ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Von Willebrand factor and gastric cancer – is there an association? – A pilot study from South India

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Submission: 02-01-2024 Revision: 08-02-2024 Publication: 01-03-2024

ABSTRACT

Background: Gastric adenocarcinoma in Indian patients' is on the rise. A high level of plasma von Willebrand factor (vWF) is associated not only with the development of cancer-associated thrombosis but also with the degree of malignancy, the rate of metastasis, and cancer prognosis. **Aims and Objectives:** The aim of this study was to study the association of vWF with tumor location, TNM staging, and grades of tumor differentiation in gastric adenocarcinoma and to assess the usefulness of circulating vWF levels as a potential biomarker for gastric adenocarcinoma. **Materials and Methods:** This study is a prospective observational study done in the Department of Surgical Gastroenterology, Madurai Medical College, Madurai, over a period of 2 years between September 2020 and August 2022. Fifty cases of gastric cancer and 50 controls were recruited and vWF levels were analyzed. **Results:** Among the 50 gastric cancer patients, 8% belonged to Stage II, 62% belonged to Stage III, and 30% belonged to Stage IV. vWF was elevated in Gastric Cancer patients compared to control group. Mean value of vWF in Cases and Control population was 3.68 ng/ml and 0.58 ng/ml respectively (p value 0.0005). Pertaining to Stage, the vWF value was, Stage II – 4.7, Stage III – 3.3, and Stage IV – 4.1 ng/mL. With respect to tumor differentiation, the vWF values were, poorly differentiated – 4.7, moderately differentiated – 3.0, and well differentiated – 3.9 ng/mL. **Conclusion:** vWF values were significantly elevated in gastric cancer patients. Based on the results, vWF can be used as a surrogate marker to predict the tumor differentiation in patients, thereby prognosticating the disease. Stage-wise, there was no significant difference in the vWF values.

Key words: Gastric cancer; von Willebrand factor; Early screening

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v15i3.61361

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Gastric cancer is the fifth most common cancer among men and sixth most common among women in India. The incidence of gastric cancer is low in India, when compared to the global scenario. Majority of the gastric cancer patients, in India, present to the outpatient department at an advanced stage.[1] This directly translates into a decrease in 5-year survival rate when compared to countries where screening facilities are available for early detection. The 5-year survival rate for patients undergoing surgical resection in India is reported to be only 27%.[2] von Willebrand factor (vWF), a macromolecular plasma glycoprotein, is thought to be exclusively synthesized in endothelial cells, megakaryocytes and platelets. The plasma level of vWF has been widely used as a marker for endothelial perturbation and propensity for thrombosis and thromboembolism.[3] vWF forms an integral link between tumorigenesis and angiogenesis, thereby having a potential role in cancer dissemination. A high level of plasma vWF is associated not only with the development of cancer-associated thrombosis but also with higher degree

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of malignancy, increased rate of metastasis, and poorer prognosis. However, its role in gastric cancer have not yet been fully characterized.

Objectives

Primary objective

The primary objective of this study was to study the association of vWF with tumor location, TNM staging, and grades of tumor differentiation in gastric adenocarcinoma.

Secondary objective

The secondary objective of this study was to assess the usefulness of circulating vWF levels as a potential biomarker for gastric adenocarcinoma.

MATERIALS AND METHODS

Type of study

This study was a prospective observational Study.

Place of study

This study was conducted in the Department of Surgical Gastroenterology, Madurai Medical College, Madurai.

Period of study

This study was conducted over a period of 2 years, September 2020–August 2022.

Sample size and population

The sample size was 100 (50 cases and 50 controls). Patients attending the Surgical Gastroenterology OPD in Madurai Medical College were taken as cases and healthy volunteers were taken as controls.

Inclusion criteria

The following individuals were included in the study:

1. Patients aged more than 18 years with gastric cancer identified by upper gastrointestinal endoscopy examination and proven on histopathology were included in the study
2. Healthy volunteers were taken as controls.

Exclusion criteria

The following individuals were excluded from the study:

1. Patients with gastric malignancy diagnosed as second primary
2. Patients who have undergone surgery, chemotherapy, or radiotherapy for gastric cancer before the study
3. Patients who do not consent for the study
4. Patients with comorbidities known to increase plasma levels of vWF such as diabetes, hypertension, heart failure, renal dysfunction, pregnancy, and hyperlipidemia.

Study procedure

Endoscopic examination

All patients who had a growth in the stomach were recorded and tissue samples were sent for histopathological examination. Biopsy-proven patients were taken as cases.

