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CD235a (Glycophorin-A) Is the Most Predictive Value Among Different Circulating Cellular Microparticles in Thrombocytopenic Human Immunodeficiency Virus Type 1

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Background: This study was conducted to assess different cellular microparticles (MPs) in thrombocytopenic human immunodeficiency virus type 1 and their significance as disease activity markers. *Methods:* Thirty-five thrombocytopenic human immunodeficiency diseases and 25 healthy controls with matched age and sex were selected. Viral load was quantitated by COBAS real-time polymerase reaction (PCR) assessment of absolute T-cell subsets CD4, CD8 as a disease progress marker. Platelet MPs, platelet-derived monocyte MPs (CD42a, CD61), erythrocyte MP (CD235a), monocytic MP (CD14), and platelet activity MPs (CD62P, PAC-1) were assessed by multicolor flow cytometry FACSCalibur, while platelet functions were assessed by platelet function analyzer (PFA-100). CD42a, CD61, and platelet activity index represented by PAC-1 and CD62. *Results:* P-selectin in HIV-infected patient samples were significantly greater ($P < 0.001$) than among controls. There was a negative correlation between the proportion of PAC-1 and CD62 P-selectin-positive MPs and levels of $CD4^+$ T-cell counts ($r = -0.403$, $P = 0.016$; $r = -0.438$, $P = 0.008$), respectively. There was a negative correlation between collagen-ADP and levels of $CD4^+$ T-cell counts ($r = -0.368$, $P = 0.03$). There was a significant high expression level of CD14 monocyte MPs in patients than controls ($P < 0.0001$), overexpression of CD235a ($P < 0.0001$), and no correlation between CD14 and CD4, whereas there was a significant negative correlation with CD235a ($r = -0.394$, $P = 0.019$). A linear regression analysis of CD4 as a disease progression marker with other variable indicators in HIV patients showed that CD235a could be the most sensitive predictor similar to CD4. *Conclusion:* Different cellular MPs and platelets activated in HIV patients could have a role in thrombotic events in these patients. J. Clin. Lab. Anal. 30:235–243, 2016. © 2015 Wiley Periodicals, Inc.

Key words: cellular microparticles; glycophorin-A; CD235a; thrombocytopenia; HIV

INTRODUCTION

Thrombocytopenia is an important and serious complication, and an independent strong predictor of mortality in patients infected with human immunodeficiency virus type 1 (HIV-1) (1). It appears that blood platelets, in addition to their major role in hemostasis and wound healing, may also participate in inflammatory and immunological processes (2). Thus, platelets have been shown to modulate the activity of neutrophils and

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monocytes (3). In vitro, platelets or platelet-derived products have been shown to modulate production of reactive oxygen species, phagocytosis, adhesion to endothelial cells (ECs), and degranulation of granulocytes (4, 5), as well as the production of chemokines and activation of the transcriptional factor NFκB in monocytes (6).

Currently, interest for cell-derived microparticles (MPs) has emerged, pointing out their essential role in hemostatic response and their potential as disease markers, but also their implication in wide range of physiological and pathological processes (7). These are derived from different cell types including platelets—the main source of MPs—but also from red blood cells, leukocytes, and ECs, and circulate in blood. Despite difficulties encountered in analyzing these MPs and disparities in results obtained by a wide range of methods, MP- generation processes are now better understood (8).

A population of platelet-derived MPs (PMPs) is generated during platelet activation, whereas other PMP populations are derived from megakaryocytes during megakaryopoiesis (9) or quiescent circulating platelets, or might result from platelet apoptosis (10). MPs from other cells may be released during cell activation, cell injury, or following cell activation independent processes, including senescence and apoptosis. Carrying markers from their parental cells, MPs are used as investigation and diagnostic tools (11, 12).

MPs are submicron fragments shed from the plasma membrane of stimulated or apoptotic cells (13). An MP could act as transcellular effectors. It is elevated in clinical situations where the thrombotic risk is increased (14). Procoagulant potency of MP is demonstrated in patients suffering from pathologic or drug-induced thrombocytopenia (15). Under most pathophysiologic conditions, PMP appears the main procoagulant circulating species, leukocytic or endothelial origins being less represented. Actual data are suggestive of a beneficial pharmacological control of circulating MP levels in thrombotic disorders (16), and more surprising in the management of hemophilia (17).

