Original article

Prognostic information of gastric carcinoma using Goseki system in relation to nuclear organizer region (AgNOR) and proliferating cell nuclear antigen (PCNA) expression

Ghosh S[1], Ghosh TK[2], Dewasi N[3], Das K[4]

Abstract:

Objective: The present study was undertaken to find out the relationship of Goseki grading system (I-IV) with existing classification of WHO, Lauren and tumour differentiation of gastric carcinoma and its prognostic information in relation to AgNOR & PCNA expression. ***Materials and methods:*** To assess the reproducibility and usefulness of Goseki grading system thirty five gastric carcinoma were selected from January 2007 to July 2009 in the department of Pathology, Burdwan Medical College in West Bengal, India and analyzed in relation to existing grading system by chi-squared testing. Mean AgNOR count & mean PCNA leveling index quantities of different tissue sections were assessed according to different classification system of gastric carcinoma and interobserver variations of all data were evaluated from Spearman Rank-order Correlation Coefficient (r_s). ***Results:*** Highly significant predictable correlation of Goseki grading system for existing classification of gastric carcinoma was obtained statistically. Increasing values of mean AgNOR count and PCNA leveling index (2.35% & 15.14%, 2.91% & 21.32%, 3.08 %& 24.76% and 3.2% & 25.12 respectively) were observed from Grade I – IV of Goseki grade. Mucin rich (3.05% & 23.22%) and tubule poor (3.14% & 24.76%) tumours higher values than mucin poor (2.71% & 19.95%) and tubule rich (2.63% & 18.23%) tumors. No significant correlations were observed in other grading system. ***Conclusion:*** Following Goseki grading system increasing expression of proliferating marker in mucin rich than mucin poor tumours and tubules poor than tubules rich tumors indicate poor prognosis and tumour behavior. Simple system may help to select patients for adjuvant therapy.

Key words: Goseki grade; gastric carcinoma; AgNOR; PCNA.

Introduction

Gastric carcinoma is highly variable lesion. Many different classification systems have been proposed for the morphological, histological classification, grading and staging system to find out tumour prognosis to patient survival. None of them are satisfactory. The surgical status of the specimen resection is highly variable due to palliative as well as curative operations. Thus the pathologist has an important role in determining the cancer progression and depends on thorough tissue sampling as well as meticulous microscopic examination. In 1992 Goseki [1] proposed a classification system of carcinoma of stomach based on tubular differentiation and intracytoplasmic mucin production and was thought superior to other classification system, such as those of World Health Organization (WHO), Lauren system and tumour differentiation. According to Goseki four grades depending on tubule formation and intracellular mucin production by the tumour cells categorized

1. *Sulekha Ghosh, Associate Professor,
2. Tapan Kumar Ghosh, Demonstrator,
3. Nikhilesh Dewasi, PGT,
4. Krishnendu Das, PGT,
Department of Pathology, Burdwan Medical College, West Bengal, India.

Corresponds to: Dr. Tapan Kumar Ghosh, 40, Kotalhat, Burdwan-2, Burdwan-713102, West Bengal, India.
Email: ghosh.drtapan@gmail.com, tapan_58@rediffmail.com.

as Grade I tubules well formed and mucin poor; II tubules well mucin rich; III tubules poor differentiated mucin poor; IV tubules poor mucin rich. Significant reliability with interobserver agreement with this grading system was observed by different workers [2, 3] and was proposed the only system with prognostic value in additional to TNM staging[4].

The clinical significance of these classifications was limited, only the Lauren and perhaps the Goseki classifications were proposed to have prognostic assessments[5]. However, the Lauren classification has been the most successful system, as it defines two distinct histological entities, which clearly exhibit different clinical and epidemiological characteristics, even in advanced gastric cancers[6].

The prognosis of gastric carcinoma is related to depth of tumour invasion, size, histological type, DNA ploidy, cell proliferation, tumour grading & staging. The cell proliferation indices like AgNOR (Nucleolar organizer regions) counting and PCNA LI (Proliferate cell nuclear antigen labeling index) have been found to be a prognostic marker along with histological classification and tumour grade of different neoplastic lesions. Various study showed increased numbers of AgNORs count in nuclei from normal mucosa to gastric carcinoma and significantly higher in intestinal type than diffuse type gastric carcinomas in Lauren type[7.] PCNA LI and AgNOR expressions were significantly increased from normal to dysplasia to gastric adenocarcinoma[8]. Another study did not found any evidence for prognostic value with PCNA in gastric carcinoma[9].

Nucleolar organizer regions (NORs) are the DNA loops which are transcribed to rRNA and ultimately to protein namely C_{23} and B_{23}[10] are responsible for silver staining of nucleoli and are easily visible under light microscope by staining with freshly prepared silver colloidal developer solution. AgNOR dots are black dots within the nucleus usually increased in number of rapidly proliferating cells and acts as a proliferate activity of tissues, particularly in neoplastic cells. AgNOR quantity was measured by counting number of dots per nucleus in 100 tumour cells and average was taken as mean AgNOR count.

