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MEAN PLATELET VOLUME AND ITS RATIO TO ITS COUNT IN CHRONIC HEPATITIS C PATIENTS WITH HEPATOCELLULAR CARCINOMA

By

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ABSTRACT

Background: Mean platelet volume (MPV) is a parameter routinely provided by the automated blood analyzers. In the last few years, MPV and MPV/platelet count (PLT) ratio were investigated in some medical conditions such as sepsis, myocardial infarction, pulmonary embolism and lung cancer. **Objective**: This work aimed to study the significance of MPV and MPV/PLT ratio in chronic hepatitis C patients with hepatocellular carcinoma (HCC). **Patients and Methods**: Mean platelet volume and MPV/PLT ratio were determined in 60 patients with chronic hepatitis C virus (HCV) infection divided into 3 groups: patients with chronic hepatitis, patients with cirrhosis and patients with HCC as well as 10 healthy subjects were included as control. **Results**: Mean platelet volume significantly increased in HCC group compared to control group, patients with chronic hepatitis and patients with cirrhosis. Mean platelet volume/platelet count ratio significantly increased in HCC group compared to control group and patients with chronic hepatitis only. In receiver operating characteristic (ROC) curve analysis, a cut-off point of 10.5 fl of MPV was found to be 100% sensitive and 55% specific for detection of HCC (the specificity rose to 92.5% when combined to alpha feto protein (AFP)), a cut-off point of 0.05 (fl/(10^3/ul)) of MPV/ PLT ratio was found to be 100% sensitive, and 47.5% specific for detection of HCC (the specificity rose to 92.5% when combined to AFP). **Conclusion**: Mean platelet volume and MPV/PLT ratio are independent or adjunctive, easy and cheap markers that may be used in detection of HCC in patients with HCV infection.

INTRODUCTION

Hepatitis C virus (HCV) related liver disease is a major health issue as it shows a steady progress to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) which represents a major cause of death from cancer and the most frequent primary malignant tumor of the liver ***(Asaoka et al., 2014 and Zeeneldin et al., 2015)***. Mean platelet volume (MPV) is a parameter and one of the platelet function tests routinely provided by the automated blood analyzers. In the last few years, MPV and MPV/platelet count (PLT) ratio were investigated in some medical conditions such as sepsis, myocardial infarction, pulmonary embolism and lung cancer ***(Azab et al., 2011, Inagaki et al., 2014, Ates et al., 2015 and Yardan et al., 2015)***. In this work, we studied the possible relation between MPV and/or MPV/PLT ratio, and the detection of HCC in HCV patients.

SUBJECTS AND METHODS

During the period between September 2015 and June 2016, this study was conducted on 70 subjects at the Out Patient Clinic and Department of Internal Medicine, Benha University Hospital. An informed consent was taken from each participant enrolled in this study. They were classified into 4 groups: The control group (group I) included 10 apparently healthy subjects. The chronic HCV group (group II) included 20 patients with chronic HCV infection documented by positive HCV antibody without evidence of complications. The cirrhotic group (group III) included 20 patients with chronic HCV infection with evidence of cirrhosis upon abdominal ultrasound and /or fibroscan (***Bates, 2004 and Nezam, 2013)***. The HCC group (group IV) included 20 cirrhotic patients with chronic HCV infection with evidence of hepatocellular carcinoma according to the European Association for the Study of the Liver (EASL) depending on typical radiological hallmarks in dynamic contrast-enhanced imaging apart from biomarkers ***(EASL, 2012)***. Patients with other diseases affecting PLT were excluded. Also, obese individuals and patients with other diseases affecting MPV as hyperlipidemia, atrial fibrillation, metabolic syndrome, diabetes, fatty liver disease, rheumatic and chronic inflammatory diseases, acute myocardial infarction, acute ischemic stroke, acute infections and other neoplastic disorders were excluded ***(Kurt et al., 2012 and Varol, 2015)***. For complete blood count (CBC) analysis including hemoglobin (Hb), white blood cell count (WBC), PLT and MPV, samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Measurements were performed within 2 hours on Sysmex XT-1800i, an automated hematology analyzer (Sysmex corporation), and the MPV/PLT ratio **(**fl/(10^3/ul)**)** was calculated. Reference values of our Sysmex equipment was 9 - 13 fl for the MPV and 150-450 10^3/ul for PLT. Alfa feto protein (AFP) was estimated using enzyme linked immunosorbent assay.

