Mean platelet volume as a novel surrogate marker for early Non-Alcoholic Fatty Liver Disease

Walid Shehab-Eldin[1], Alaa Efat[1], Somaia Shehabeldeen[2], Abdallah Essa[2], Mohamed Shereef Abdou[3], Hazem Omar[4] and Dena Elazab[5]

[1]Internal Medicine Department, Faculty of Medicine, Menoufia University, Egypt
[2]Tropical Medicine Department, Faculty of Medicine, Menoufia University, Egypt
[3]Clinical Pathology Department, National Liver Institute, Menoufia University, Egypt
[4]Radiology Department, National Liver Institute, Menoufia University, Egypt
[5]Pathology Department, National Liver Institute, Menoufia University, Egypt
waleedshehab2000@gmail.com.

Abstract: Background: Non Alcoholic Fatty Liver Disease (NAFLD) is a chronic symptomless disease with deleterious outcome. The gold standard method of its diagnosis is liver biopsy (LB). LB, however, is invasive and not safe. **Aim of the work:** Prediction of early NAFLD. **Subjects and methods:**38 (24 males, 14 famales) volunteers assigned to liver donation were included in the study. Ultrasonography and LB were done during their routine pre-donation workup. Subjects were divided into 2 groups according to LB; control and NAFLD groups. All subjects underwent physical examination and anthropometric measures. Multiple indicators of NAFLD were assessed including blood glucose, lipid profile, and liver function tests. In addition Mean Platelet Volume (MPV) was assessed during routine CBC. **Results:** In a univariate logistic regression analysis waist circumference, leucocytic count, cholesterol, ALT and MPV were associated with NAFLD. In a multivariate regression analysis, only MPV was significant (p=0.017). ROC curve analysis detected MPV of 10.8 fl as the best cutoff level to predict NAFLD with sensitivity of 87%, specificity of 80% and AUC of 0.864. No significant difference was found between MPV and ultrasonography ROC curves to diagnose NAFLD (p=0.546). **In Conclusion:** MPV is an independent surrogate marker for the detection of early NAFLD.
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1. Introduction:

Non Alcoholic Fatty Liver Disease (NAFLD) is a disease characterized by excessive deposition of fat (steatosis) within the hepatocytes [1]. This disease comprises a wide range of pathological changes in the liver according to the amount of the deposited fat. It ranges from steatosis to Non Alcoholic Steatohepatitis (NASH) with inflammation of the liver [2]. NAFLD is the most common liver disease affecting 20-30% of the general population [3]. Although it is a symptomless disease, it shortens the life span of the patients. The most common cause of death in patients with NAFLD is cardiovascular death accounting for 48% of mortality whileliver related deaths occur only in 7% of the patients [4].

The definition of NAFLD is a pathological definition hence Liver Biopsy (LB) is the gold standard method for its diagnosis[5]. So, the diagnosis of NAFLD without liver biopsy is challenging and controversial. Meanwhile, the invasiveness and possible complications of LB limits its wide use on asymptomatic patients. That is why a lot of non-invasive biomarkers were tested for indirect diagnosis of NAFLD [6, 7].

The increased cardiovascular death in patients with NAFLD is attributed to the same risk factors predisposing to both diseases. Traditional risk factors are age, hypertension, diabetes, physical inactivity, smoking, hyperlipidemia, metabolic syndrome and diet. Non-traditional markers for inflammation (e.g., hsCRP, lipoprotein (a), homocysteine), markers of fibrinolytic and hemostatic function (e.g., fibrinogen, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1) antigens) are also elevated in both diseases [8].

For a long time, platelet count has been linked with liver diseases. This link is evidenced by theuseof platelet in several scoringsystems, such as the age-platelet index (AP index) [9], aspartate aminotransferase (AST)-to-platelet ratio index (APRI) [10], NAFLD fibrosis score [11], and the FIB4 index [12], to predict various liver diseases.
Mean Platelet Volume (MPV) is an indicator of platelet function and activation. It is a simple test that can be done during routine Complete Blood Count (CBC)[13]. High MPV is a predictor of coronary artery disease and risk stratification [14].

