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Original Research Article

Expression of AgNOR in histopathologically diagnosed gastric carcinoma patients

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ABSTRACT

Background: Worldwide, gastric cancer is the second leading cause of cancer-related fatalities, after lung cancer. Identifying stomach cancer without relying entirely on histopathology is critical. The Silver-stained nucleolar organizer regions (AgNOR's) reflect the aggressiveness of the tumor, according to the study. To establish the effectiveness of these approaches in detecting stomach cancer, the present study was done in order to test their efficacy. The aim of the study was to observe expression of AgNOR in histopathologically diagnosed gastric carcinoma patients.
Methods: This cross-sectional analytical study was carried out in the department of pathology, Sir Salimullah Medical College, Dhaka, Bangladesh from July 2017 to June 2019. A total of 60 adults who were histopathologically diagnosed patients with gastric carcinoma in gastric endoscopic biopsies and resected samples.
Results: More than one-third of the patients (38.3%) belonged to the age group of 61-70 years. The age range of the participants was 40-82. 55% were male and 45% were female, 63.3% of the cancers were in the pylorus region, 33.3% were in the body and 3.3% were in the cardiac end. 80% of the GC were intestinal type and 20% were of diffuse type. 50% of the cancers were moderately differentiated, 36.67% were poorly differentiated, and 13.33% were well differentiated. The association between histopathological grading of gastric carcinoma and AgNOR was statistically significant.
Conclusions: The growth of the tumor is highly variable. The AgNOR stain is also a good marker to detect the proliferation of tumor cells.

Keywords: Expression, AgNOR, Histopathology, Gastric carcinoma

INTRODUCTION

Gastric carcinoma, or gastric cancer, is a malignant tumor of the stomach. It can develop in any part of the stomach and can spread to other parts of the body as well. Gastric cancer was the primary cause of cancer related deaths among American men, and the third common cause among women back in 1930.[1] Due to medical advancements, there has been a large decline in the incidence rates of Gastric carcinoma (GC), but it still holds the position of the second

most common cause of cancer related deaths worldwide.[2] Presently, it is the fourth most commonly diagnosed cancer worldwide.[3] The global distribution of stomach cancer varies significantly between geographical locations, reflecting the plethora of factors linked with the disease's incidence, survival, and mortality.[4]

Cancer has affected humans throughout recorded history. Some of the ancient evidence of cancer is found among fossilized bone tumors in human mummies of ancient Egypt and dates back to approximately 1600 BC. Cancer of any kind constitutes an enormous burden on society in more and less economically developed countries alike.[5] Although cancer incidence rates are much lower in the developing countries compared to the developed countries, the mortality rates from cancer remain similar in both cases. This is mainly because although the incidence is lower, cancer survival becomes much harder in developing countries due to lack of proper medical facilities. A substantial proportion of the worldwide burden of cancer could be prevented through the application of existing cancer control knowledge, early detection and treatment, as well as public health campaigns promoting physical activity and a healthier dietary intake.

Significant efforts are being made to elucidate risk factors and an etiological aspect underlying the disease, and to identify potential diagnostic and prognostic predictors that can be translated into prevention, early detection and cure of the malignancy. This, however, has been a difficult task due to the multi factorial causality and complexity of this group of diseases.[6]

Understanding of cancer thus represents one of the major challenges for the scientific community in the present century. The detection of GC falls among the criteria of one of these factors. Gastric cancer is generally diagnosed by an upper endoscopy, which is an invasive and painful procedure. Many researches are being conducted to find better and non-invasive methods of GC. Stomach cancer is often either asymptomatic or it may cause only nonspecific symptoms. By the symptoms occur, the cancer has often reached an advance stage and may have metastasized, which is one of the reasons for its poor prognosis. Advancement in the treatment of gastric carcinoma, including combination chemotherapy, has resulted in improved overall survival compared to single agent chemotherapy alone.[7]

Additional therapy aimed at specific targets in cancer has shown a survival benefit in certain tumors. But a severe need still exists for methods to determine and diagnose GC in a non-invasive manner. The Nucleolar organizer region (NOR) technique has recently been applied to several cancers.