Statistical analysis: The comparison between groups with qualitative data was done using ***Chi-square test***. Comparison between more than two independent groups regarding quantitative data was done using ***One Way Analysis of Variance (ANOVA)*** followed by post hoc analysis using least significant difference (LSD) test. ***Spearman correlation coefficients*** were used to assess the correlation between variables and the receiver operating characteristic (ROC) curve analysis was done to evaluate the diagnostic performance. The statistical analysis was performed with Statistical Package for Social Science of International Business Machines (IBM SPSS) version 20. P values <0.05 were considered statistically significant.

RESULTS

Sixty patients with HCV infection, divided into chronic hepatitis, cirrhosis and HCC groups, as well as ten healthy subjects, the control group, were included in this study. Demographic data and CBC findings were presented in table (1).

There were significant differences in MPV an MPV/PLT ratio between all the studied groups.

Table (1): Demographic data and CBC of all the studied groups.

Parameters / Groups		Group I	Group II	Group III	Group IV	P value						
		No.= 10	No.= 20	No.= 20	No.= 20	*P1	*P2	*P3	*P4	*P5	*P6	*P7
Age (years)	Mean ± SD	39.60 ± 18.80	52.50 ± 18.01	56.65 ± 8.40	59.25 ± 5.27	0.002	>0.05	0.002	0.001	>0.05	>0.05	>0.05
	Range	12 – 76	20 – 84	45 – 79	47 – 68							
Sex	Female	4 (40.0%)	9 (45.0%)	6 (30.0%)	5 (25.0%)	>0.05	–	–	–	–	–	–
	Male	6 (60.0%)	11 (55.0%)	14 (70.0%)	15 (75.0%)							
Hb (g/dl)	Mean ± SD	13.56 ± 2.09	13.84 ± 2.12	12.42 ± 1.79	12.31 ± 2.10	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	Range	11.4 – 16.7	9.6 – 16.7	9.5 – 15.7	8.7 – 16.5							
WBC (10^3/l)	Mean ± SD	6.91 ± 2.53	7.06 ± 2.87	5.41 ± 1.89	5.57 ± 2.26	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	Range	2.36 – 10.59	3.6 – 10.75	2.43 – 9.03	2.64 – 10.8							
PLT (10^3/ul)	Mean ± SD	254.00 ± 41.85	203.80 ± 49.64	129.10 ± 77.09	110.95 ± 47.40	0.001	0.011	0.001	0.001	0.001	0.001	>0.05
	Range	197 – 327	113 – 290	49 – 317	51 – 198							
MPV (fl)	Mean ± SD	9.38 ± 0.68	10.00 ± 0.65	11.03 ± 0.88	11.94 ± 0.83	0.001	0.022	0.001	0.001	0.001	0.001	0.002
	Range	8.4 – 10.1	8.2 – 11.5	8.5 – 12.3	10.7 – 13.5							
MPV/PLT Ratio (fl/(10^3/ul))	Mean ± SD	0.04 ± 0.01	0.05 ± 0.02	0.11 ± 0.06	0.13 ± 0.05	0.001	0.010	0.001	0.001	0.001	0.001	>0.05
	Range	0.03 – 0.05	0.03 – 0.09	0.03 – 0.25	0.06 – 0.24							

*P1=P value between all groups *P2=P value between group II and group I *P3=P value between group III and group I *P4=P value between group IV and group I *P5=P value between group II and III *P6=P value between group II and IV *P7 =P value between group III and IV

Also, there were statistically significant differences in MPV and MPV/PLT ratio (Figure 1) between control and each of chronic HCV, cirrhosis, and HCC group.

As regard patients groups, the comparison between the chronic HCV and each of cirrhosis and HCC group showed that there were statistically significant differences in MPV and MPV/PLT ratio, and there was a statistically significant difference in MPV only in comparing between cirrhosis and HCC group (Table 1). As regard MPV/PLT ratio, it was higher in HCC group than cirrhotic group however with no statistically significant difference.