PCNA is a nonhistone nuclear protein (36 KD) has a role in initiation of cell proliferation[11] and the level of PCNA increased in the nucleus during late G1 phase immediately prior to the onset of DNA synthesis and maximum in S phase of cell cycle. Immunolocalization of PCNA in tumour used as proliferate activity of tumour and acts as proliferative tumour marker. The progressive growth of the tumour is determined by excess of cell proliferation over cell death and major fractions of transformed cells are in proliferative pool.

Two recent studies found that the Goseki histological classification was predictive of survival in patients with gastric cancer [3, 12], although two other studies did not get any predictive value [13, 14] and thus the prognostic value of the system remains controversial [15].

Purpose of the present study was to find out a comparison of Goseki's grading system for existing classification systems of gastric adenocarcinoma along with expression of mean AgNOR count and PCNA labeling index (PCNA LI) and an assessment of their reproducibility.

Material and methods

The study was done in the year of January 2007 to July 2009 from the tissues received in the Department of pathology; Burdwan Medical College and Hospital, West Bengal, India. 35 cases of tissue paraffin blocks of primary gastric adenocarcinoma were included in this study after taking

permission of ethical committee. The tissue sections of 5 micron size were taken on microscopic glass slides precoated with 3% gelatin from routinely processed paraffin embedded blocks. The sections were then stained with routine haematoxxylin & eosin stain and alcian blue PAS pH 2.5 to detect intracytoplasmic mucin production for classification of Goseki grade.

The tumors were categorized according to WHO classification into tubular, papillary, mucinous, signet ring cell type, adenosquamous, and small cell and undifferentiated type determined by prominent component of the tumour. According to Lauren as intestinal and diffuse type and few tumors not categorized were designated as mixed (tumors with equal quantity of intestinal and diffuse type). Tumors were also categorized as well, moderate and poor according to differentiation. In the Goseki system four categories were graded into Grade I tubules well differentiated and mucin poor; II tubules well mucin rich; III tubules poor differentiated mucin poor; IV tubules poor mucin rich gastric carcinoma. The tumors not categorized in any systems were excluded in this study.

AgNOR staining and counting

The deparaffinised tissue sections were first thoroughly washed with double distilled water for 15-20 minutes. Working solution was freshly prepares mixture of one volume of 2% gelatin in 1% formic acid solution and two volume of 50% aqueous silver nitrate solution in a dark room condition. Working solution was poured over the sections kept in a dark place for 45 minutes at 37^0 C. After washing with double distilled water all the sections were mounted in D.P.X. AgNOR dots were counted within the nucleus by Crocker's method[16] both single dot and in clusters (counted as single dot) under oil immersion. Total dots were counted in 100 tumour cells and average was taken as mean AgNOR count.

PCNA immunostaining

Immunohistochemistry were done in all tissue sections by standard procedure by using anti PCNA antibodies (PC 10 DAKOPATTS) at a dilution of 1 in 50 using ABC technique with AEC as chromogen and mounted in glycerin jelly. High grade breast carcinoma was taken as positive control. The PCNA positive cells showed reddish colour nucleus with AEC chromogen. The percentage of cells positive for PCNA among 500 tumour cells nuclei were counted under oil immersion as Proliferating Cell Nuclear Antigen Lebiling Index (PCNA LI).

Both AgNOR count and PCNA LI of all tissue sections were performed by two different observers to avoid inter-observer variation.

Statistical evaluation

The relationship of Goseki's grade to the other classifications and grading system was performed from the results of contingency table X^2 statistical test (Chi-squared testing).The results of AgNOR counts and PCNA LI of two different observers were evaluated from Spearman Rank-order Correlation Coefficient (r_s). Multivariate analyses of different Goseki grades were analyzed by 'F' test.

Results and analysis:

The results of this study consists of 35 primary adenocarcinoma of stomach whose ages ranged from 33 to 79 years of ages with 23 male and 12 female patients. The AgNOR dots were ranged from 1-5 brownish black dots per tumour cell nucleus and mean AgNOR count in different tumors were 1.2-5.1%. The PCNA immunostaining showed nuclear positivity. Few foci in some tumors also showed mild cytoplasmic positivity and mean PCNA LI were 3-42.6%. The frequency of observations in the four Goseki grade for other histological classification and differentiation were tabulated in Table I. According to Goseki's

grading 6(17.14%), 13 (37.14%), 11(31.42%) and 5(14.30%) tumors were found in grade I, II, III, and IV respectively. Good tubular differentiation of Goseki grade I and II were reflected in the relationship of WHO classification, intestinal type of Lauren classification and histological differentiation. 84% of WHO tumour of tubular differentiation were fell in Goseki grade I and grade II of tubule rich category. Similarly 94.73% of intestinal type of Lauren and well and moderately differentiated tumors were fallen in same category. Goseki's tubule poor grade III and IV showed 75% of poorly differentiated tumors. However 14.28% of WHO tubular tumors and 2.5% of intestinal tumour of Lauren type were found in tubular poor of Goseki's grade III and grade IV. In mucin content tumour all 4 of WHO type were in Goseki II and III grade and one signet ring cell type was categorized in grade IV Goseki system. High significant correlation obtained on Chi- squared testing of Goseki's grading system with WHO system, Lauren system and tumour differentiation and Degree of freedom & probability were 56.7(18) &0.000, 23.8(6) &0.001 and 32.0(6) &0.000 respectively.