The Silver staining technique of NOR (AgNOR) is based on argyrophilia of NOR associated proteins and can be performed at room temperature on paraffin embedded tissues. AgNOR count can help distinguish benign from malignant lesions. Many studies have shown that increased AgNOR counts are frequently associated with malignancy and may have prognostic usefulness.[8]

The aim of the study was to observe the correlation between the expression of AgNOR and histopathological diagnosed gastric carcinoma patients.

Objective

The objective of this study was to observe the expression of AgNOR in histopathologically diagnosed gastric carcinoma patients.

METHODS

This was a cross-sectional analytical study conducted at the department of pathology, Sir Salimullah Medical College Mitford Hospital in Dhaka city during the period of two years, from July 2017 to June 2019. Purposive and convenient sampling was used to select 60 adult patients who were histopathologically diagnosed with GC in gastric endoscopic biopsies and resected samples.

Using a mathematical formula, the initial sample size was found to be 384, but due to time constraints and funding problems, the present study was conducted with 60 patients. Informed written consent was taken from all the study participants, and ethical approval was obtained before the beginning of the study, by submitting the research protocol to the Institutional ethical committee of SSMC, Dhaka.

Both endoscopic biopsied and resected samples were collected and preserved for each participant. Inclusion criteria was patients with histopathologically diagnosed gastric carcinoma and patients older than 35 years of age. Mentally ill, patients declining consent, patients who were treated with radiotherapy or chemotherapy and patients having metastatic cancer were excluded.

The number of AgNORS dots was counted in the nuclei, using oil immersion in 1000 magnifications. The overall Mean AgNORs (mAgNOR) per nucleus was obtained by counting the AgNOR dots in 100 nuclei.

Data were collected by using a pre-tested structured questionnaire by fact-to-face interview and it was checked, verified and edited using Statistical package for the social sciences (SPSS) for data analysis.

RESULTS

It was observed that more than one-third of the patients (38.3%) belonged to the age group of 61-70 years. 21.67% were from the age group of 51-60 years, another 21.67% were from the age group of younger than 51 years, and the oldest age group of 71 and older had only 18.33% of participants. The mean age was 61.05-11.99 years, and the age of the participants ranged from 40 to 82 years.

In this study, it was observed that more than half (55.0%) of the patients were male and (45.0%) were female. The male: female ratio was 1.22:1. Almost two-thirds (63.3%) of patients had cancer in the pylorus region, 20 (33.3%) patients had cancer in the body and 2 (3.3%) had cancer in the cardiac end. It was observed that over three quarter (80%) of the study patients had intestinal type of adenocarcinoma and 12 (20.0%) had diffuse type of adenocarcinoma.

It was observed that half of the patients (50%) had moderately differentiated gastric carcinoma. Of the rest, 13.3% had differentiated gastric carcinoma and 36.7% had poorly differentiated gastric carcinoma.

It was observed that in 75% of patients with well differentiated gastric carcinoma, the tumors were located in the pyloric region, and the remaining 25% well differentiated gastric carcinoma were located in the body. In the case of moderately differentiated gastric carcinoma, 60% were located in the pyloric region, 33.3% in the body, and 6.7% in the cardiac end. In the case of the poorly differentiated GC, 63.6% were located in the pyloric region, and 36.4% in the body. No statistically significant association was found between the sites of tumor and the histopathological grading.

ANOVA followed by the Bonferroni test was performed to compare between groups. The mean score found among the three grades were 2.34±0.11 in well differentiated, 2.63±0.25 in moderately differentiated, and 2.76±0.27 in poorly differentiated. When the mean AgNOR scores were compared with three gradings of gastric adenocarcinoma, it was found statistically significant (p=0.005). When AgNOR scores were compared in between three groups such as A vs B (0.003) and A vs C (0.001) they were also found significant (p<0.05). However, when compare between B vs C (0.079) it was found not significant.

The mean AgNOR was 2.63±0.28 in intestinal type of GC and 2.67±0.29 in diffuse type. The difference was statistically not significant (p>0.05) between two groups.

Table 1: Distribution of the study patients by age (n=60).