P-selectin, one of the platelet activity markers involved in leukocyte rolling, is upregulated during venous thrombosis and promotes vein wall inflammation. The prominent role of platelet-borne P-selectin in MP recruitment through interactions between P-selectin glycoprotein ligand 1 (PSGL-1) and tissue factor (TF) bearing MP was demonstrated in a laser-induced arteriole endothelial injury model, showing their colocalization on the leading edge of the thrombus (18). PMP and/or leukocyte-derived MP would thus concentrate TF activity above a threshold to allow blood coagulation to be triggered. Therefore, interactions between P-selectin and PSGL-1 may have a dual function, first the generation of procoagulant MP, second the recruitment of MP to the growing thrombus (19, 20). Many studies have focused on platelet MPs of HIV patients, however no evidence of red cells or monocyte MPs was found. Our research focused on different patterns of cellular MPs, its significant relation to thrombocytopenia, and disease activity markers.

Aim

This study assesses the prognostic significance of different cellular MPs in HIV-1 patients with thrombocytopenia and its relation to platelet hyperactivity and CD4 T-helper cell as HIV-1 disease progress.

METHODS

Peripheral blood was drawn after taking informed consent from HIV-infected subjects ($n = 35$) and healthy controls ($n = 25$). Study was conducted in Tropical Unit at Mansoura University Hospital. Subjects were phlebotomized after fasting overnight and took complete rest prior to early morning to avoid circadian variation in platelet response. Blood was drawn using a 19-gauge needle. The first 2 ml blood was discarded, and the remaining blood (10 ml) was collected in 4.5 ml tubes containing 3.2% sodium citrate for platelet function assay; EDTA tube was used for automated cell counting and real-time PCR, and another citrated tube was used for flow cytometry.

Routine Cell Counting for Platelets and Absolute Neutrophils by Automated CBC Hematological Analyzer Using Sysmex-XT-2000 I (Sysmex Corporation, Kobe, Japan)

Platelet function assay by PFA-100 (Siemens Healthcare Diagnostic, Germany)

Platelet aggregation in response to ADP and epinephrine was recorded using a platelet function analyzer (PFA-100) with both Collagen-ADP (CADP) and Collagen-epinephrine (CEPI) cartridges. After checking internal controls, 800 μl of fresh citrated whole blood sample was loaded into each specific test cartridge, the membrane was coated with collagen that is a subendothelial protein generally believed to be the initial matrix for platelet attachments—platelets adhered to the collagen-coated membrane. Platelets got activated and released their granule contents on contacting agonist such as ADP or epinephrine. Platelet thrombus at the aperture was formed due to the platelet aggregations, thereby arresting the blood flow. The results were reported as closure time by PFA systems, which is an indicator of platelet functions when compared to reference range of each value. The PFA-100 assay was performed in duplicate with citrated whole blood according to the manufacturer's instructions.

Quantification of HIV-1 RNA by Real-Time PCR for Viral Load

COBAS Ampli Prep COBAS TaqMan, version 2.0 (Roche Diagnostics, Indianapolis, IN, USA), permits automated specimen preparation followed by automated reverse transcription, PCR amplification, and detection of HIV-1 target RNA and quantitation standard (QS) armored RNA for specific quantitation of group M subtypes of HIV-1. Generic silica-based capture technique is commonly used. Eight hundred fifty microliters of plasma was lysed by incubation, at elevated temperature, with a protease and chaotropic lysis/binding buffer that releases nucleic acids; unbound substances and other cellular impurities was removed by washing. Then reverse transcription and PCR amplification reaction was performed with Thermus specie DNA Polymerase (Z05), which had both reverse transcriptase and DNA polymerase activity. Selective amplification was performed using AmpErase (uracil-N-glycosylase) enzyme that catalyzed the destruction of DNA strands containing deoxyuridine33, but not DNA-containing deoxythymidine. Specific oligonucleotide probes were hybridized with a reporter dye and quencher dye. When these probes were intact, the fluorescence of the reporter dye was suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. Once the reporter and quencher dyes were released and separated, the fluorescent activity of the reporter dye was increased. The amplification of HIV-1 RNA and HIV-1 QS RNA was measured independently at different wavelengths. This process was repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes. The PCR cycle, where a growth curve started exponential growth, was related to the amount of starting material at the beginning of the PCR.

