

## IMMUNOHISTOCHEMICAL EXPRESSION OF THYMIDYLATE SYNTHASE IN DIFFERENT GRADES OF COLORECTAL CARCINOMA

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### ABSTRACT

**Objective:** To Evaluate the Immunohistochemical Expression of Thymidylate Synthase in different grades and histological types of Colorectal Carcinoma.

**Methods:** It was Cross sectional, Descriptive study conducted from June 2019 to November 2020 in the Department of Pathology at Postgraduate Medical Institute Lahore / Lahore General Hospital. Forty resected gut specimens diagnosed on light microscopy as Colorectal adenocarcinoma were included on the basis of non-probability, convenience sampling technique. Cases were reviewed and histological typing and grading was done separately by two competent Histopathologists. Immunohistochemical staining for Thymidylate Synthase was performed. Results of Thymidylate Synthase immunostaining was interpreted by the two observers based on the cytoplasmic staining of tumor cells. Thymidylate Synthase expression was quantified through a visual grading system depending upon the staining intensity and percentage of stained cells.

**Results:** Out of 40 cases of adenocarcinoma, 18 of the patients (45%) belong to the age range of 51-60 years, with the mean age of 51.55 years. There were 31(77.5%) male patients while 9(22.5%) were females. 28 (22%) cases were classified as low grade while 12 (11%) cases were reported as high-grade carcinomas. Amongst these Thymidylate Synthase expression was seen in 78.6% of low-grade carcinoma and 91.6% of high-grade carcinomas. Regarding histological subtyping, 23(57.5 %) cases were identified as non-mucinous adenocarcinomas, (9)22.5% as mucinous adenocarcinoma and (8) 20% as signet ring adenocarcinoma. Thymidylate Synthase expression was found to be similar i.e., 78% in non-mucinous as well as mucinous carcinomas however, 100% positivity was noticed in signet ring carcinomas. The overall positivity for Thymidylate Synthase was found to be 82.5%.

**Conclusion:** Colorectal carcinoma was prevalent among males of 51-60 years of age. 82.5% cases of colorectal carcinoma expressed Thymidylate synthase receptors and its expression was found to be higher in high grade tumors.

**Key words:** Thymidylate Synthase (TS), Adenocarcinoma, Colorectal.

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### INTRODUCTION

Cancer is the second major reason of death around the globe with colorectal cancer being the 2<sup>nd</sup> major cause of cancer related deaths.<sup>1</sup> Although Pakistan is included in a low-risk zone for colorectal cancer however a considerable rise is being seen especially in individuals over 50 years of age.<sup>2</sup> Unfortunately, the incidence of

colorectal carcinoma also appears to be increasing in patients who are less than 50 years of age.<sup>3</sup> In Pakistan, Colorectal carcinoma is found to be the '4th' most frequent and '5th' most regularly diagnosed cancer. Numerous genetic alterations including mutations in TP53, APC, SMAD4, PTEN, β-catenin and KRAS play an important role in pathogenesis. Dietary factors contribute to almost half of all colorectal cancer cases. Appropriate weight maintenance and exercise along with reduction in intake of red meat, alcohol and smoking can protect against colorectal cancer.<sup>4</sup>

According to American Cancer Society, colorectal cancer may remain asymptomatic initially however alteration in bowel habits usually becomes the first symptom. Direct endoscopic colonoscopy and flexible sigmoidoscopy provides effective visualization of gut and biopsy whenever required. Histopathological evaluation is the standard criterion for the confirmatory diagnosis of colorectal carcinoma in biopsied and resected specimens. Histologically, the most common type of colorectal carcinoma is adenocarcinomas comprising almost 95% of all colorectal cancer cases. Depending upon the percentage of glandular elements the WHO AJCC 8th edition update has divided colorectal carcinoma as Low grade (well differentiated and moderately differentiated tumors) and high-grade tumors (poor/ undifferentiated)<sup>5</sup>.

Studies are being carried out to ascertain prognostic markers of colorectal cancer and to figure out best treatment modalities.<sup>6</sup> Apart from diagnosis of primary and secondary cancer, the goal of utilization of genetic diagnostics is to unveil new prognostic markers and their use in treatment modifications. Biomarkers prove to be important in this regard. Traditionally, MSI, KRAS and BRAF, CA 19.9, TAG-72 and TPS are being evaluated. However, no single marker has an absolute and accurate diagnostic capability.<sup>7</sup>

Thymidylate synthase (TS) is a fundamental enzyme for formation of thymidylate; a key precursor for DNA biosynthesis.<sup>8</sup> Tumors which exhibit enhanced levels of TS seem to have a poor survival.<sup>9</sup> However, TS acts as a target for chemotherapeutic agents like Fluorouracil (FUra); a TS inhibitor. There is some evidence that the chemosensitivity of 5 FU for high TS expressing tumors is better than those with low expression of TS.<sup>10</sup>

As colorectal carcinomas with high TS expression may respond better than the tumors with low levels of TS expression to 5 FU chemotherapeutic drugs.<sup>11</sup> therefore this study was undertaken with the objective of evaluating the expression of TS in our local patient population of colorectal carcinoma so that the chances of their response to chemotherapy may be utilized to improve their survival.