Table I: Relationship of Goseki grade for other classification of gastric carcinoma

	Goseki Classification				**n = 35**	**%**
	Grade I Tubule rich Mucin poor	Grade II Tubule rich Mucin rich	Grade III Tubule poor Mucin poor	Grade IV Tubule poor Mucin rich		
WHO						
Tubular	3	9	2	0	14	40
Papillary	1	3	0	0	4	11.43
Mucinous	0	1	0	3	4	11.43
Signet ring cell	0	0	0	1	1	2.86
Adenosquamous	2	0	0	0	2	5.71
Small cell	0	0	2	1	3	8.57
Undifferentiated	0	0	7	0	7	20
Lauren						
Intestinal	6	12	1	2	21	60
Diffuse	0	1	8	3	12	34.28
Mixed	0	0	2	0	2	5.72
Differentiation						
Well	4	1	0	0	5	11.43
Moderate	2	11	2	2	17	48.57
Poor	0	1	9	3	13	40

Frequency of mean AgNOR count and PCNA LI were calculated in all types of different tumors classifications system against Goseki grade and tabulated according to different types of gastric adenocarcinoma system including Goseki's scheme. The Spearman Rank-Order Correlation Coefficient (r_s) of two different observers was highly significant (Table II).

Analysis of Mean AgNOR and PCNA LI count in different classifications (Table IV) and their correlation falling in Goseki grading system as follows. Mean AgNOR count varies from 1.78 to 4.11% (fig I&II) and Mean PCNA LI vary from 7.1% to 29.13% (fig III&IV).

Table II: Spearman Rank-Order Correlation coefficient (r_s) of AgNOR & PCNA LI values expressed in different grades of gastric carcinoma

Grading System	Mean AgNOR count %			AgNOR r_s	Remarks	Mean PCNA LI %			PCNA LI r_s	Remarks
	Obser.-I	Obser - II	Mean			Obser - I	Obser - II	Mean		
WHO										
Tubular (14)	2.61±1.01	2.88±o.92	2.73	0.74	*	17.72±13.37	17.64±10.02	17.67	0.95	*
Papillary(4)	2.65±0.37	2.92±0.29	2.78	0.63	**	24.87±17.62	23.25±13.84	24.06	1.0	*
Mucinous(4)	3.3±0.4	3.38±0.64	3.43	0.54	**	28.5±17.14	27.45±13.46	27.97	0.8	*
Signet ring (1)	2.2	1.5	1.85	--	single	7.9	6.3	7.1	--	single
Ad squamou.(2)	2.45±1.77	2.4±0.71	2.42	1	*	25.0±25.45	22.65±20.29	23.82	1.0	*
Small cell (3)	4.13±0.57	4.1±0.17	4.11	0		30.3±20.19	27.97±18.88	29.13	0.5	**
Undiff. (7)	2.27±0.63	2.9±0.78	2.79	0.84	*	24.77±15.09	23.36±12.61	24.06	0.67	**
Lauren										
Intestinal(21)	2.71±0.89	2.89±0.70	2.8	0.73	*	21.67±15.16	20.62±12.23	21.14	0.96	*
Diffuse(12)	2.97±0.84	3.15±0.86	3.06	0.89	*	22.49±16.02	21.56±13.40	22.02	0.86	*
Mixed(2)	2.05±2.33	3.35±2.47	3.20	1	*	29.45±19.02	28.45±12.94	28.95	1	*
Differentiation										
Well(4)	2.13±0.98	2.72±0.72	2.42	0.95	*	14.45±14.90	13.25±11.10	13.85	1	*
Moderate(17)	3.15±0.91	3.17±0.88	3.15	0.83	*	25.60±15.37	24.28±12.33	24.95	0.92	*
Poor(14)	2.63±0.84	2.89±0.89	2.75	0.90	*	20.74±15.1	20.21±12.46	20.46	0.91	*
Goseki grade										
Grade I (6)	2.1±0.96	2.6±0.64	2.35	0.76	*	15.27±14.81	15.03±12.35	15.14	0.94	*
Grade II (13)	2.88±0.68	2.97±0.62	2.91	0.80	*	21.7±14.54	20.93±10.8	21.32	0.94	*
Grade III (11)	2.97±1.10	3.19±1.09	3.08	0.91	*	25.51±15.36	24.04±12.93	24.76	0.90	*
Grade IV (5)	3.22±0.86	3.18±1.10	3.2	0.89	*	25.9±18.9	24.34±16.20	25.12	0.9	*

*High degree of similarity. ** Moderate degree of similarity. Obser—Observer.