MPV may be a new surrogate marker of NAFLD. Moreover it may be a link explaining in part

the increased cardiovascular death in patients with NAFD.

The aim of the present work is to study different risk factors in patients with early stages of NAFLD.

2. Subjects and methods:

This study was conducted on future liver donors at our liver transplantation center. Local ethical committee approval was obtained. All subjects assigned to liver donation workup during the period from 1/1/2014 to 28/6/2014and approved to take part in this study were included after signing an informed consent. Patients with hypertension, heart, kidney, liver or chronic inflammatory diseases were excluded from this study. Patients who drink alcohol were also excluded. Volunteers who are taking any drugs affecting platelet function (Aspirin or warfarin) were also excluded. Patients with NASH with elevated liver enzymes were excluded from the study as well.

All subjects were assigned to a 75 gm Oral Glucose Tolerance Test (OGTT) and anthropometric measures after 8 hours of overnight fasting. Five patients were excluded after the diagnosis of new onset Diabetes Mellitus by OGTT. On another occasion patients were asked to come after overnight fasting for 12 hours. Blood was withdrawn and divided into two tubes. One blood sample were collected for the assay of prothrombin time and CBC directly within 2 hours from sample withdrawal using auto hematology analyzer (Mindray®BC-3000, Plus). EDTA was not added to this tube to avoid platelet swelling [15] or affecting prothrombin time. Another sample were collected from each volunteer and stored in EDTA containing tubes for estimation of blood glucose, liver function, and kidney function and lipid profile. Only 38 (24 males and 15 females) future donors were included in the study.

Percutaneous ultrasound guided liver biopsy:

Under complete aseptic technique Percutaneous US guided liver biopsy was done by an interventional ultrasonography expert. Uncorrected coagulopathy is the only absolute contraindication for liver biopsy. The presence of as cites, liver anatomy evaluation and adjacent organs assessment was checked before the procedure. The patient was then asked to lie on the back with the right elbow to the side and the right hand under the head. Local infiltrative anesthesia was administered. After choosing the location using ultrasound, an incision is made using an 11-blade scalpel. Then, an automated spring-loaded core-biopsy needle (18 gauge) passed across the hepatic capsule in the direction shown by ultrasound and fired. The sample is then fixed with formalin and embedded in paraffin for histological evaluation. Post-biopsy ultrasound examination was done for documentation and confirmation of the absence or presence of capsular or sub-capsular hematoma. In addition to biopsy, ultrasonography reported patients with hyper echogenic bright liver.

The histologic examination:

The histologic findings of the liver biopsies were evaluated by an experienced liver pathologist, blinded to the radiological and laboratory findings. Serial sections 4um thick from the formalin-fixed, paraffin embedded liver tissue were cut and placed on three different slides. They were stained with hematoxylin and eosin to evaluate pathological changes. Masson trichrome was used to assess fibrosis and Pearl stain was used to detect iron deposits. The degree of steatosis, hepatitic changes and portal fibrosis were made. The macrovesicular steatosis were graded as minimal (1% -10%),mild > 10% up to 30%, moderate > 30% up to 60% and marked > 60%[16]. Steatohepatitis was determined by the presence of ballooning degeneration of hepatocytes with necro-inflammation (lobular inflammation formed if mixed lymphocytes, macrophages and neutrophil).

For the purpose of this study future donors were assigned to either a control group with steatosis less than 10% (11 males and 4 females) or the NAFLD group with hepatic steatosis more than 10%. Patients with NASH were excluded from the study.

Statistical analysis:

Data were calculated using SPSS version 21 and presented as mean $\pm$ SD. Group differences were analyzed by Student t test, Mann-Whitney test and X^2 for non-normally distributed, normally distributed and non-continuous variables respectively. ROC curve was plotted and calculated using medcalc software.

3. Results:

According to the liver biopsy, all volunteers were assigned to either the control **or** the NAFLD groups. The control group included 15 (11 males and 4 females) subjects while the NAFLD group included 23(13 males and 10 females) subjects.