Methods for the Preparation of Cell Suspensions

Lysed whole blood technique was used by FACSCalibur flow cytometry (Becton Dickinson, San Jose, CA). Blood samples were stained with the following FITC-labeled, phycoerythrin (PE) labeled, or PerCp-labeled panels of monoclonal antibodies (mAbs): CD45, CD3, CD4, and CD8. Negative isotype matched controls were used for determining the nonspecific binding. Ten microliters of mAb were added to 100 μl of blood. This mixture was incubated in the dark for 20 min at room temperature, then washed with 2 ml FACS lyse (1×), and again incubated in the dark for 10 min at room temperature. The mixture was then centrifuged at 300 × *g* for 5 min, discarded supernatant was washed thrice with Phosphate Buffered Saline (PBS). Centrifuged and discarded supernatants were then washed and resuspended in 300 μl PBS, and FACS was acquired. At least 10,000 cells per sample were acquired; and an appropriate lymphocytes cell gate based on both forward and side scatter, or CD45 expression and side scatter, was selected and analyzed by CellQuest software (Becton Dickinson). Mononuclear cells (10),000) were collected on FACScan flow cytometer (Becton Dickinson) equipped with a 15 mW air-cooled 488 nm argon-ion laser. The FACScan was calibrated with Calibrite beads (plastic microspheres) and AutoCOMP software.

Preparation of Platelet-Free Plasma for Detection of MPs

- Collect 10 ml whole blood in a BD Vacutainer blood collection tube containing acid citrate dextrose solution.
- In a 15 ml polypropylene tube, centrifuge the sample at 2,600 × *g* for 15 min at 10°C.
- Transfer approximately 4.5 ml of platelet-poor plasma into a microcentrifuge tube.
- Centrifuge this a microcentrifuge tube at 9,900 × *g* for 5 min at 10°C .
- Transfer the supernatant (platelet-free plasma, PFP) into a tube, which is ready for MPs detection.

Labeling of MPs

For the detection of MPs, the following panel of mAb was used: PE-labeled anti-CD14 (MFP9, IgG2b) versus anti-CD42b-PE (HIP1, IgG1, kj), and anti-CD41-FITC (5B12, IgG1, Kj) versus antiglycophorin A (CD235)-PE (JC159) that was purchased from Dako (Glostrup, Denmark). Anti-P-selectin (CD62P) FITC versus PE PAC-1 are recognized as epitopes on activated GPIIb-IIIa (BD Bioscience, San Jose, CA].

The sample acquisition includes the following steps:

- After gently mixing the resuspended MPs, transfer 50 μL of MP into three tubes.
- To each tube containing 50 μL of the MP suspension, add 5 μL of the two different antibodies, each conjugated to a different fluorescent tag (FITC- and PE-conjugated antibodies).
- Simultaneously in a separate tube, prepare samples labeled with relevant isotype controls.
- In the dark, incubate all tubes for 20 min at room temperature.
- After this incubation, centrifuge samples at 19,800 × *g* for 10 min at 10°C . Remove the supernatant, leaving 100 μL in the tube. This step is repeated twice.
- Add 500 μL of PBS and resuspend the pellet. Thus, the samples are ready for acquisition.

Flow Cytometry of MPs

Three-color flow cytometry was performed on a FACSCalibur flow cytometer equipped with CellQuestPro software (Becton Dickinson). MPs should be analyzed in a protocol with both forward scatter (FSC) and side scatter (SSC) sets in the logarithmic mode. Double fluorescence plots from flow cytometric analysis demonstrated the presence of MPs of different cellular origin in each sample.

Type of Flow Cytometer (Manufacturer, Model) Analytical Software

BD FACSCalibur flow cytometer was purchased from Becton Dickinson, and SN Eg7501146 from BD Bioscience. CellQuestPro software was used for acquisition and analysis of samples stained with CD4, CD8, and CD3 and the rest of other MP markers.

Laser Lines and Optical Emission Filters Used for Each Fluorochrome

Optical filters for cytofluorimetric analysis with 488 nm excitation of fluorescein-stained cells (maximum emission, 520 nm) and green beads (maximum emission, 538 nm) were used for each fluorochrome.

Technique of Multicolor Compensation

Labeled calibrite beads and Comp software were used for calibration. After calibration and checking the laser beam, the samples were prepared as described below.

Number of Events

For data processing, 10,000 positive events were analyzed each time using the CellQuest program (Becton Dickinson).This program includes the method used for defining gates, and the gating scheme was used for scatter dot-blot histogram.