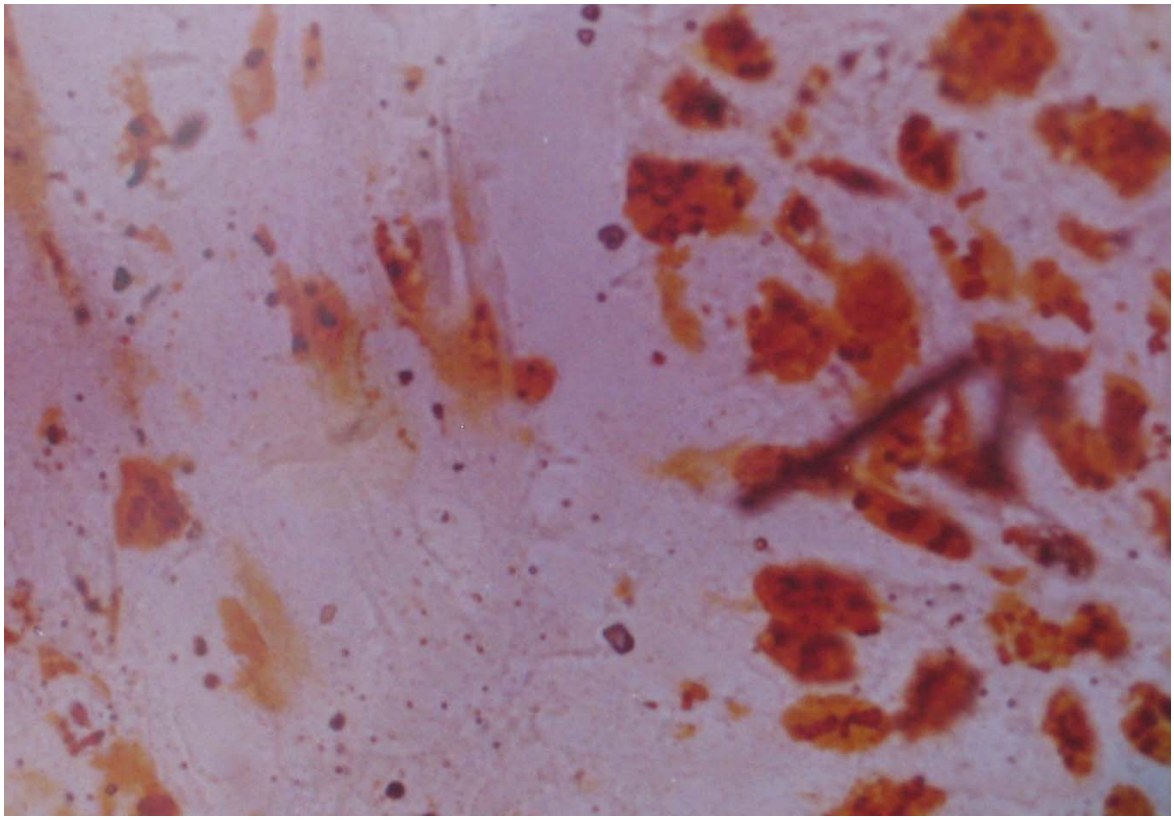

Figure I: Photomicrograph showing multiple AgNOR dots within nucleus of tubules of Gastric Carcinoma (x1000)