Age (in year)	N	%
≤50	13	21.67
51-60	13	21.67
61-70	23	38.33
>70	11	18.33
Mean±SD	61.05±11.99	

Table 2: Distribution of the study patients by histological types of gastric adenocarcinoma (n=60).

Type of tumor	N	%
Intestinal	48	80
Diffuse	12	20

Table 3: Histopathological grading to gastric carcinoma (n=60).

Histopathological grading	N	%
Well-differentiated	8	13.33
Moderately differentiated	30	50.00
Poorly differentiated	22	36.67

Table 4: Association between sites with histopathological grading of the study patients (n=60).

Sites	Histopathological grading						P value
	Well differentiated		Moderately differentiated		Poorly differentiated		
	N	%	N	%	N	%	
Pylorus	6	75	18	60	14	63.6	
Body	2	25	10	33.3	8	36.4	0.652^{ns}
Cardiac end	0	0	2	6.7	0	0	

Note: ns= not significant, p value reached from Chi-square test.

Table 5: Relation of AgNOR with histopathological grading (n=60).

Histopathological grading	AgNOR		P value
	N	Mean±SD	
Well differentiated (A)	8	2.34±0.11	

Continued.

Histopathological grading	AgNOR		P value
	N	Mean±SD	
Moderately differentiated (B)	30	2.63±0.25	
Poorly differentiated (C)	22	2.76±0.27	
Statistical analysis			
A vs B vs C			0.005[s]
A vs B			0.003[s]
A vs C			0.001[s]
B vs C			0.079[ns]

Note: s= Significant, ns= Not significant.

Table 6: Association between types of tumors with AgNOR of the study patients (n=60).

Type of tumor	AgNOR		P value
	Mean±SD	Min-max	
Intestinal	2.63±0.28	2.15-3.15	0.636[ns]
Diffuse	2.67±0.29	2.35-3.05	

Note: ns= Not significant, p value reached from unpaired t-test.

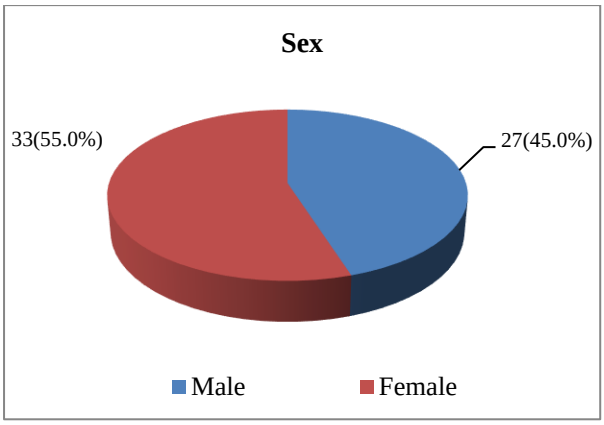

Figure 1: Pie chart showing sex of the study patients (M>F).

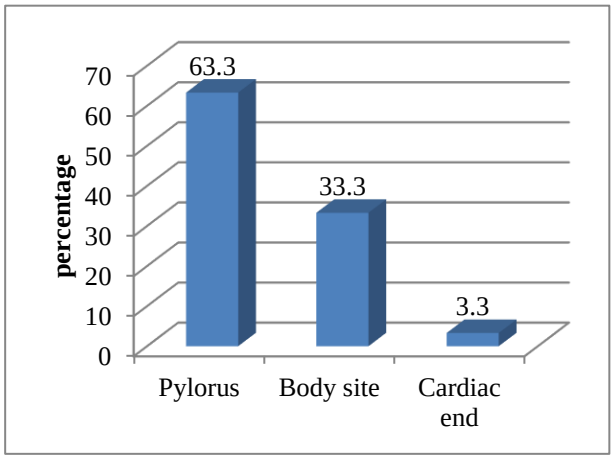

Figure 2: Bar diagram showing site of tumors in the study patients.