Platelets, from platelet-enriched citrated plasma, were identified on FSC versus SSC plots in plasma of small size and low complexity. The platelet MPs were selected by expression of the surface marker glycoprotein 1b (CD42b) using a PE-conjugated mAb (Dako).

STATISTICAL METHODS

The statistical analysis of data was conducted using SPSS (SPSS, Inc., Chicago, IL) program statistical package for social science version 17. The normality of data distribution was tested by Kolmogorov–Smirnov test, as most of the data were nonnormally distributed. The data were described in the form of median and range for quantitative data, and in frequency and proportion for qualitative data. The data were analyzed to test statistical significant difference between groups.

Mann–Whitney test was used for comparing qualitative data between two groups. Spearman correlation test was used for testing the association between CD4 and other parameters in HIV-infected patients. Significant results from univariate analysis were entered in multivariate linear regression analysis to predict the significant predictors for CD4. P value was significant if ≤ 0.05 at confidence interval 95%.

RESULTS

The median ages for the patient and control populations were 34 years (range 21–72) and 36 years (range 18–58), respectively ($P = 0.38$). Male percentage was 68.5% and 64% for patients and control groups, respectively. Forty-one percent of patients had plasma RNA levels >400 copies/ml. The median plasma HIV RNA level for the entire patient population was 8,000 copies/ml (range = 0.00–164,000 copies/ml) and the median $CD4^+$ T-cell count was 324.0 cells/μl (range = 82.00–1,121 cells/μl). We studied surface expression of CD42a, CD61, CD62 P-selectin, and PAC-1 by flow cytometry in 35 HIV-infected donors and 25 healthy controls (Table 1).

Platelet Count in HIV-Infected Individuals and Its Relation to Platelet Function Assay

Platelet count in HIV-infected samples was significantly lower ($P < 0.0001$) than among controls. Platelet function assays as collagen-ADP and collagen-epinephrine were significantly greater in HIV-infected patients ($P < 0.0001$) than among controls (Table 1).

Platelet Surface Marker and Its Activity Index by Multicolor Flow Cytometry

The proportions of platelets expressing CD42a and CD61 in HIV-infected patient samples ($P < 0.001$) as well as the proportions of platelet activity index expressing PAC-1 and CD62 P-selectin in HIV-infected patient samples were significantly higher ($P = 0.001$) than among controls (Table 1).

Evidence of Red Cells and Monocyte MPs in HIV Patients

There was a significant increase in CD14 monocyte MPs ($P < 0.0001$) and red cell marker (CD235a;

TABLE 1. Demographic, Hematological, Immunological Data for Patients and Control

Variable	Cases ($n = 35$) Median [minimum–maximum]	Control ($n = 25$) Median [minimum–maximum]	Z	P
Age (years)	34 [21–72]	36 [18–58]	−0.87	0.38
CD3 (cells/μl)	1,445 [204–2,903]	1,982 [876–2,482]	−1.75	0.08
CD4 (cells/μl)	324 [82–1,121]	960 [488–1,820]	−5.87	0.001***
CD8 (cells/μl)	816 [109–2,496]	606 [316–920]	−2.27	0.02*
CD14 (cells/μl)	2.14 [1.34–3.43]	0.88 [0.46–1.08]	−6.56	0.001***
CD42a (cells/μl)	28.2 [18.2–84.1]	9.7 [7.68–12.68]	−6.56	0.001***
CD61 (cells/μl)	33.4 [14.2–77.8]	10.68 [6.4–14.24]	−6.55	0.001***
CD62P (cells/μl)	1.24 [0.65–2.95]	0.74 [0.18–1.12]	−5.07	0.001***
CD235a (cells/μl)	82.4 [59.3–97.3]	26.4 [16.24–38.62]	−6.56	0.001***
Collagen-ADP (s)	152 [104–88]	98 [74–118]	−6.22	0.001***
Collagen-epi (s)	69 [48–96]	134 [104–160]	−6.56	0.001***
ANC (cells/μl)	1.8 [0.8–4.4]	4.2 [2.2–6.8]	−6.03	0.001***
PAC-1 (cells/μl)	1.08 [0.74–1.98]	0.98 [0.24–1.42]	−2.21	0.001***
Platelets ($\times 10^6$/μl)	83 [27–154]	363 [184–520]	−6.56	0.001***
Viral load (copies/ml)	8 [0–164]	0 [0–0]	−6.17	0.001***
WBC ($x10^3$/μl)	4.02 [1.64–6.52]	7.03 [4.23–10.2]	−5.89	0.001***

Results were presented as median, range. P significant if ≤0.05. CD, cluster differentiation; WBC, white blood cell count; ANC, absolute neutrophils count; collagen-ADP, collagen-adenosine diphosphate; collagen-epi, collagen-epinephrine. ***highly significant.