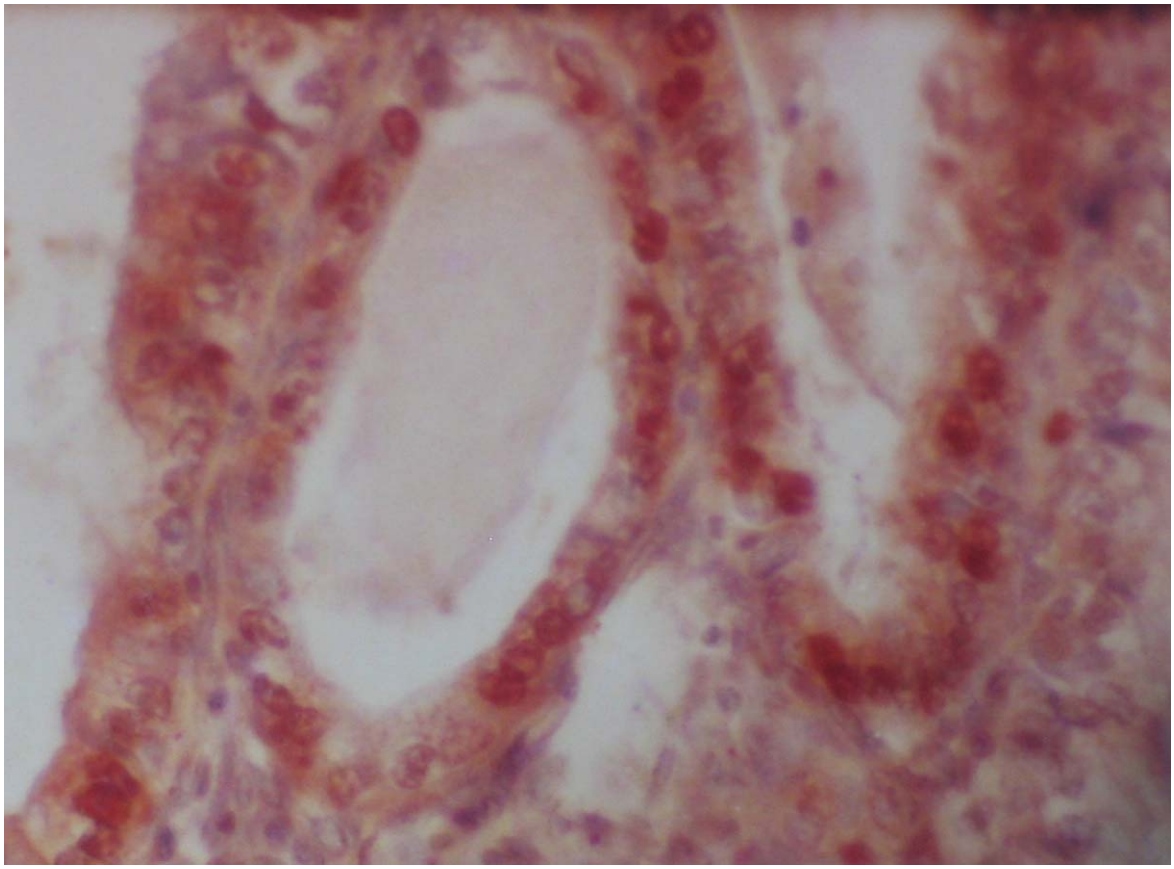

Figure II: Photomicrograph of same case Fig I showing PCNA nuclear positivity (x1000)

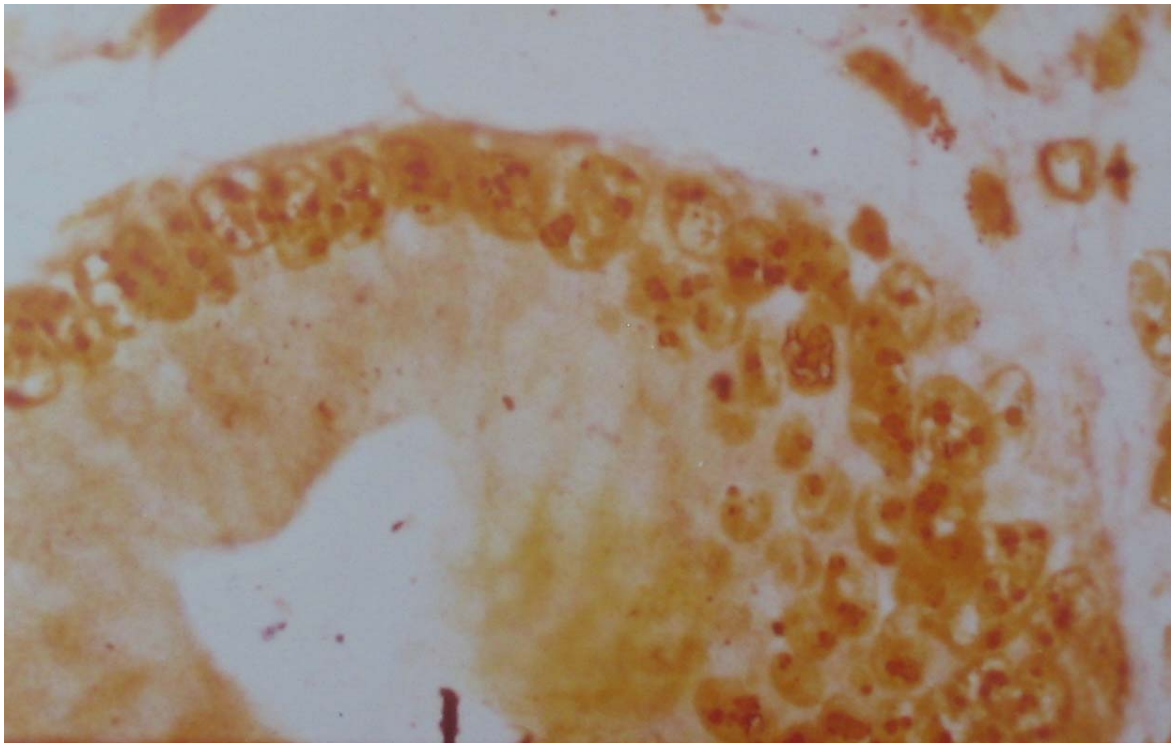

Figure III: Photomicrograph showing multiple AgNOR dots within nucleus of tubule poor Tumour of Gastric Carcinoma(x1000)