Comparison between the Control and the NAFLD groups: (Table 1)

As regarding the demographic and anthropometric measures of both groups, The NAFLD group showed a significantly higher waist circumference. No significant difference between both groups as regarding age, sex or BMI. As regarding the CBC, both MPV and Total Leucocytic Count (TLC) were significantly higher in the NAFLD group than the control group. On the other hand, no significant statistical difference were found between both groups as regarding the haemoglobin or platelet count.

As regarding the lipid profile and blood glucose levels, the NAFLD group showed a significantly higher total cholesterol (p=0.023). No significant difference between both groups as regarding LDL cholesterol, HDL cholesterol, Triglycerides, fasting blood glucose or postprandial blood glucose.

As regarding liver functions, a highly significant ALT level were found in the NAFLD than the control group (*P*=0.000). Similarly, AST was higher in the NAFLD group. No statistical significant difference between both groups as regarding serum albumin, prothrombin and either total or direct bilirubin. Similarly, renal function as represented by urea and creatinine was statistically equal in both groups.

Ultrasonography showed bright echo pattern in the NAFLD group compared to the control group with high statistical difference (*P*=0.000).

Univariate logistic regression analysis between both groups: (Table 2)

In addition to age and sex, all parameters that showed statistical difference between the NAFLD and Control groups were tested separately in a univariate logistic regression analysis to show its association with NAFLD. Waist circumference, TLC, total cholesterol, ALT and MPV showed a significant association. On the other hand, age, sex and AST didn't show a significant association.

Multivariate logistic regression analysis between both groups: (Table 3)

All markers that still significant in the univariate logistic regression analysis were included in the multivariate logistic regression analysis. Only MPV remain significant with an odd ratio of 3.856 at 95% confidence interval of 1.271 to 11.698 (*P* value = 0.017).

Receiver Operating Characteristic (ROC) curve between MPV and NAFLD: (Fig. 1)

To detect the threshold of MPV that diagnose NAFLD, ROC curve was plotted to detect the sensitivity and specificity of each value of MPV to detect NAFLD. MPV of 10.8 fl was the best cutoff value ($p < 0.0001$) with a sensitivity of 87% and specefecity of 80% and Area Under the Curve (AUC) of 0.864.

Comparison between MPV and ultrasonography ROC curves: (Fig. 2)

Comparison between MPV as a surrogate marker of NAFLD with ultrasonography as a common non-invasive radiological method to diagnose NAFLD showed no superiority of one method over the other ($p = 0.546$).

Table 1: Descriptive and biochemical data of the control and NAFLD groups

Parameter	Normal	NAFLD	*p* value
Subjects (No.)	11	23	
Age (Years)	30.66±8.24	31.22±4.25	0.778
Gender (M/F)	11/4	13/10	0.289
BMI (Kg/M^2)	27.27±3.81	29.22±2.65	0.071
Waist Circumference (Cm)	94.27±8.04	100.83±8.07	0.019*
Hemoglobin (gm/dl)	13.13±1.45	13.43±1.34	0.517
TLC /µl	6.56±1.66	7.99±1.69	0.015*
Platelet Count /µl	263.47± 63.64	290.30±79.60	0.280
MPV(fl)	9.90±1.11	11.37± 0.94	0.000**
FBS(mg/dl)	96.73 ±16.36	101.30±19.51	0.458
PPBS(mg/dl)	129.27±24.24	140.13±26.27	0.207
Total Cholesterol(mg/dl)	140.47±36.09	177.87±53.27	0.023*
LDL Cholesterol(mg/dl)	98.73±17.14	112.00±28.39	0.113
HDL Cholesterol(mg/dl)	42.53±17.95	45.91±24.79	0.652
Triglycerides(mg/dl)	134.27±60.12	138.52±68.19	0.845
ALT(U/L)	17.60±8.87	27.96±8.84	0.001*
AST(U/L)	19.33±5.33	22.39±3.69	0.043*
Albumin(g/dl)	4.55± 0.34	4.58± 0.25	0.710
Prothrombin Con. (%)	95.07±4.74	93.09±5.00	0.232
Total Bilirubin(mg/dl)	0.63± 0.24	0.56± 0.21	0.328
Direct Bilirubin(mg/dl)	0.14 ± 0.05	0.14± 0.05	0.963
Urea(mg/dl)	24.20±4.72	21.13±5.49	0.084
Creatinine(mg/dl)	0.83± 0.13	0.96± 0.23	0.067
Ultrasonography	11/0	1/22	0.0001*

