RESEARCH ARTICLE

Mean Platelet Volume and Platelet Distribution Width Are Associated with Gallbladder Cancer

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Abstract

Gallbladder cancer (GBC) represents the most common biliary tract malignancy. Activated platelets play an essential role in cancer development and progression. Mean platelet volume (MPV) and platelet distribution width (PDW) are commonly used indexes of activated platelets in clinical practice. The aim of the current study was to investigate the association of MPV and PDW with GBC. 104 GBC patients and 109 normal control subjects were entered in this study between January 2015 and December 2015. We collected all participants' clinical and laboratory characteristics at initial diagnosis. The odds ratios (ORs) for GBC were calculated using multivariate logistic regression analysis after adjusting for confounding variables across MPV and PDW quartiles. MPV levels were markedly lower and PDW levels were remarkably higher in GBC patients than control subjects. A significant correlation between PDW and lymph node metastasis was detected. In addition, after adjusting for other risk factors, the ORs (95% CIs) for GBC in each MPV quartile were 5.117 (1.939-13.506), 2.444 (0.917-6.516), 3.718 (1.381-10.007), and 1.000, respectively. The ORs (95% CIs) for GBC in each PDW quartile were 1.000, 2.063 (0.825-5.162), 3.070 (1.108-8.507), and 12.108 (4.243-34.553), respectively. In conclusion, decreased MPV and elevated PDW were independently associated with GBC. Our findings suggest that MPV and PDW are available parameters for early detection of GBC.

Keywords: Gallbladder cancer- mean platelet volume- platelet distribution width- diagnosis

Introduction

Gallbladder cancer (GBC) represents the most common biliary tract malignancy. Due to the lack of typical symptoms, GBC is often detected at advanced stage when the tumor is unresectable or metastatic. Therefore, identification of novel markers to find GBC patients in early stage is urgently needed.

Platelets play a key role in cancer progression and metastasis. An intimate cross-talk between platelets and tumor cells causes tumor growth, angiogenesis, metastasis, and dissemination(Bambace and Holmes, 2011; Goubran et al., 2014). Much of the research has revealed that increased platelets are associated with a poor prognosis in various types of cancer, including pancreatic cancer, gastric cancer, colorectal cancer, endometrial cancer, and ovarian cancer (Chadha et al.,2015; Feng et al., 2016; Heng and Benjapibal, 2014; Josa et al., 2015; Li et al., 2014). However, platelet count is determined by the balance between the rate of production and consumption of platelets. A normal platelet count could conceal the presence of highly hypercoagulative and pro-inflammatory cancer phenotypes in the presence of

efficient compensatory mechanisms(Seretis et al.,2015).

Mean platelet volume (MPV) is a marker of activated platelets and is related to different inflammatory conditions (Gasparyan et al.,2011). Changed MPV levels were observed in gastric cancer, ovarian cancer, lung cancer, colon cancer, and breast cancer (Gu et al., 2015; Kemal et al., 2014; Kilincalp et al., 2014; Kumagai et al., 2015; Li et al., 2014). Platelet distribution width (PDW), another platelet index, indicates variation in platelet size. However, the clinical implication of PDW in cancer is unclear.

The relationship between platelet indices and GBC has not yet been studied. The aim of the current study was to investigate the association of MPV and PDW with GBC.

Materials and Methods

A total 104 GBC patients (mean age 60.3 ± 10.5 years, range 36-84 years) and 109 normal control subjects (mean age 60.2 ± 3.6 years, range 54-71 years) participated in the present study. All patients undergone surgical resection in Harbin Medical University Cancer Hospital between January 2015 and December 2015. The diagnosis of

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GBC was confirmed by histology. None of the patients received chemotherapy or radiotherapy prior to surgery. The exclusion criteria included hematological disorders, coronary artery disease, hypertension, diabetes mellitus, and medical treatment with anticoagulant, statins, and acetylic salicylic acid. The control subjects were recruited from Harbin Medical University Cancer Hospital, Harbin Medical University. They were matched for age, gender, body mass index (BMI), and smoking status.

This study was approved by the Institutional Review Board of Harbin Medical University Cancer Hospital and all subjects provided written informed consent.