TABLE 2. Spearman Correlation Test for CD4 T-Helper Prognostic Marker With Other Variable Parameters in HIV Patients

	r	P
Age	−0.098	0.575
CD8	0.249	0.148
CD14	−0.066	0.704
CD42a	−0.214	0.218
CD61	−0.241	0.162
CD62P	−0.438	0.008
CD235a	−0.394	0.019
Collagen-ADP	−0.368	0.03
Collagen-epinephrine	0.371	0.028
ANC	0.360	0.034
PAC-1	−0.403	0.016
Platelets	0.369	0.029
Viral Load	−0.664	0.001***
WBC	0.325	0.057

P is significant if ≤0.05 (***highly significant).

$P < 0.0001$) in HIV-infected patients than among controls (Table 1).

Correlation Between Platelet Count and Platelet Function of CD4 T-Helper Cell As a Disease Progression Marker in HIV-Infected Individuals

There was a positive correlation between platelet count and levels of $CD4^+$ T-cell counts ($r = 0.369$, $P = 0.029$). Regarding platelet function, there was a negative correlation between collagen-ADP and levels of $CD4^+$ T-cell counts ($r = -0.368$, $P = 0.03$), while there was a positive correlation between collagen-epinephrine and levels of $CD4^+$ T-cell counts ($r = 0.371$, $P = 0.028$; Table 2).

Platelet Activation MPs Were Differentially Related With CD4 T-Cell Counts

There was a negative correlation between the platelet activation index represented by proportion of PAC-1 and CD62 P-selectin-positive MPs and levels of $CD4^+$ T-cell counts ($r = -0.403$, $P = 0.016$; $r = -0.438$, $P = 0.008$). On the other hand, there was no relationship between other PMPs—CD42a, CD61—and levels of $CD4^+$ T-cell counts (Table 2).

CD235 Showed a Significant Negative Correlation With CD4 T-Cell Counts

No correlation between CD14 and CD4 as disease progression marker was found, while there was a significant negative correlation between CD235a ($r = -0.394$, $P = 0.019$), as red cells activity, and CD4 (Table 2).

Prognostic Significance of Different Cellular MPs

A linear regression analysis of CD4 as a disease progression marker with other variable indicators in HIV patients, as well as CD4, showed that erythrocyte marker, CD235a, is the most sensitive predictor, while linear regression analysis with viral load as a disease progression test showed no significance (Table 3).

DISCUSSION

HIV-1-infected patients have an increased risk of a hypercoagulable state. The incidence of thrombotic events

TABLE 3. Linear Regression Analysis Test of CD4 and Other Parameters

Model	Unstandardized coefficients		Standardized coefficients			95% Confidence interval for B	
	B	Standard error	Beta	*t*	Significant	Lower bound	Upper bound
(Constant)	612.044	492.456		1.243	.225	−402.187	1,626.276
Viral load	−.738	1.049	−.116	−.703	.488	−2.898	1.423
CD62P	−49.684	66.133	−.134	−.751	.460	−185.888	86.520
CD235a	−6.599	2.542	−.345	−2.597	.016	−11.834	−1.365
Collagen-ADP	−.633	1.480	−.066	−.428	.673	−3.682	2.416
Collagen-epinephrine	2.980	2.731	.153	1.091	.286	−2.644	8.603
PAC-1	−22.170	129.884	−.032	−.171	.866	−289.671	245.332
Neutrophil	−4.350	48.916	−.013	−.089	.930	−105.095	96.396
Platelets	1.576	1.004	.245	1.569	.129	−.492	3.644

Linear regression analysis shows that the most sensitive predictor in HIV patients is erythrocyte microparticle CD235a.

in HIV-infected patients is rising, but the underlying pathogenic mechanisms remain uncertain.

MPs are submicron fragments shed from the plasma membrane of stimulated or apoptotic cells (13). They are released from different cell types and carry membrane proteins from their donor cells to normal individuals under basal physiological condition (21).