Plasma collection and vWF analysis

After enrolment of the patient, 2 mL of blood sample was taken between the hours of 8 am and 10 am. Samples were drawn into prechilled tubes containing EDTA, put on ice right away, and then quickly centrifuged at 4°C. Plasma was separated and kept at a temperature of −80°C. ELISA, a two-step sandwich assay with antibody-coated micro plates, was used to quantify vWF as per manufacturer protocol (R&D Systems, Inc. cat No DY2764-05). This DuoSet ELISA Development kit contains the basic components required for the development of sandwich ELISAs to measure natural and recombinant vWF-A2. Assay ranges from (46.9 to 3000 pg/mL).

Ethical approval

The study was approved by the Institutional Ethics Committee of Madurai Medical College, Madurai, and performed as per the standards laid down by the Declaration of Helsinki for medical research involving human subjects.

Statistics

The results were analyzed with IBM SPSS Statistics for Windows, Version 29.0 (Armonk, NY: IBM Corp). To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean, median, and standard deviation were used for continuous variables. To find the significant difference between the bivariate samples in Independent groups, the Independent sample t-test was used. For the multivariate analysis, the one-way ANOVA was used. To find the significance in qualitative categorical data, Chi-square test was used similarly if the expected cell frequency is <5 in 2×2 tables, then the Fisher's exact was used. In all the above statistical tools, the probability value (P)<0.05 was considered as significant.

RESULTS

In this study, 50 patients diagnosed to have gastric cancer and 50 controls who were healthy volunteers were recruited. Of the 50 cases, 20 (40%) were female and 30 (60%) were male. The median age of gastric cancer was 55.26±10.6 years. There were 8% (4) patients having Stage II disease, 62% (31) of patients belonging to Stage III, and 30% (15) patients belonging to Stage IV. None of the patients had

Stage I disease. Antropyloric growth was seen in 25 (50%) of patients, 17 (34%) patients harbored malignancy in the body of the stomach, 6 (12%) patients had proximal gastric cancer, and 2 (4%) had diffuse gastric cancer. With respect to grade of the tumor, 11 (22%) patients had well-differentiated tumor, 24 (48%) had moderately differentiated tumor, whereas 15 (30%) had poorly differentiated tumor. Mean value of vWF among cases was 3.68±0.34 ng/mL and in control population vWF value was 0.58±0.20 ng/mL (Figure 1), with P<0.0005. Pertaining to Stage, the vWF value was, Stage II – 4.7±1.3 ng/ml, Stage III – 3.3±1.1 ng/mL, and Stage IV– 4.1±2.5 ng/mL (Figure 2). With respect to tumor differentiation, the vWF values were, poorly differentiated – 4.7±2.8 ng/mL, moderately differentiated – 3.0±2.1 ng/mL, and well differentiated – 3.9±1.8 ng/mL, with P=0.098 (Figure 3).