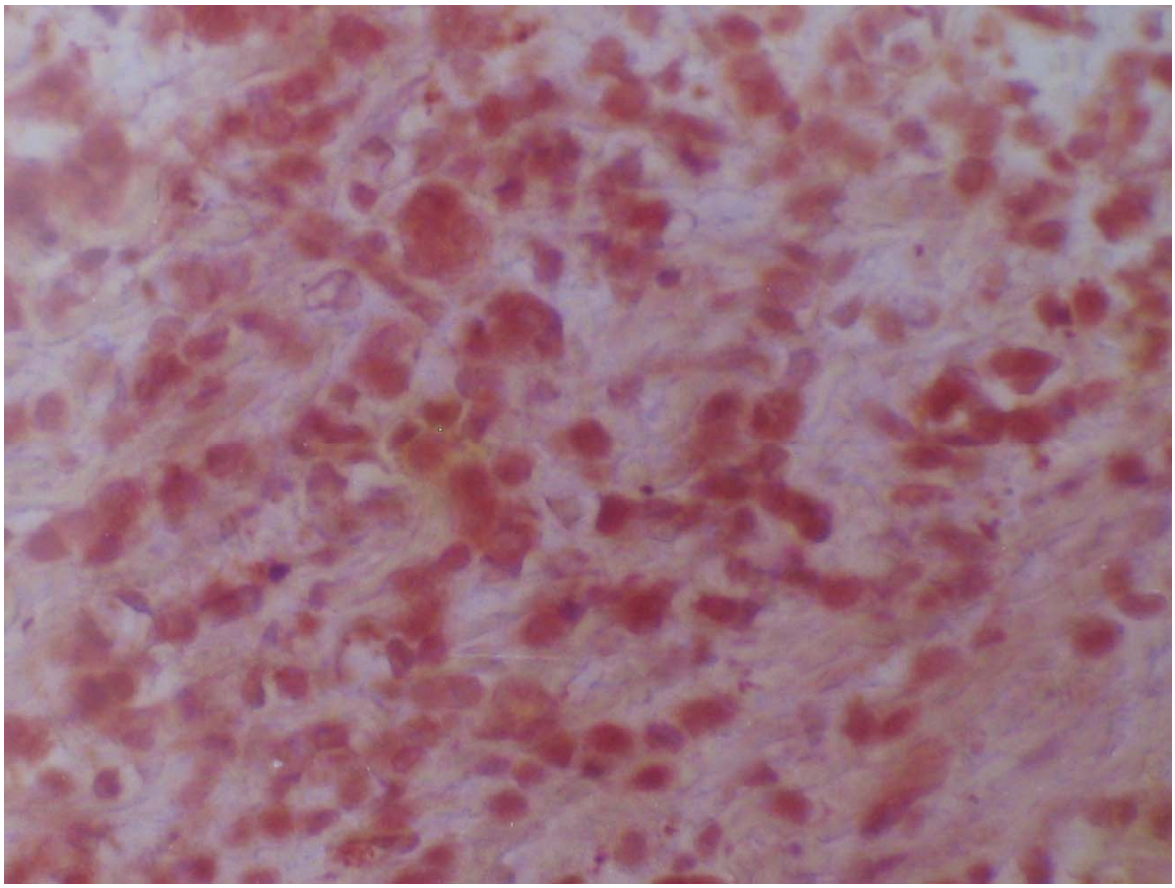

Figure IV: Photomicrograph of same case Fig III showing PCNA nuclear positivity (x1000)

WHO classification

The maximum AgNOR dots were found in mucinous (3.43%) and small cell type (4.11%). The mean PCNA LI was maximum in small cell type (29.13%) and mucinous type (27.97%). The tubular type of tumors (40%) did not show any significant result. No correlations were found among different tumors of this classification.

Lauren system

In this system 21 (60%) tumors were intestinal type and 12(34%) were diffuse type. Mean AgNOR intestinal type was 2.8% and 3.06% in diffuse type. Mean PCNA LI in intestinal type was 21.14% and 22.02% in diffuse type. The 5.72% tumors showed both diffuse and intestinal pattern (mixed) and found higher mean AgNOR count (3.2%) & PCNA LI (28.95%) than other category. Mucin-rich & mucin-poor tumors and tubules rich & tubules poor tumors of Goseki's grade were distributed across both Lauren types. In intestinal type 66.66% were mucin-rich tumors while in diffuse type 33.33% were mucin-rich and mean AgNOR count were higher in diffuse type (3.02%) than intestinal type (2.74%) Mucin poor tumors of intestinal type showed higher values of mean AgNOR and PCNA LI (3.22% and 27.52%) than mucin-poor tumors of diffuse type (2.91% and 21.88%). Intestinal type of tubule rich tumors showed lower values than tubule poor tumors of both types. Tubules poor of intestinal type showed higher AgNOR & PCNA LI (3.31% & 31.11%) than diffuse type (3.28% & 24.31%).

Tumour differentiation

Mean AgNOR count were 2.43%, 3.15% and 2.75% in well, moderately and poorly differentiate tumours respectively. Similarly the mean PCNA LI were 13.85%, 24.95% and 20.46% in well, moderate and poorly differentiated tumors. The moderately differentiated tumors (48.57%) showed higher values than remaining types. Tubule rich (Goseki grade I&II) & tubule poor (Goseki grade III&IV) tumors and mucin rich (Goseki grade II&IV) & mucin poor (Goseki grade I&III) of different gastric carcinoma classification systems were tabulated. Mean AgNOR count 2.88% and PCNA LI 20.46% of poorly differentiated carcinomas showed higher values than well and moderately differentiated carcinomas. Mucin rich tumors of different differentiation falling in Goseki II and IV showed higher mean AgNOR (3.16%) and PCNA LI(25.91 %) than mucin poor of Goseki grade I and III (AgNOR 3.2% and 25.82%). Tubule poor tumors of Goseki's III and IV grade showed higher mean AgNOR and PCNA LI (3.81% and 31.97%) than tubule rich type of grade I and II (2.86% and 22.81%).