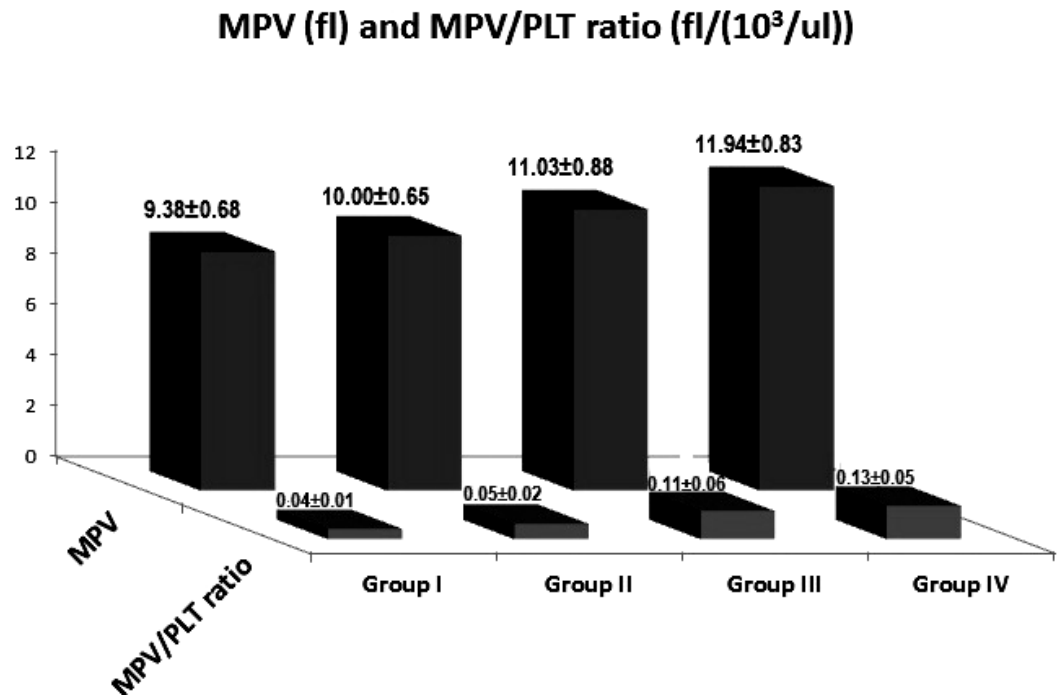

Figure (1): MPV and MPV/PLT ratio results of the study groups.

Table (2): Correlation of MPV and MPV/PLT ratio with duration of illness as known by the patients*.

Groups / Parameters	Duration of illness (months)							
	All patients groups		Group II		Group III		Group IV	
	r	p-value	r	p-value	r	p-value	r	p-value
MPV (fl)	0.236	0.060	0.285	0.224	0.035	0.888	0.309	0.198
MPV/PLT Ratio (fl/(10³/ul))	0.236	0.074	0.116	0.625	-0.251	0.300	0.432	0.065

*Most of patients in the study discovered their illness accidently or the first presentation of their illness was development of complications of cirrhosis.

There was no significant correlation of either MPV or MPV/PLT ratio with duration of illness as known by the patients (Table 2).

As regard analysis of ROC curves (figure 2), a cut-off point of 12.6 IU/mL of serum AFP was found to be 82.35% sensitive and 95% specific for detection of HCC, a cut-off point of 10.5 fl of MPV was found to be 100% sensitive and 55% specific for detection of HCC (the specificity rose to 92.5% when combined to AFP), a cut-off point of 0.05

(fl/(10^3/ul)**)** of MPV/ PLT ratio was found to be 100% sensitive and 47.5% specific for detection of HCC (the specificity rose to 92.5% when combined to AFP) (Table 3).