Clinical examination and biochemical measurements

Clinical data including smoking status, medical history and medication use were recorded for each subject. Preoperative blood samples after a 10-hour overnight fasting were collected from the individuals. White blood cell (WBC), haemoglobin, and platelet indices were analyzed by an autoanalyzer (Sysmex XE-2100, Kobe, Japan). The whole blood samples were collected in EDTA-containing tubes, and were processed within 30 minutes after blood collection. The inter- and intra-assays coefficients of variation (CVs) of all these assays were below 5%. All the measurements were performed blinded to the clinical data.

Statistics

Data for continuous variables were presented using the number of subjects, the means ± SD or median values. Significance differences of clinicopathological parameters between groups were determined with the Student's t test, Mann-Whitney U test, or Chi-square test based on the type of the data. The odds ratios (ORs) and 95% confidence intervals (95% CIs) for GBC were calculated after adjusting for confounding variables across MPV and PDW quartiles using multivariate logistic regression analysis. $P < 0.05$ was considered to indicate a statistically significant difference. All data analyses were performed using SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

The study consisted of 104 GBC patients, and 109 control subjects between January 2015 and December 2015. Of the 213 participants enrolled, 106 (49.8%) were men and 107 (50.2%) were women. The mean ages were 61.0 ± 6.3 and 59.6 ± 9.0 years, respectively.

The characteristics of GBC patients, and control subjects are presented in Table 1. GBC patients had lower MPV and haemoglobin levels and higher PDW compared to control subjects (p<0.001). However, there was no difference in age, gender, smoking status, alcohol intake, body mass index, fasting plasma glucose, platelet count, and white blood cell in two groups.

Correlations between clinicopathological characteristics and platelet indices in GBC are summarized in Table 2. Platelet count correlated with pathologic T stage, lymph node metastasis and TNM stage. PDW correlated with lymph node metastasis. However, there were no correlations between platelet count and MPV and

Table 1. The Characteristics of the Participants According to GBC Status

Variables	With GBC	Without GBC	P value
N	104	109	
Age (years)	60.3±10.5	60.2±3.6	0.927
Gender (male, %)	48 (46.2)	51 (53.2)	0.303*
Smoker (n, %)	38 (36.5)	40 (45.0)	0.212*
BMI (kg/m²)	23.5±3.8	23.9±2.8	0.384
FPG (mmol/L)	5.10 (4.80-5.81)	5.29 (5.06-5.62)	0.088**
WBC (×10⁹/L)	6.71±2.04	6.59±1.89	0.655
Haemoglobin (g/dl)	130.0±17.3	138.3±12.7	< 0.001
Platelet (×10⁹/L)	243.1±78.5	236.7±61.9	0.508
MPV (fL)	9.4±1.4	10.0±1.4	< 0.001
PDW (%)	16.3±2.1	15.0±2.2	< 0.001

Values are shown as mean ± standard deviation or median (IQR) or percentage. GBC, gallbladder cancer BMI, body mass index; FPG, fasting plasma glucose; WBC, white blood cell; MPV, mean platelet volume; PDW, platelet distribution width. p value was calculated by Student's t test; *p value was calculated by chi-square test. **p value was calculated by Mann-Whitney U test.

tumor size, tumor differentiation, and perineural invasion.

To understand the relationship between MPV and PDW levels and the prevalence of GBC, the subjects were classified into quartiles by their MPV and PDW levels. The prevalence of GBC in participants with different
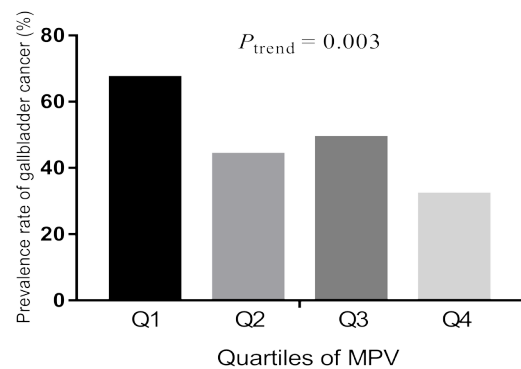

Figure 1. Prevalence Rate of Gallbladder Cancer in the Subjects with Different Quartiles of Mean Platelet Volume.
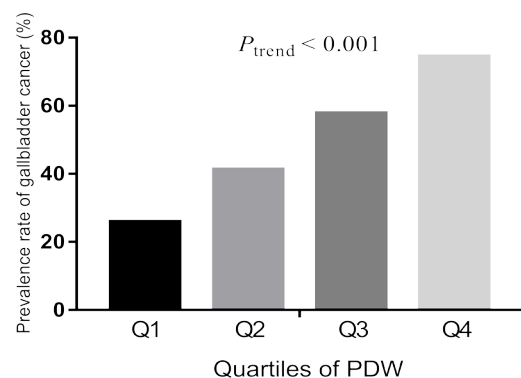

Figure 2. Prevalence Rate of Gallbladder Cancer in The Subjects with Different Quartiles of Platelet Distribution Width.