**METHOD**

It was a cross sectional analytical Study carried out in Postgraduate Medical Institute Lahore and Lahore General Hospital from June 2019 to November 2020. As, no direct clinical details of patients were disclosed, therefore, waiver of informed consent was taken from the IRB. Sample size was calculated using the formula for determining the specificity and sensitivity as per WHO "Sample size determination in Health studies" software<sup>12</sup> and found to be 40 as follows:

$$n = \frac{Z^2 \times P(1 - P)}{d^2}$$

Z= 1.96 at 95% (Confidence level).

P= 88% (Anticipated Sensitivity)

d = 10% (Desired precision)

n = 40 (sample size)

Non-probability, convenience sampling technique was used. Specimen of male and female patients of all ages with histologically diagnosed Colorectal Carcinoma (Adenocarcinoma) were included, however samples of those patients who were already taking chemotherapy, radiotherapy or hormone therapy were excluded.

Formalin fixed tissue sections were processed in an automated processor, embedded, and stained with Hematoxylin and eosin as per protocol. Cases were examined, diagnosed and followed by histological typing and grading. It was done initially separately by two experienced consultants Histopathologists, and later on discussed in intra departmental consultation. Immunohistochemical staining for Thymidylate Synthase was performed. Results of Thymidylate Synthase immunostaining was interpreted by these observers based on the cytoplasmic staining of tumor cells. Their interpretation reflected a consensus opinion. Thymidylate Synthase expression was quantified through a visual grading system depending upon the staining intensity and percentage of stained cells. Positive and negative control for TS was established to improve reliability of results. Percentage and intensity score was determined by examining 10 consecutive HPFs.

Table 1: Scoring for Percentage of Thymidylate Synthase Immunostaining

PERCENTAGE OF CELLS STAINING	SCORE
<5%	0
6-25	1
26-50	2
51-75	3
>75%	4

Figure I: Association of Histological grade of tumor with TS positivity

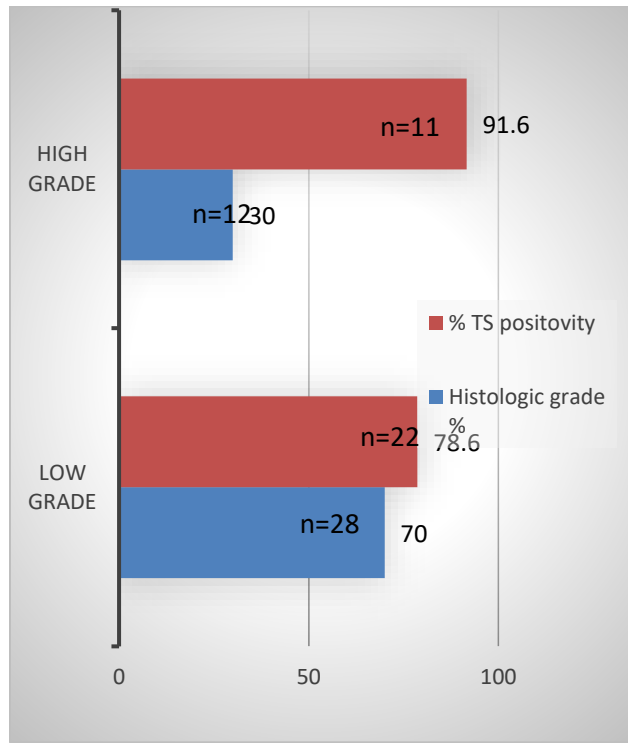


Table 2: Scoring for Intensity of Thymidylate Synthase Immunostaining

INTENSITY OF CYTOPLASMIC STAINING	SCORE
Negative	0
Weak	1+
Moderate	2+
Strong	3+

The final score was obtained by multiplying the percentage and intensity of cytoplasmic/cell expression (Numeric Score= Percentage × Intensity). If the resultant score generated was 04 or higher; the tissue/tumor was considered as TS positive and if it was less than 04; it was marked as TS negative.<sup>10</sup> Data was analyzed with SPSS (social sciences software) version 23. For continuous quantitative variables like age, mean and standard deviation were calculated. The expression of TS was described as percentage and frequency.

**RESULTS**

Majority of the patients 18 (45%) were between 50 to 59 years of age, 10 patients (25%) were forty or less than forty years of age and only 3 patients (7.5%) were above 70 years. The mean age was 51.55 and standard deviation was found to be 12.087. Male patients were

31(77.5%) and female patients were only 07(22.5%). Male to female ratio was 3.4: 1.

The immunohistochemical analysis of the cases showed 33 (82.5%) were stained positive for TS and only 07 (17.5%) were negative.

TS positivity was higher in younger patients mostly ≤ 40 years (90%) and in age groups between 50 to 69 years (83%). However, the difference was statistically non-significant (P >0.05). P-value = 0.89 (non-significant). TS positivity was higher in male patients (84%) as compared to female patients (78%). However, the difference was statistically non-significant (P >0.05). P-value = 0.76 (non-significant).