Table (3): Sensitivity and specificity of AFP, MPV, MPV/PLT ratio for the diagnosis of HCC

Variables	Cut off point	Sensitivity	Specificity
MPV (fl)	>10.5	100.00	55.00
MPV/PLT ratio (fl/(10^3/ul))	>0.05	100.00	47.50
AFP (IU/ml)	>12.6	82.35	95.00
MPV (fl) + AFP (IU/ml)	-	85.00	92.50
MPV/ PLT ratio (fl/(10^3/ul)) + AFP (IU/ml)	-	70.00	92.50

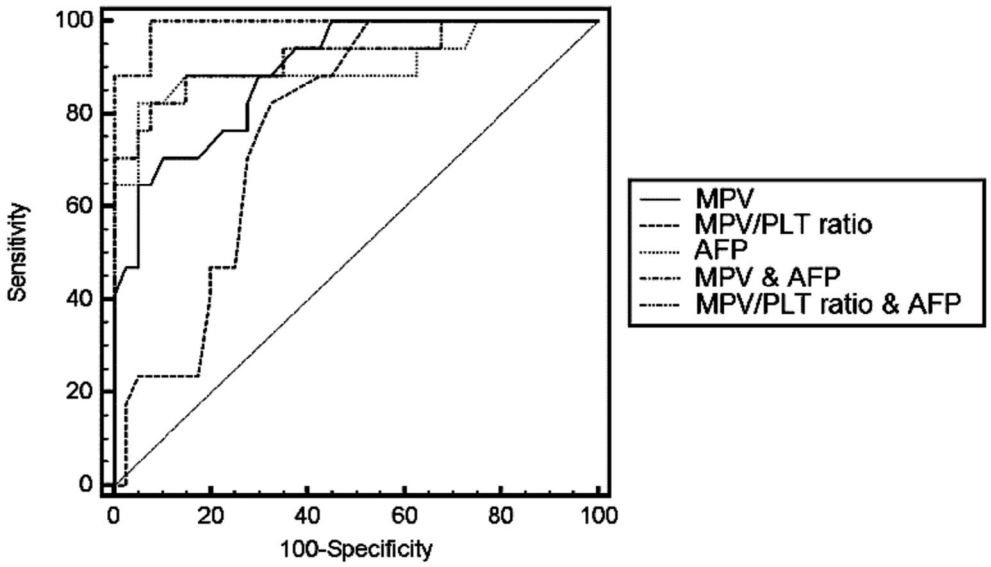

Figure (2): ROC curve analysis for MPV, MPV/PLT ratio and AFP.

DISCUSSION

The results of this study showed that there were significant positive changes in values of MPV and MPV/PLT ratio not only in relation to HCC but also to other stages of the HCV.

In the present study, both MPV and MPV/PLT ratio were significantly higher in patients than control group while the PLT was significantly lower than the control.

Purnak et al. (2013) and Omar et al. (2014) found that a statistically significant increase in MPV values was observed in chronic hepatitis C patients compared to healthy controls.

Also, in this study, both MPV and MPV/PLT ratio were significantly higher in each of the patients group in comparison to control while the platelet count was significantly lower than the control.

As the PLT was significantly lower and MPV was higher, the resultant MPV/PLT ratio was significantly higher.

Also, there was an inverse relationship between MPV and PLT in order to preserve hemostasis by maintaining a constant platelet mass (PLT * MPV), i.e. the lower the PLT, the higher the MPV. As thrombocytopenia is a common complication in these patients, the higher MPV is understandable (***Gasparyan et al., 2011)***.

Another possible cause of high MPV in these patients, in all stages of the disease, is the chronic inflammatory process with increased release of proinflammatory cytokines including the monocyte chemoattractant protein and tumor necrosis factor (TNF) and this process is increased as the disease progress (***Omar et al., 2014)***.

Other cytokines which have influence on thrombopoiesis and the release of platelets into circulation such as interleukine (IL)-1, TNF-alpha, and IL-6 are suggested to play a role ***(Gasparyan et al., 2010)***.

Specifically, as regard HCC, this study demonstrated that both MPV and MPV/PLT ratio were significantly higher in HCC group in comparison to control group.

Kurt et al. (2012) and Cho et al. (2013) found that there was significant difference between control and HCC group as regard MPV. ***Cho et al. (2013)*** found also that the MPV/PLT ratio in patients with HCC significantly higher than control group.

In patients with HCC, thrombopoietin (TPO) may play a role as it is synthesized mainly in hepatocytes and noticed to be elevated in HCC (***Hwang et al., 2004 and Cho et al., 2013)***.

Interleukine-6 levels were found to be higher in patients with HCC in comparison to cirrhotic patients without HCC ***(Porta et al., 2008)***. Interleukine -6 is capable of progressively augmenting platelet diameter as it, along with IL-3, may (directly or indirectly) modify maturation of megakaryocytes ***(Deutsch and Tomer, 2006)***.