Table 2. Correlations between Clinicopathological Features and Pre- Operative Platelet Indices in GBC

Variables	N	Platelet (×10⁹/L)	P value	MPV (fL)	P value	PDW (%)	P value
Age (years)			0.159		0.202		0.102
< 60	49	231.6 (70.8)		9.5 (1.4)		16.7 (2.0)	
≥ 60	55	253.4 (84.1)		9.2 (1.4)		16.0(2.3)	
Gender			0.554		0.003		0.406
Male	48	238.1 (75.4)		8.9 (1.4)		16.5(1.9)	
Female	56	247.3 (81.5)		9.7 (1.3)		16.2(2.3)	
Tumor size			0.431		0.360		0.830
≤ 2.5cm	33	234.2 (74.8)		9.2 (1.4)		16.3(2.1)	
> 2.5cm	71	247.3 (80.4)		9.4 (1.4)		16.3(2.2)	
Tumor differentiation			0.450		0.992		0.530
Well	23	230.2 (71.3)		9.4 (1.3)		16.7(1.9)	
Moderate	27	257.9 (75.0)		9.4 (1.2)		16.3(2.1)	
Poor	54	241.2 (83.2)		9.3 (1.5)		16.1(2.2)	
Pathologic T stage			0.006		0.969		0.205
T1-T2	45	219.2 (74.3)		9.4 (1.3)		16.6(2.2)	
T3-T4	59	261.3 (77.3)		9.3 (1.5)		16.1(2.1)	
Lymph node metastasis			0.005		0.976		0.029
Negative	43	225.9 (76.9)		9.4 (1.5)		16.7(1.9)	
Positive	61	269.6 (74.3)		9.3 (1.3)		15.7(2.4)	
Perineural invasion			0.334		0.094		0.263
Negative	61	249.4 (81.9)		9.5 (1.3)		16.1(2.2)	
Positive	43	234.2 (73.5)		9.1 (1.5)		16.6(2.0)	
Clinical stage			0.001		0.721		0.173
I/II	38	211.0 (70.4)		9.4 (1.4)		16.7(2.2)	
III/IV	66	261.6 (77.4)		9.3 (1.4)		16.1(2.1)	

quartile levels of MPV and PDW were analyzed. For MPV, the prevalence of GBC in Q1, Q2, Q3, and Q4 was 67.2% (39/58), 44.0% (22/50), 49.1% (27/55), and 32.0% (16/50), respectively (Figure 1). For PDW, the prevalence of GBC in Q1, Q2, Q3, and Q4 was 25.9% (14/54), 41.3% (26/63), 57.8% (26/45), and 74.5% (38/51), respectively (Figure 2). The results indicated that the prevalence of GBC decreased as MPV quartiles increased (ptrend < 0.001) and increased as PDW quartiles

Table 3. ORs and 95% CIs for GBC According to MPV and PDW Quartiles

Variables	β	OR (95% CI)	p value
MPV (fL)			0.007
Q1 (≤ 8.8)	1.633	5.117 (1.939-13.506)	0.001
Q2 (8.9-9.8)	0.894	2.444 (0.917-6.516)	0.074
Q3 (9.9-10.6)	1.313	3.718 (1.381-10.007)	0.009
Q4 (≥ 10.7)		1 (reference)	
PDW (%)			< 0.001
Q1 (≤ 13.8)		1 (reference)	
Q2 (13.9-16.5)	0.724	2.063 (0.825-5.162)	0.122
Q3 (16.6-17.0)	1.122	3.070 (1.108-8.507)	0.031
Q4 (≥ 17.1)	2.494	12.108 (4.243-34.553)	< 0.001

Adjusting for age, gender, smoking status, body mass index, fasting plasma glucose, white blood cell, and haemoglobin. β, partial regression coefficient. OR, odds ratio; CI, confidence interval

increased (ptrend < 0.001).