Figure 1: vWF value in Cases and Control group

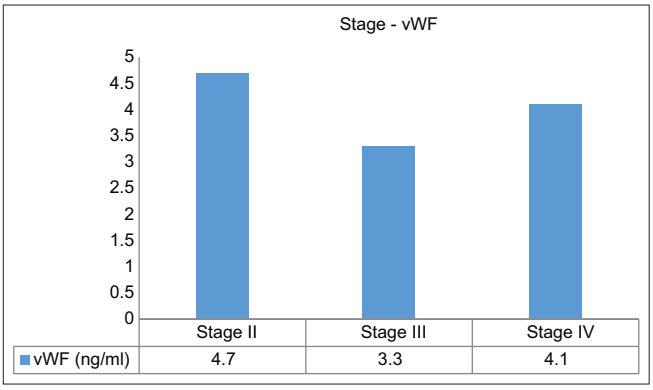

Figure 2: vWF value - Stage wise

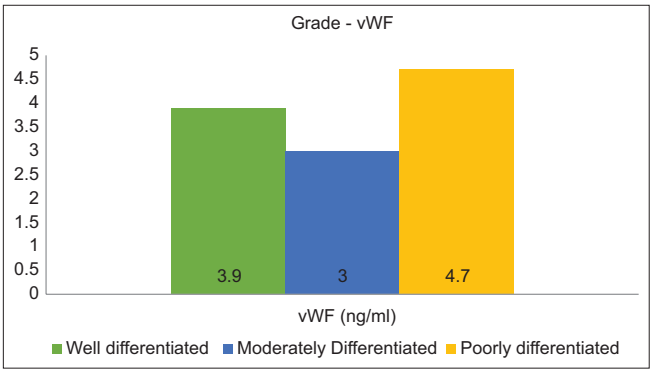

Figure 3: vWF value - Grade wise

DISCUSSION

Despite the low incidence of gastric cancer in Indian population, the advanced nature of the disease at the time of presentation poses a humongous burden to the health care and patient fraternity. vWF levels' association with malignant tumors has been studied in both human and animal models. There is significant increase in Vwf levels in hematological and non-hematological malignancies, which includes colorectal, gastric, and prostate cancers, when compared to healthy controls.[4] The tumor secretomes by acting on endothelial cells, induce inflammatory cytokines, matrix metalloproteinases, and vascular endothelial growth factor-A (VEGF-A). This pathway leads to raised circulatory vWF factor levels. Colon carcinoma and gastric cancer display increased vWF expression in their tumor stroma, which is mediated by increase in the tumor vasculature and potent vWF gene expression. Accessibility to vasculature plays an important determinant in cancer progression.[5]

VWF has been described as having both pro- and anti-angiogenic roles in different tissue beds and in specific disease settings. Both gastric and colon cancer secreted VEGF-A and was shown to upregulate vWF expression and secretion from endothelial cells *in vitro* in a dose and time-dependent manner. Circulating platelets play an important role in tumor metastases. This phenomenon is related to the accelerated endothelial synthesis together with its tumor-dependent angiogenesis. Hematogenous metastasis is the predominant factor that determines VWF-mediated cancer dissemination, rather than lymphatic metastasis.[5] This phenomenon is supposedly mediated by the formation of "platelet-taxi" or by interacting directly with tumor cells.[3]

Interestingly, animal experiments have shown that antiplatelet and anti-vWF antibodies can substantially reduce the occurrence and number of metastasis. These results may be used for analyzing the use vWF antibodies in mitigating the progression of the disease. There have been studies that embark on the fact that, vWF is not only a biomarker in Gastric cancer but also a mediator for cancer metastasis and a new therapeutic target for cancer. As evidenced by the role of vWF in cancer and its progression, the present study has been undertaken to assess the role of vWF as a potential biomarker in carcinoma stomach and its correlation toward disease progression.

In the present study, vWF values were significantly elevated in gastric cancer patients compared to normal controls. The association between vWF levels and gastric carcinoma was statistically significant, with value of P<0.0005. Majority of the patients, 24 (48%) had moderately differentiated tumor, whereas 15 (30%) had poorly differentiated tumor. Since both well differentiated and poorly differentiated tumors had vWF values higher than that of moderately differentiated tumors, the association between vWF values and grade of differentiation of the tumor was not significant.

Studies on gastric cancer have shown enhanced levels of vWF activity in plasma with a strong association between vWF and the disease severity. In the present study, on analyzing the association between the levels of vWF and the progression of the disease, it was noted that the vWF values did not correlate with the progression of the disease as iterated in animal and human studies. Although gastric cancers in Indian subcontinent have a low incidence, the mortality rates are still high, with majority of the patients presenting at an advanced stage, as evident from this study. An effective biomarker is needed for screening and to prognosticate the disease. vWF values were significantly elevated in gastric cancer patients in comparison to controls. Based on these results, it is evident that vWF can be used as a surrogate marker to predict the differentiation status of patients, thereby paving way for a prognostic tool. Despite the rise in vWF in gastric cancer patients, stage-wise there was no significant difference in the vWF values.

Limitations of the study

Low sample size in the study.

CONCLUSION

The association between vWF and gastric cancer is evident from the present study. However, stage-wise progression of the disease and grade of differentiation did not significant correlation with vWF values. Being a pilot study, it has a low sample size, which may hamper the extrapolation of data onto the general population. Further studies are needed to emphasize the usefulness of vWF factor levels, as a screening tool for early detection of gastric cancers and also for identifying disease recurrence.

ACKNOWLEDGMENT

We acknowledge our Dean, Nodal officer of Multi-Disciplinary Research Unit at, Madurai Medical College, Madurai, Tamil Nadu, India, under Department of Health Research, Government of India, for the support of this study and also acknowledge and thanking Mrs K. Vennila and B Punithavathi laboratory technicians for processing the samples.

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Authors Contribution:
KS- Concept, design, clinical protocol; **NA-** Literature survey, prepared first draft of manuscript, data collection, data analysis, manuscript preparation and submission of article, and preparation of figures; **SA-** Design of study, editing, and manuscript revision; **VR-** Review manuscript and editing; **R-** Definition of intellectual content, data collection; **PS-** Review manuscript; **RG-** Implementation of study protocol and interpretation; **MK-** implementation of study protocol and interpretation.

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Source of Support: The study project was funded by the Department of Health Research, Ministry of Health and Family Welfare, Government of India through MRU, Madurai Medical College (No. V25011/464/2015/HR), **Conflicts of Interest:** None declared.