Procoagulant potency of MP was demonstrated in patients suffering from acute coronary syndrome with eptifibatide drug induced thrombocytopenia (22). PMPs are the main procoagulant circulating species with leucocytes or endothelial origins being less represented in mycocardial infarction studies treated by primary percutenous transluminal coronary angioplasty (23). Endothelial MPs, platelets, and leucocytes activation was elevated in patients with venous thrombosis (24). Abnormal levels of MPs (PMPs) were associated with several prothrombotic conditions (25).

Blood MP provides procoagulant phospholipid surface for the assembly of the clotting enzymes complexes promoting thrombin generation. Their catalytic property relies on a procoagulant anionic aminophospholipid, phosphatidylserine (PhtdSer), which is made accessible by membrane remodeling and shedding processes occurring after cell stimulation (26), and by the possible presence of TF that is the cellular initiator of coagulation expressed in stimulated monocytes and ECs. PhtdSer considerably enhances TF procoagulant activity. MP carries or harbors pro-inflammatory lipids and membrane glycoproteins (27).

In our study of 35 thrombocytopenic HIV patients and 25 healthy controls, as reference controls with matched age and sex in patients, we found enhanced platelet MPs expression of CD42a, CD61, which was directly related to clinical indices of HIV disease—plasma viremia. This increase of platelet MPs had a higher significance and was explained by previous studies as a result of apoptosis when HIV virus infects megakaryocytes (28), or lacks clearance from cells of monocytes and macrophages lineage (29), which is the main scavenger of apoptotic microvesicles with reduction of its clearance and over-release of MPs (30).

The proportions of CD62 P-selectin in HIV-infected patient samples ($P < 0.001$) and platelet MPs expressing PAC-1 in HIV-infected patient samples ($P = 0.001$) were significantly higher than among controls. We found a significant positive correlation between platelet expressions of PAC-1-positive platelets/PMPs and levels of plasma viremia ($r = 0.452$, $P = 0.006$) in our patient populations, while there was a negative correlation between the proportion of PAC-1 and levels of $CD4^+$ T-cell counts ($r = -0.369$, $P = 0.028$).

Expression of P-selectin (CD62P) on platelets is a well-recognized indicator of platelet activation (31). PAC-1 is also considered as an index of platelet granularity release and represented as a marker of platelet activity. We assume that selectins of leukocyte, platelets, or endothelial origin become potent contributors to thrombus initiation and propagation through platelets and leucocyte rolling to endothelial wall, then platelet activation and release of granule contents are detected by increased CD62P and PAC-1 in our study. Circulation of platelets or leukocytic origin MPs modulates cellular interactions through the upregulation of cytokines and cytoadhesins in ECs. This was proved by Myers et al. who concluded that P-selectin was involved in leukocyte rolling, upregulated during venous thrombosis, and promoted vein wall inflammation (32).

There was a negative correlation between the proportion of CD62 P-selectin-positive PMPs and levels of $CD4^+$ T-cell counts ($r = -0.438$, $P = 0.008$). This finding was similar to Holme et al. (33) who observed a significant

negative correlation between P-selectin expression on the platelet surface and the CD4 lymphocyte counts in peripheral blood ($r = -0.49$, $P = 0.03$).

Falati et al. explained a prominent role of platelet-borne PSGL-1 and TF-bearing MP, in a laser-induced arteriole endothelial injury model, showing their colocalization on the leading edge of the thrombus (18).

Other study by Himber et al. (34) had established the contribution of leukocytes in venous thrombosis induced by collagen. TF-bearing MP of platelet origin played an important role in the initiation and propagation of coagulation in vitro. Muller et al. (35) showed that platelets and/or leukocyte-derived MP would thus concentrate TF activity above a threshold to allow blood coagulation to be triggered. Wakefield et al. concluded that high PMP levels combined with high D-dimer and P-selectin levels correlate with the diagnosis of deep venous thrombosis. This could be the result of procoagulant phosphatidyle serine and TF exposure on MPs. Selectins recruited MPs and amplified thrombosis in an animal model of inferior vena cava thrombosis (36).

In our research, we observed a significant increase in platelet activity of collagen-ADP and -epinephrine in HIV-infected patients when compared to healthy controls. More interesting finding is the significant negative correlation of collagen-ADP with disease prognostic activity markers CD4, while no correlation was found with collagen-epinephrine. We therefore hypothesize that a significant increase in platelet P-selectin measures platelet glycoprotein IIb/IIIa reflecting granular activity of platelets and correlated with higher collagen/ADP; and enhanced platelet activation in HIV-1-infected patients has been reported and shown to strongly correlate with plasma viral load and CD4 progression marker.