TLC ….Total Leucocytic Count; MPV……Mean Platelet volume; FBS…. Fasting Blood Suger;
PPBS…. Postprandial Blood Suger; ALT….. Alanine Aminotransferase; AST…. aspartate aminotransferase

Table 2: Univariate logistic regression analysis of significant parameters with liver biopsy

Parameter	B	P value	OR	95% CI
Age (years)	0.015	0.781	1.016	0.911 – 1.133
Sex	0.749	0.298	2.115	0.516 – 8.668
Waist Circumference (Cm)	0.103*	0.031	1.109	1.010 – 1.218
TLC /µl	0.503*	0.023	1.654	1.072 – 2.553
AST(U/L)	0.166	0.055	1.181	0.996 – 1.399
ALT(U/L)	0.122*	0.004	1.130	1.039 – 1.229
T. Cholesterol (mg/dl)	0.020*	0.038	1.020	1.001 – 1.039
MPV (fl)	1.269*	0.002	3.556	1.577 – 8.018

TLC ….Total Leucocytic Count; MPV……Mean Platelet volume; ALT….. Alanine Aminotransferase;
AST…. aspartate aminotransferase

Table 3: Multivariate logistic regression analysis of significant parameters with liver biopsy

Parameter	B	P value	OR	95% CI
Waist Circumference (Cm)	0.091	0.082	1.096	0.988 – 1.215
TLC /µl	-0.078	0.336	0.925	0.788 – 1.085
ALT(U/L)	0.530	0.154	1.699	0.821 – 3.517
T. Cholesterol (mg/dl)	0.012	0.294	1.012	0.990 – 1.035
MPV (fl)	1.350*	0.017	3.856	1.271 – 11.698
Constant	-14.355		0.00	

TLC …… Total Leucocytic Count; ALT……Alanine Aminotransferase; MPV……Mean Platelet volume

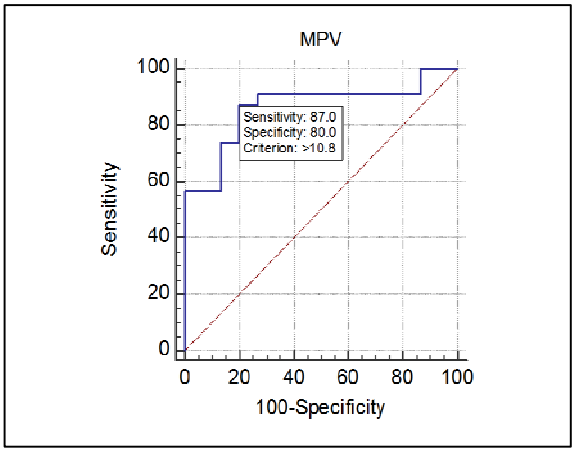

Figure 1: Receiver Operating Characteristic (ROC) curve between MPV and NAFLD
MPV ……. Mean Platelet volume;
NAFLD…. Non-Alcoholic Fatty Liver Disease.

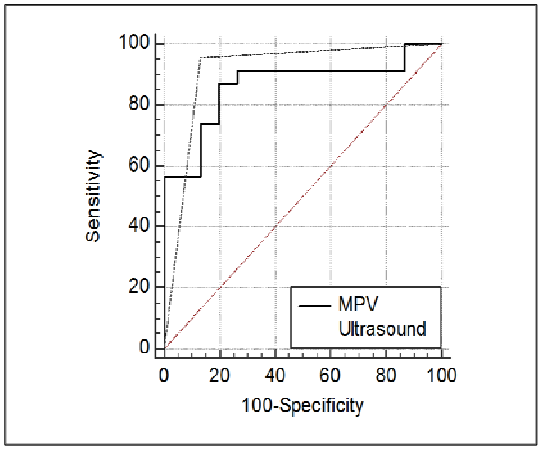

Figure 2: Comparison between MPV and ultrasonography ROC curves
MPV ……. Mean Platelet volume.