The histological examination of these 40 cases of colorectal carcinoma showed that there were 28/40 (70%) low grade and 12/40 (30%) high grade tumors.

The histological typing revealed 23/40 (57.5%) non mucinous adenocarcinoma, whereas 09/40 (22.5%) cases of mucinous and 08/40 (20%) cases of signet ring adenocarcinoma. TS positivity was higher in high grade colorectal carcinomas (91.6%) as compared to low grade carcinomas (78.6%). However, the difference was statistically non-significant P-value = 0.76 TS positivity was highest in signet ring carcinoma (100%) as compared to mucinous and non-mucinous adenocarcinomas (78%).

Table 3: Association of Histological grade of tumor with TS positivity

Histological Grade	No. of cases	TS positivity	
		N=Positive	%
Low grade	28	22	78.6
High grade	12	11	91.6
Total	40	33	
p-value	P-value = 0.76 (non-significant)		

Table 4: Association of Histological type with TS positivity in cases of Colorectal Adenocarcinoma

Histological Types	No. of cases	%age	TS positivity	
			N= positive cases	%
Non mucinous	23	57.5	18	78
Mucinous	09	22.5	07	78
Signet ring	08	20	08	100
p-value	P-value = 0.90 (non-significant)			

**DISCUSSION**

This study reflected the fact that most of the patients having colorectal carcinoma presented between the age group of 50 to 59 years. Male to female ratio was 3.4: 1 with male predominance. These results are similar to a study

done at Khyber Teaching Hospital, Pakistan, where they found 66% of cases were males and 34% females.<sup>13</sup>

Regarding histological type, out of 40 cases included in our study; 57.5 % were diagnosed as non-mucinous adenocarcinomas, 22.5% as mucinous and 20% as poorly differentiated signet ring adenocarcinomas. Evidence from literature reveals mucinous carcinoma constitutes about 10-20% of colorectal cases in most of the studies including meta-analysis studies.<sup>14</sup>

Regarding adenocarcinoma colon, 'Poor histological differentiation' remains the most adverse histopathological factor for predicting clinical course of the disease and prognosis<sup>15</sup>. Regarding the grades, 28 cases of our study (70%) were categorized as low grade while 12 (30%) were labeled as high-grade carcinomas. Yamaguchi, T et al found 70% of colorectal carcinomas in their study as low grade while 30% high-grade adenocarcinomas<sup>16</sup>. According to a study conducted in Agha Khan University, Pakistan, 16.47% Colon adenocarcinomas were graded as grade I, 71.76% as grade II and 11.76% as grade III.<sup>17</sup> When a two-tiered grading system is recommended, based on the WHO classification: low grade include the well differentiated and moderately differentiated tumors while high grade tumors include poorly differentiated and undifferentiated ones. The two-tiered grading system is much more reproducible and more prognostically representative.<sup>18</sup>

In another study at University of Health Sciences, Nabi U et al found 75% of Colon adenocarcinomas to be low grade tumors while 25% were labeled as high-grade including signet ring carcinoma and most of mucinous carcinomas.<sup>19</sup>

Overall positivity for TS was found to be 82.5% in the present study. TS expression was 78.6% in low grade tumors whereas it was positive in 91.6% in tumors with high grades. 100 % positivity was found in signet ring carcinomas. These results depict that as the grade advances, the expression of TS gets higher. (p-value = 0.76 but it was not statistically significant).

TS has become an important platform for targeted therapy against colorectal carcinoma and newer compounds with lesser side effects and improved efficacy are continually being tested and tried.<sup>20,21</sup> Though some previous studies have proved better survival for TS negative tumors in contrast to TS positive group, some studies demonstrate better treatment response to 5 FU and other similar chemotherapeutic agents in cases that are positive 5-FU metabolic enzymes, TS being one of them, making it a ray of hope for these cancer patients.<sup>22,23</sup>

## CONCLUSION

The results of our study concluded that Colorectal carcinoma is more prevalent among middle aged males. Most cases of colorectal carcinoma express Thymidylate synthase receptors. The TS expression was found to be higher in high grade colorectal carcinoma including all signet ring carcinomas. Immunohistochemical Expression of Thymidylate synthase may be used as a prognostic marker for colorectal carcinoma and can help in deciding the treatment modality.

## LIMITATIONS OF STUDY

The present study was conducted on a small sample due to financial constraint. Considering the small sample size, the chances that results might not be truly representative, can't be excluded.

## RECOMMENDATIONS

No study has been done in Pakistan so far regarding the expression of Thymidylate synthase over paraffin embedded sections of colorectal carcinoma. A cross-sectional study with a greater number of cases will conclusively verify the findings of TS expression in colorectal carcinoma. Moreover, a prospective cohort study will be more appropriate to confirm the relationship of TS expression and survival rate of patients with colorectal carcinoma.

*Ethical Approval: Submitted*

*Conflict of Interest: Authors declare no conflict of interest.*

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