CONCLUSIONS AND RECOMMENDATIONS

Mean platelet volume (MPV) and MPV/PLT ratio are easy and cheap markers and as they increased in patients with HCC. These may be used in routine follow up along with the other markers as they give valuable information about severity and stage of liver disease in patients with HCV.

In spite of the MPV and the MPV/PLT ratio have low specificity for detection of HCC in chronic HCV patients, it may be combined with other markers like AFP in order to be more valuable.

In further studies, prospective follow-up of the chronic hepatitis C patients with MPV values (≥10.5 fl) and MPV/PLT ratio **[**≥ 0.05 fl/(10^3/ul)**]**to observe the development of HCC would provide more reliable data. Also, follow up for at least six months is recommended in future studies in order to detect the significant changes in MPV and MPV/PLT ratio during the disease progression.

The future studies should focus on and reveal the exact underlying mechanisms of the MPV and MPV/PLT ratio changes and magnifying its usefulness as an indicator of presence of HCC as well as investigate their significance in patients with other chronic liver diseases.

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متوسط حجم الصفائح الدموية و نسبة متوسط حجمها إلى عددها فى مرضى إلتهاب الكبد المزمن سي الذين يعانون من سرطان خلايا الكبد

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خلفية البحث: يعد متوسط حجم الصفائح الدموية واحدا من مقاييس وظائف الصفائح الدموية التي تظهر بشكل روتيني بتحليل الدم الآلي ، وقد تمت في السنوات القليلة الماضية دراسة متوسط حجم الصفائح الدموية ومتوسط حجمها نسبة إلى عددها في بعض الحالات الطبية مثل تعفن الدم، وإحتشاء عضلة القلب والإنسداد الرئوي وسرطان الرئة.

الهدف من البحث: دراسة أهمية متوسط حجم الصفائح الدموية و نسبة متوسط حجمها إلى عددها فى مرضى إلتهاب الكبد المزمن سي الذين يعانون من سرطان خلايا الكبد. **المرضى وطرق البحث**:تم تحديد متوسط حجم الصفائح الدموية و نسبة متوسط حجمها إلى عددها فى ستين مريضا من المصابين بفيروس إلتهاب الكبد سي وتم تقسيمهم إلى ثلاث مجموعات تشمل إلتهاب الكبد المزمن وتليف الكبد وسرطان خلايا الكبد ، بالإضافة إلى عشرة أصحاء تضمنتهم الدراسة كمجموعة ضابطة.

النتائج: قد كان متوسط حجم الصفائح الدموية زائدا بصورة كبيرة في مجموعة سرطان خلايا الكبد مقارنة بالمجموعة الضابطة وبمجموعة المرضى بإلتهاب الكبد المزمن وبمجموعة المرضى بتليف الكبد. وقد كانت نسبة متوسط حجم الصفائح الدموية إلى عددها زائدة بصورة كبيرة مجموعة سرطان خلايا الكبد مقارنة بالمجموعة الضابطة وبمجموعة المرضى بإلتهاب الكبد المزمن فقط. وفي تحليل منحنى إستقبال الخصائص التشغيلية تم تحديد النقطة الفاصلة 10.5 فيمتولتر لمتوسط حجم الصفائح للكشف عن سرطان خلايا الكبد بحساسية قدرها 100٪ ونوعية قدرها 55٪ (إرتفعت النوعية إلى 92.5٪ عند الجمع بين البروتين ألفا الجنيني و متوسط حجم الصفائح)، وتم تحديد النقطة الفاصلة 0.05 فيمتولتر/(10^3/ميكرولتر) لنسبة متوسط حجم الصفائح / عددها بحساسية قدرها 100٪ و نوعية قدرها 47.5٪ للكشف عن سرطان خلايا الكبد (إرتفعت النوعية إلى 92.5٪ عند الجمع بين البروتين ألفا الجنيني و نسبة متوسط حجم الصفائح/ عددها).

الإستنتاج: متوسط حجم الصفائح الدموية ونسبة متوسط حجمها إلى عددها تعد علامات مستقلة أو مساعدة وسهلة ورخيصة يمكن إستخدامها في الكشف عن سرطان خلايا الكبد فى مرضى إلتهاب الكبد المزمن سي.