4. Discussion:

NAFLD is a strong significant predictor of coronary artery disease [17]. The gold standard method to diagnose NAFLD is LB which is an invasive procedure. Non-invasive efforts should be made to predict and early diagnose NAFLD, hence the disease can be managed early reducing the cardiovascular mortality.

Predictors of any disease are related to either the risk factors leading to this disease or the changes resulting from this disease. In the present work, we investigated the risk factors leading to NAFLD such as age, gender, blood glucose and lipid profile in addition to changes that may result from NAFLD such as liver function, ultrasonography and the new surrogate marker; MPV. Although many factors are associated with NAFLD in the univariate regression analysis (Table 2), only MPV is higherin the NAFLD patients compared with the control group in the present study (Table 3). This means that MPV is an independent predictor of early NAFLD.

These results agree with Ozhan *et al.*, who showed that NAFLD is an independent predictor of MPV [18]. Another study on Korean population demonstrated the significant association between NAFLD and high MPV on 628 obese volunteers [19]. However, our study is more valuable than these studies as we depended on LB, the gold standard

method for the diagnosis of NAFLD, while these studies dependedon ultrasonography which is an indirect and inaccurate method for the diagnosis of NAFLD.

Another study reached to a similar result and concluded that MPV is an independent predictor of NAFLD in biopsy proven NAFLD subjects[20]. However, this study included patients with NASH and elevated liver enzymes while in our study we included only early stages of NAFLD with normal aminotransferases. Patients with advanced NAFLD are easy to be diagnosed by ultrasonography and elevated liver enzymes and no need for prediction in such population. Moreover, the early diagnosis allow for earlier management and better outcome before inflammation of the liver ensues.

On the other hand, kilciler et al., failed to show this association between MPV and NAFLD [21]. Their population is different as they included patients with persistently elevated liver enzymes while our patients have normal liver enzymes. Recently, Kocabay et al., didn't find any association between MPV and NAFLD [22]. However, they didn't depend on LB in patients with early NAFLD and only advanced NASH patients underwent the biopsy. They explained that by the unaccepted concept of performing LB in patients with mildly elevated liver enzymes. In our study, we made benefit from the routine liver biopsy to apparently normal persons assigned to liver donation.

The cause of increased MPV in patients with NAFLD is not clear. Actas et al., explained it on the base of low grade inflammatory state induced by hepatic steatosis leading to platelet activation [23]. Activated platelets have a bigger size with increased MPV[15]. This platelet activation may be the reason behind the increased thrombosis in patients with higher MPV [24, 25].

To the best of our knowledge, the present study is the first to detect a cutoff value of MPV to predict NAFLD patients. The best cutoff value is 10.8 fl with area under the curve of 0.864 (Fig. 1). Comparison between MPV as a surrogate marker for diagnosis of early NAFLD and ultrasonography by ROC analysis showed no superiority to either test over the other (Fig. 2). As long as MPV is elevated in many other conditions, its addition to ultrasonography increase the specificity of either test to diagnose early NAFLD.

Although MPV is a cheap and simple test, it is usually neglected by clinicians. Its importance in patients with NAFLD is not only in the prediction of the disease but also in the prediction of cardiovascular mortality in patients with already diagnosed NAFLD[26]. Our data explain in part the increased cardiovascular mortality in patients with NAFLD[27].

Corresponding Authour:
Dr. Walid Abd-Elmohsen Shehab-Eldin.
M.B.B.Ch., M.Sc., M.D. Internal Medicine. Lecturer of Internal Medicine, Faculty of Medicine, Menoufya University, Egypt. Assistant Professor of Emergency Medicine, Alghad International Colleges for Applied Medical Sciences, KSA.
E mail: waleedshehab2000@gmail.com.

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