DISCUSSION

GC is one of the most common cancers, accounting for the second highest number of cancer deaths globally.[9] Diagnostic and therapeutic procedures have improved during the previous decade, but the prognosis for GC patients remains bleak, particularly those from the advanced stage.[10] Tumor biomarkers represent the pathogenic properties of tumor cells, and as a result, they frequently have diagnostic or prognostic value. When it comes to guiding patients' therapy, the expression level of several prognostic indicators is connected with patient survival. AgNOR expression in gastric carcinoma should therefore be evaluated for its diagnostic accuracy and utility, as well as its usefulness in grading stomach carcinoma in individuals who have been histopathologically diagnosed as having gastric carcinoma. The purpose of this study was to provide some light on the utility of detecting histopathologically diagnosed stomach cancer using AgNOR expressions. In the current study, more than one-third (38.3%) of the patients were between the ages of 61 and 70, 21.7% were between the ages of 40 and 50, 21.7% were between the ages of 51 and 60, and 18.3% were between the ages of 71 and 82. As people aged above 70, the rate of GS incidence began to fall.

Another study found that stomach cancer is rare before the age of 40, but its frequency continuously increases afterward and peaks in the seventh decade of life.[11] More over half (33, 55%) of the 60 patients were men, with the remainder (27, 45%) being women. The male population of this study had a slightly increased incidence of GS, with a male: female ratio of 1.22:1. This was similar to few other studies where the male prevalence was higher than the female in varying degrees.[12,13] After observing the anatomic site of GC among the participants, the pylorus was the most common location of GS, with 63.3 percent of subjects having it. Following that, 33.3 percent developed GC in the body, and 3.3 percent had cancer in the cardiac end. These findings were consistent with another study published in 2015, which found that the most prevalent anatomic site of gastric cancer was the antrum in 47.5

percent of patients, the body in 38.8 percent, and the cardiac end in only 13.7 percent.[14]

However, a few studies found that the most prevalent site of GC was the stomach's body as well as the cardiac end.[13,15] This difference might be the result of geographical and cultural differences, along with food habit. Distribution of the patients according to the type of carcinoma showed that 80% were intestinal types, and only 20% were diffuse types. Only 13.33 percent of the GC patients had well differentiated cases, 50 percent had moderately differentiated cases, and 36.67 percent had poorly differentiated cases, according to histopathological grading. Following an assessment of the relationship between cancer sites and histological grading, it was discovered that all of the cardiac end GC were of the moderately differentiated grading.

Seventy-five percent of individuals with well-differentiated GC had pyloric sites, with the remaining 25 percent having sites elsewhere in the body. 60 percent of moderately differentiated gastric carcinomas were found in the pyloric area, 33.3 percent in the body, and 6.7 percent in the cardiac end. The poorly differentiated GC was seen in 63.6 percent of cases in the pyloric area and 36.4 percent in the body. There was no statistically significant relationship established between tumor location and histological grading. In terms of the relationship between AgNOR and histopathological grading of the study patients (N=60), the mean AgNOR was 2.340. 11 in well differentiated, 2.630. 25 in moderately differentiated, and 2.760. 27 in poorly differentiated. The difference between the three groups was statistically significant ($p<0.05$). Individual comparisons between well differentiated and moderately differentiated AgNOR scores ($p=0.003$), and between the two groups with the least amount of differentiation ($p=0.001$), also revealed significant results. There was no statistical significance revealed in the comparison between moderately differentiated and badly differentiated ($p=0.079$).

Dividing the AgNOR score by type of GC, the mean AgNOR was 2.63±0.28 in intestinal type of gastric carcinoma and 2.67±0.29 in diffuse type. The difference of AgNOR scores between the two types were statistically insignificant. Tumors that are poorly differentiated, such as gastric carcinomas, tend to grow and spread more swiftly and widely than those that are well or moderately differentiated. On the basis of the results of a previous study, poorly differentiated carcinomas had a higher mean AgNOR count (2.88 percent) than did well and moderately differentiated carcinomas.[16]

Limitations

The study was conducted in a single hospital with small sample size. So, the results may not represent the whole community.

CONCLUSION

The growth of the tumor is highly variable. The AgNOR stain is also a good marker to detect the proliferation of tumor cells. AgNOR scores expressed correlation with diagnosis and prognosis of gastric carcinoma. Results of present study indicate an association between AgNOR count with histopathological grading and differentiation of gastric carcinoma.

Recommendations

Study recommend multi-centre study with large sample size. Which can give a more reliable scenarios of the problems.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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