In our research, we observed a significant increase in monocyte MPs as CD14 in HIV-infected patients than controls. These monocytes have an important role in directing cytokines release toward anti-inflammatory and are participative in thrombus generation. It was explained by KÖppler et al. who studied transfer of MP and found that monocytes taking MPs showed a significant decrease in production of Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Tumour Necrosis Factor (TNF-α) in comparison to monocytes that had not taken up MPs. In contrast, the transfer of MPs to monocytes clearly increased the release of IL-10 ($P < 0.01$) (37). MPs exposed to serum activate complement resulting in a deposition of iC3b on MPs. This enables the uptake of MPs by human B cells. Transfer of MPs to B cells is almost completely mediated by the complement receptor CD21. The transfer of MPs alters the cellular functions of acceptor cells toward an anti-inflammatory phenotype. In addition, Morelli et al. showed that the uptake of iC3b-coated apoptotic cells by dendritic cells markedly suppressed the secretion of pro-inflammatory cytokines (38). Hoffmann et al. observed that in vivo interaction of phosphatidylserine-containing liposomes with the "phosphatidylserine-receptor" suppresses inflammation in tissues by mediating the release of the anti-inflammatory cytokine transforming growth factor β (TGF-β) (39).

Fortunately, we found another significant increase in red cell MPs (glycophorine-A) CD235a, this observation could have a role in initiation of thrombosis. This erythrocyte MP increased in number due to excess red cells in circulation, and its activity may lead to an increased apoptosis and more release of those microvesicles. In our study, we supposed a significant role of erythrocyte MPs as well as platelet MPs in the initiation of thrombus through endothelial wall, while few studies found that RBCs adherence to platelet may lead to cell–cell infection and maintain virus infection for long potency. This red cell marker has negative correlation with CD4 T cell, which in turn negatively correlate with collagen-ADP, while positively correlate with collagen-epinephrine; this observation could be explained by the fact that the red cell MPs may have a strong relation with platelet functions and activation. Some studies proposed that RBCs could be the carrier of HIV-1 and bind virus to red cells containing blood group antigen known as Duffy that is a receptor for chemokines (40, 41). In addition, recent studies reported that HIV-1 binds to and is internalized by platelets and red cells in vitro (42, 43). Beck et al. found that erythrocyte-bound platelet could bind HIV-1 to cause cell–cell infection to peripheral blood mononuclear cells (44).

Linear regression analysis showed CD235a could be a predictor for disease activity and hypercoagulability state index. This statistical finding may give critical insights to the value of erythrocyte MPs and will help in pharmacological control of this MP.

Nomura et al. (45) explained that this circulation of blood contained a variety of MPs derived from platelets, leukocytes, and ECs, which activated endothelium and, moreover transferred chemokines to it. Thus, the stimulation of ECs by PMPs, in vitro, results in cytokines release and expression of adhesion molecules. Furthermore, it was demonstrated by Mause et al. that PMPs contained substantial amounts of regulated cells on activation, normal T-cells expressed and secreted (RANTES). This pro-inflammatory cytokine can get deposited on activated endothelium. Thus, this transcellular delivery of RANTES promotes leukocyte recruitment to murine atherosclerotic carotid arteries (46). Pfister explained that arachidonic acid was subsequently metabolized to thromboxane A2 that induced artery contraction. Methacholin-induced contractions were even increased by preincubation with PMPs (47). Finally, Amabile et al. showed that

endothelial MPs affected endothelial nitric oxide (NO) synthesis, but did not change NO synthase expression (48).

Finally, our study showed high expression of PMPs (CD41a, CD61), platelet activation markers (CD62P, PAC-1), as well as monocytes (CD14) in combination with erythrocyte MPs (CD235a) These different cellular patterns may interact with each other to initiate thrombus propagation and activate endothelial wall through different pathway. These MPs significantly correlate with platelet activity markers and functions, this association increased the risk of morbidity and mortality in those patients.

CONCLUSIONS

This study concluded that activated platelets may have a role in thromboembolic events that are more common in HIV-infected persons than among the general population. High expression of different cellular MPs may act as a storage pool, interacting with each other to impair essential functions as inflammatory response, hemostasis, vasomotricity, vascular remodeling, cell survival, and apoptosis. The pharmacological control of MP-mediated vascular responses constitutes the next challenging issue for those patients and different diseases associated with these MPs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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