associated with the grade of the tumor. Positivity was seen in mostly well differentiated tumors with score 1+.The finding were similar to the study done by Suma S et al [32]. Seo AN et al., in their study also showed no correlation with the differentiation of the tumor and other clinicopathological parameters [33]. In our study, Her 2 expression was associated with the stage of the tumor. Similar results were reported Ingold Heppner et al. In addition, the expression was also related to lymphnode metastasis and local growth. Zahra Heidri et al., in their study has shown no association with HER 2 expression and stage of the tumor [34]. They did not find any relationship with lymphnode metastasis and expression of the marker. In our study, only stage T3 tumors showed score 3+ expression. Suma S et al., has shown that majority of Stage B cases showed positivity in their study group. In our study, among rectal adenocarcinomas, HER 2

was expressed in 86.6% of the tumors. Similar findings were reported by Marshall J et al [35]. According to Mohammed E Salem [36], amongst the tumors of large intestine, tumors of rectum expressed more of Her 2compared to other tumors. In study conducted by ConradiLc et al., the percentage of expression of HER 2 in rectal cancers was 26.7% [37]. In our study, there was association between the grade of the tumor and expression of HER 2.Conradi LC et al., also showed association of the grade of the tumor with expression of the protein. Rameez Hasan et al., has reported that the marker was expressed more in well differentiated tumors than moderately and poorly differentiated tumors [38]. According to Ji-Lin Li et al., there was correlation between the expression of the marker and grading of the tumor [39]. In our study, the stage of the tumor is not associated with the expression of the marker. Where as Richman SD et al., and Valtorta E et al., have reported the association of HER2 with the stage of the tumor [40,41].

P53:

In our study, P53 was expressed in 86.6% of the Gastric adenocarcinomas. The expression was nuclear in all the tumors. Young- EunJoo et al., in their study also had P53 positivity in all the gastric tumors and was nuclear positivity [42]. They have described lot of heterogenisity in gastric tumors but in our study the staining was homogenous. Shashikanth et al., has reported P 53 positivity in 72% of the gastric cancers in their study [43]. Ygomyo et al., has reported 65% positivity of P53 in gastric carcinomas [44]. In our study, expression of P53 was associated with the histologic grade of the tumor as well as stage of the tumor. It was similar to the study done by Tushar Hiralal et al., and Teiichiro Honda et al., where in the expression of the marker was associated with the histologic grade of gastric carcinoma as well as TNM staging of the tumor [45,46]. In our study, P53 expression was negative in all the benign neoplasms of colon. In malignant neoplasms,90% of the tumors showed P53 positivity with majority of the adenocarcinomas showing score 2+.Several studies have given variable positive percentage of P53 in colonic adenocarcinoma. In the study done by Kavitha Mardi et al., P53 was expressed in 70% of the tumors [47]. Ghavam-Nasiri et al., had reported 40% positivity of P53 in colonic adenocarcinomas in their study [48]. In our study, we have observed that the expression of P53 was associated with the tumor grade and stage of colonic adenocarinomas which was similar to the study done by Hye Seung Han et al. [49]. On the contrary, P53 expression was not associated with grade and stage of the tumor in the study done by Kavitha Mardi et al. In our study, 96.6% of the rectal adenocarcinomas showed P53 positivity. Márcia Teresinha Jurach et al., has reported 41% positivity of P53 in rectal adenocarcinomas [40]. In our study, the expression of P53 was associated with the grade and stage of the rectal adenocarcinomas. Marcia Teresinha Jurach has observed that there was no correlation between the expression of the marker with the stage and grade of the tumor. Mohammad-Reza Ghavam-Nasiri MD et al., in their study, has found that there was no association between the expression of the marker and stage or grade of the rectal carcinomas.

Ki 67:

Ki67 was negative in all the benign neoplasms of Stomach but was positive in all the adenocarcinoma. It also showed correlation with the stage and grade of the tumor. Similar findings were reported in the study done by H.Amrani et al., wherein Ki 67 expression was associated with the grade and stage of the tumor[51]. In our study, colonic adenocarcinoma expressed Ki 67 and it was associated with the grade and stage of the tumor. O Fluge et al., has reported similar findings wherein they have observed association of the marker with the grade and stage of the tumor [52]. Also in our study we didn't find any association between the marker and stage of rectal adenocarcinoma but there was an association between the grade of the tumor and expression of Ki 67.

5. CONCLUSION:

Gastrointestinal tumors are one of the most extensively studied tumors. Marked geographical variations have been identified in the phenotypic characters of the tumors and understanding of the pattern of expression of prognostic as well as predictive markers shall be helpful in better analysis of the tumor behavior. Numerous studies done throughout the world had given varied results regarding the expression of these particular markers and this makes it necessary to standardize the data in sub-population groups to overcome this geographical variation. Also newer therapeutic drugs have evolved which would target these proteins and inhibit the tumor cell proliferation and survival. Hence, study of the expression of these markers in gastrointestinal tumors would help in identifying the patients who would benefit from using these drugs in therapeutic regimes. In the present study we have studied only the expressed proteins in the tumor cells but further evaluation by studying the genetic expression of these markers would give added information on the geneis of these markers and also accentuate the value of these markers in their role as predictive and prognostic markers.

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