The risks for GBC according to MPV and PDW quartiles are listed in Table 3. In multiple logistic regression model, after adjusting for age, sex, smoking status, body mass index, fasting plasma glucose, white blood cell, and haemoglobin, the corresponding ORs (95% CI) for GBC across MPV quartiles were 5.117 (1.939-13.506), 2.444 (0.917-6.516), 3.718 (1.381-10.007), and 1.000, respectively. Similarly, the ORs (95% CI) for GBC across PDW quartiles were 1.000, 2.063 (0.825-5.162), 3.070 (1.108-8.507), and 12.108 (4.243-34.553), respectively.

Discussion

In this study, we provided evidence that platelets is activated in GBC using a simple, relatively inexpensive, almost universally obtained test. The GBC patients had lower MPV and higher PDW compared to the controls. Furthermore, MPV and PDW were independently associated with GBC.

Platelets act as a key modulator in tumor development, angiogenesis, and metastasis. Several reports found that thrombocytosis is associated with poor prognosis in various types of cancers, including lung cancer, ovary cancer, endometrium cancer, rectum cancer, renal cancer, gastric cancer, pancreas cancer, and breast cancer. Some

clinical studies have found the altered indicators of platelet activation, such as soluble P-selectin, CD40 ligand, and β-thromboglobulin (β-TG) in patients with cancer (Al-Mondhiry, 1983; Ay et al., 2008; Huang et al., 2012). However, these parameters reflecting platelets activation were not routinely used in clinical practice and can not be easily evaluated prior to treatment. MPV levels are routinely recorded in the clinical setting and can be easily estimated prior to treatment. Thus, MPV might be useful in early detection and in creating an individual patient therapy plan.

However, the change of MPV and PDW in different types of cancers is inconsistent. Higher MPV was observed in gastric cancer and ovarian cancer (Kemal et al., 2014; Shen et al., 2016). Moreover, increased PDW was found in gastric cancer and lung cancer (Gunaldi et al.,2016; Oncel et al., 2015). In contrast, reduced PDW was noted in thyroid cancer and breast cancer (Okuturlar et al., 2015; Yaylaci et al., 2016). The conflicting data may be due to small sample sizes, failure to rule out confounding factors, different tumor types, and selected populations. Therefore, further research on MPV and PDW levels in different cancers is warranted.

The molecular mechanisms underlying the association of MPV and PDW with GBC are poorly understood. Bone marrow cells (including megakaryocytes) dys-regulation may contribute to altered MPV and PDW. Platelet volume is determined both during megakaryopoiesis and during thrombopoiesis. Megakaryocytic maturation, platelet production and platelet size could be modulated by cytokines, such as interleukin-6 (IL-6), granulocytes colony stimulating factor (G-CSF) and macrophage colony stimulating factor (M-CSF) (Kaushansky,1998). Increased interleukin-6 (IL-6) has been verified in almost all types of tumors acting as a major cytokine in the tumor microenvironment (Lippitz and Harris, 2016). Several lines of evidence revealed that IL-6 overexpression promotes tumorigenesis by regulating apoptosis, survival, proliferation, angiogenesis, metastasis and metabolism (Kumari et al., 2016). Moreover, the cytokines G-CSF and M-CSF secreted by tumor cells could stimulate megakaryopoiesis and subsequent thrombopoiesis in cancer (Kowanetz et al., 2010). MPV was an early index of activated platelets. Decreased MPV could be a result of an enhanced consumption of large platelets in inflammatory states (Gasparyan et al., 2011). In addition, MPV has been reported to be positively associated with the levels of thrombopoietin and interleukin-6 which regulate megakaryocyte ploidy (Brown et al., 1997; Martin et al., 1983). More experiments need to be carried out to explore the molecular mechanism for the change of MPV and PDW in GBC.

Considerable evidence supports the association between activated platelets and cancer in clinical studies. In this study, we showed the association between activated platelets and GBC using a convenient and inexpensive marker. This marker may be a complement to the present biomarkers and a benefit to the early detection of GBC.

The present study has several limitations. First, we analyzed the subjects cross-sectionally and this type of study fails to indicate a causal relationship between MPV and PDW and GBC. Second, this is a study carried out in a single hospital. Therefore, prospective studies are needed to illuminate the relationship between MPV and PDW and GBC.

In conclusion, the study found that MPV was independently associated with the GBC. Further studies on the involvement of MPV in GBC are needed.

Conflict of Interest

None.

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