Goseki grading

Gradual increasing of mean AgNOR and PCNA LI from grade I-IV were 2.35% & 15.14%, 2.91% & 21.32%, 3.08% & 24.76% and 3.2% & 25.12% respectively. Mucin rich tumors (Goseki grade II and IV) show higher mean AgNOR count and PCNA LI (3.05% and 23.22%) than mucin poor (Goseki grade I and III) category (2.71 % and 19.95%). Accordingly tubule poor tumors (Goseki grade I and II) expressed higher mean AgNOR and PCNA LI (3.14 % and 24.76%) than tubule rich (Goseki III and IV) type (2.63%and 18.23%). Multivariate analysis showed statistically significant correlation between the different Goseki grades as evidenced by 'F' test.

Discussion:

In spite of different classifications of gastric carcinoma system none of them showed any definitive prognosis and treatment prediction. New classification system with prognostic value still trying by different workers. WHO group was recognized as descriptive classification and not recognized as much prognostic out coma. In this system 63.15% of the tumors fell into tubular category and 23.33%

tumors were undifferentiated (Table I). The present study did not show significant values of mean AgNOR count or PCNA LI (Table II) in any histological subtypes which helped in patient for good clinical management or survival.

Lauren classification system according to Jarvi et al[17] showed prognostic and useful in epidemiological studies. Prakash I et al[18] found higher values of mean AgNOR and PCNA LI in intestinal type than diffuse type of adenocarcinoma of Lauren system. Oshima et al[7] also found same result considering AgNOR. But opposite results were obtained in the present study (Table II). In intestinal type 66.66% and diffuse type 33.33% were mucin-rich tumors and mean AgNOR count were higher in diffuse type (3.02%) than intestinal type (2.74%). Opposite result obtained in PCNA LI expression. So diffuse type gastric tumors of Lauren classification which predict a worse prognosis showing 33.33 % of mucin rich tumors of Goseki's grade and showing higher proliferative activity of AgNOR count. Mucin poor tumors of intestinal type showed higher values of mean AgNOR and PCNA LI (3.22% and 27.52%) than mucin-poor tumors of diffuse type (2.91% and 21.88%).

Tumour differentiations of gastric carcinoma though have little prognostic significance; poorly differentiated tumors usually spread more rapidly and extensively than well and moderately differentiated carcinomas. Higher mean AgNOR count (2.88%) and PCNA LI (20.46%) of poorly differentiated carcinomas than well and moderately differented carcinomas reflecting higher proliferating activity. Mucin rich tumors of different differentiation showed higher mean AgNOR (3.16%) and PCNA LI (25.91 %) than mucin poor (AgNOR 3.2% and 25.82%) expecting poor survival. Better tubular rich carcinomas of Goseki's grade I and II were well and moderately differentiated, where tubule poor of grade III and IV were poorly differentiated carcinomas. Tubule poor tumors showed higher mean AgNOR and PCNA LI (3.81% and 31.97%) than tubule rich (2.86% and 22.81%) falling in tumor differentiation indicates increased tumour progression.

Assessment of Goseki's grading system showed increasing values mean AgNOR and PCNA LI with the increasing grade I to IV indicating the proliferative activity of different tumor character. Mucin rich tumors showed higher mean AgNOR count and PCNA LI (3.05% and 23.22%) than mucin poor category (2.71 % and 19.95%). Martin I G et al[19] observer in a study of five year survival of patients with mucus rich (Goseki II and IV) T3 tumours was significantly worse than that of patients with mucus poor (Goseki I and III) T3 tumours (18% v 53%, $p < 0.003$). Goseki grading identifies subgroups of patients with a poorer prognosis than is predicted by TNM staging alone. Accordingly tubule poor tumors (Goseki grade I and II) expressed higher mean AgNOR and PCNA LI (3.14 % and 24.94%) than tubule rich (Goseki III and IV) type (3.14 and 24.94). Thus higher proliferative activity markers AgNOR and PCNA in Goseki's mucin rich tumors and tubule poor tumors indicating tumour progression. Goseki in 1992 proposed four grading system depending on tubular differentiation and degree of intracytoplasmic mucin production by the tumour cells. Along with other authors[19, 20] Goseki suggested that mucin production is most important than tubule formation for assessment of prognosis. Goseki and his coworkers suggested from their 200 sample series that grade I tumors (tubular rich and mucin poor) had higher frequency of haematogenous metastasis, while type IV tumors (mucin rich and tubule poor) spread directly into the lymph nodes and peritoneum. In one study by Dixon et al[2]showed 55% patients with mucin-rich carcinomas died within 5 years than mucin poor patients.

In the present study, correlations of intra cytoplasmic mucin production and tubule formation by tumors falling in different histological classifications gastric adenocarcinoma (WHO, Lauren, tumour differentiation) with Goseki's grading system along with proliferating markers (AgNOR and PCNA) helps to determine the efficacy of Goseki's grading system. Mean AgNOR count and PCNA LI expression of different histological classification systems in WHO, Lauren and tumor differentiation did not show any significant correlations, probably due to tumour heterogenecity and some form of deregulated expression of cell proliferative markers from tumour to tumour, from superficial to deeper layers of advanced gastric carcinoma and different tissue sections and microscopic fields. Increasing quantifications of mean AgNORs and PCNA LI of gastric carcinoma according to Goseki grading system along with the mucin rich and mucin poor tumors together with tubule rich & poor tumors may reflect the tumour behavior and prognosis in addition to tumour staging. The present study is a cost effective simple laboratory procedure may help to select patients for adjuvant therapy in rural hospital and needs further study with larger sample size.

References

1. Goseki N, Takizawa T, Koike M. Differences in the mode of extension of gastric cancer classified by histological types: new histological classification of gastric carcinoma. *Gut.* 1992.33:606–612.
2. Dixon MF, Martin IG, Sue-Ling HM, Wyatt JI, Quirke P, Johnston D. Goseki grading in gastric cancer: comparison with existing systems of grading and its reproducibility. *Histopathology*. 1994.25:309–316.
3. Songun I, Van de Velde CJ, Arends JW, Blok P, Grond AJ, Offerhaus GJ, Hermans J, Van Krieken JH..Classification of gastric carcinoma using the Goseki system provides prognostic information additional to TNM staging. *Cancer.* 1999. 85:2114–2118.
4. IIfet Songun, Cornelis J. H. van de Velde, Jan W. Arends, Paul Block, A. Joris K. Grond, G. johan A. Offerhaus, et al 2000. Classification of Gastric Carcinoma Using the Goseki System Provides Prognostic Information Additional toTNM staging. *Cancer*; 2000. vol 85:10; 2114-2118.
5. Alekseenko SA, Timoshin SS, Avilova AA, Fleishman MI, Lamekhova VG. Effect of enalapril, lisinopril, amlodipine on the course of chronic gastritis in patients with arterial hypertension]. *Klin Med (Mosk)***.** 2004.82, 42-45.
6. Satoh S, Kawashima K, Matsumoto S, Hasegawa S, Okabe H, Nomura A, Yoshibayashi H, et al. Retrospective evaluation of sequential outpatient chemotherapy for advanced gastric cancer. *Chemotherapy*. 2007.53, 226-232.
7. Oshima CT, Forones NM. AgNOR in stomach neoplasm. *Arq Gastroenterol*. 2001. 38(2); 89-93.
8. Ozercan IH, Bahcecioglu IH, Ozercan MR, Bozlak N, Dogan C, Yekeler H. Proliferating cell nuclear antigen and nucleolar organizer region for differential diagnosis of dysplasia and adenocarcinoma in gastric biopsies. *Indian J Gastroenterol*. 2000; 19(2):57-60.
9. Mangham DC, Rowlands DC, Newbold KM, Renolds GM, Fielding JW, Hallissey MT. Expression of proliferating cell nuclear antigen (PCNA) in gastric carcinoma: no evidence for prognostic value. *Am J Pathol*. 2000; **24**(2): 167-176.
10. Lischwe MA, Smetama K,Olson MOJ, et al 1979. Proteins C23 and B23 are the major nucleolar silver staining proteins. *Life Sci*. 1979. **25**:701-708.
11. Derial J.D, Mercer J.K, Calabretta B and Baserga R.Initiation of cell proliferation by antisense oligodeoxy nucleotides to PCNA/Cyclin. *Science*; 1988.**240**: 1544-1546.
12. Martin IG, Dixon MF, Sue-Ling, Axon ATR, Johnston D.Goseki histological grading of gastric cancer is an important predictor of outcome. *Gut.* 1994. 35:758–763.

13. Guglielmi A, De Manzoni G, Tomezzoli A, Ricci F, Pelosi G, Laterza E, Di Leo A, Bonfiglio M. Prognostic value of histologic classifications of advanced stomach cancer: comparative study of Lauren's and Goseki's classifications. (In Italian). *Chir Ital*.; 1997.49:45–49.

14. Roy P, Piard F, Dusserre-Guion L, Martin L, Michiels-Marzais D, Faivre J. Prognostic comparison of the pathological classifications of gastric cancer: a population based study. *Cancer*. 1998. 33:304–310.

15. Ventura L., Chiominto A., Discepoli S., Ventura T., Di Giacomo C., Leocata P. Lack of prognostic significance in Goseki grading of gastric carcinoma. *Journal of experimental & clinical cancer research.* 2002. Vol 21.225-227.

16. Croker J, Boldy DAR, Egan MJ. How should we count AgNORs? Proposals for standardized approach. *J Pathol* 1989; 158:185.

17. Jarvi O, Nevalainen T, EKfors T. The classification and histogenesis of gastric tumours. *Excerpta Medica International Congress* Series No 354, 228-234.

18. Prakash I, Mathur RP, Kar P et al. Comparative evaluation of cell proliferative indices and epidermal growth factor receptor in gastric carcinoma. *Indian J Pathol Microbiol.* 1997; 40(4): 481-490.

19. Martin IG, Dixon MF, Sue-Ling, Axon ATR, Johnston D. Goseki histological grading of gastric cancer is an important predictor of outcome. *Gut*. 1994; 35:758–763.

20. Songun I, Van de Velde CJ, Arends JW, Blok P, Grond AJ, Offerhaus GJ, Hermans J, Van Krieken JH. Classification of gastric carcinoma using the Goseki system provides prognostic information additional to TNM staging. *Cancer*. 1999; 85:2114–2118.