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# International Journal of Medicine and Medical Sciences

## ARTICLES

*Full Length Research Paper*

# Incidence of thrombocytopenia in seropositive dengue patients

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**Dengue has become a major health concern globally in recent decades. Dengue infected patients manifest a spectrum of symptoms and in severe cases the fate is mortality. A hallmark of dengue infection is thrombocytopenia which causes concern for the patients and treating doctors. This study aimed to evaluate the incidence of thrombocytopenia in seropositive dengue patients. Bleeding manifestation was also investigated in dengue patients to evaluate its association with the severity of thrombocytopenia. In this study, 750 individuals were screened for dengue infection by detecting immunoglobulin M (IgM) against dengue virus in their serum. Enzyme-linked immunosorbent assay (ELISA) was performed for detection of IgM antibody and 250 individuals were found to be seropositive. Platelet counts were performed on whole blood of seropositive patients using Sysmex XE-5000 Automated Hematology Analyzer. Among 250 dengue patients, 2% had severe thrombocytopenia, 65.2% were found to have mild to moderate thrombocytopenia and 32.8% had normal platelet counts. Bleeding was related to the severity of thrombocytopenia as 80% of patients having platelet count lower than 25000/μl showed bleeding manifestations.**

**Key words:**  Dengue, thrombocytopenia, seropositive, immunoglobin M (IgM), enzyme-linked immunosorbent assay (ELISA), platelet count.

## INTRODUCTION

Dengue viral infection is currently amongst the most critical arthropod-borne infections from the public health view point. Concerning the incidence of dengue all over the world, the graph has risen up noticeably in recent decades and over 40% of the world's population is now at risk from dengue. It has been estimated that there may be 50 to 100 million dengue infections globally per year (World Health Organization (WHO), 2013).

 Four distinct serotypes of dengue virus are known to cause the disease (DEN-1, DEN-2, DEN-3 and DEN-4) and *Aedes aegypti* mosquito is the primary vector. Recovery from infection by one serotype offers lasting immunity against that particular serotype, but subsequent infections by other serotypes increase the risk of developing severe dengue (Centers for Disease Control and Prevention (CDC), 2000).

 Dengue fever is a severe, flu-like sickness in which high grade fever (104°F) is accompanied by severe

headache, pain behind the eyes, joint pains, vomiting and rashes on body, but is rarely fatal. However, severe dengue (previously referred as Dengue Haemorrhagic Fever) is a potentially lethal complication characterized by plasma leaking, fluid accumulation, severe bleeding, or organ impairment (WHO, 2013).

In Pakistan, dengue has been around for the past 20 years. The first major outbreak in Pakistan was reported in 1994 to 1995. During 2005 to 2006, there was an un-expected spread of virus in the country. The recent (2011 to 2012) wave of dengue fever hitting Pakistan's eastern province of Punjab killed at least 365 people and 21597 cases of dengue fever have been reported, making it the world's biggest epidemic of DF ever (Shakoor et al., 2012).

The normal range of platelet count in blood of healthy adults is 150,000 to 450,000/mm$^3$ and counts less than 150,000/mm$^3$ are referred to as thrombocytopenia (Dacie and Lewis, 2006). Thrombocytopenia is a common prob-lem in dengue, which causes concern for the patients and treating doctors (Halstead, 2007). With the advances in medical research, it is now evident that activation of immune process and direct marrow suppression by the viral particles is responsible for decline in platelets (Gubler, 2002). It has been proposed that platelets are sensitized by auto antibodies, and then are destroyed by the reticulo-endothelial system of the body. These auto antibodies against glycoproteins of the platelet membrane can be identified in 80% of the patients (Cines and McMillan, 2005).

Thrombocytopenia in dengue infection raises concerns about bleeding risk. The aim of this study was to assess the incidence of thrombocytopenia in seropositive dengue patients. Bleeding manifestation was also inspected in seropositive dengue patients to evaluate its association with the severity of thrombocytopenia

## MATERIALS AND METHODS

### Sample collection

Samples were taken from 750 individuals who presented to Excel Diagnostic Laboratory, Islamabad for serological screening test of dengue from 1st of September 2011 to 15th of January, 2012. Patient having any hematological malignancy (which may interfere with platelet counts) or bleeding disorders such as VWB disease were excluded from the study. After informed consent and written performa was taken from each patient, 2 ml of blood was drawn into gel vial for serum separation and another 2 ml was drawn into an ethylenediaminetetraacetic acid (EDTA)-filled tube for platelet count. Blood tubes were transported on ice within 2 h to the Biochemistry Department of ARID Agriculture University, Rawalpindi. Samples were processed within 6 h of the initial sample collection.

### Serological screening of dengue infection

The Calbiotech Dengue virus IgM ELISA Kit (Catalog #DE051M) was used for the detection of IgM antibody to dengue virus in serum samples. IgM ELISA was performed according to the protocol given

by the manufacturer. In brief, 100 µl of patient serum was added to wells in microtitre plate coated with purified antigen. An incubation of 20 min was given to allow specific IgM, if present in patient sample, to bind to the coated antigen. Microtitre plate was then washed to remove all unbound materials and 100 µl of the enzyme conjugate was added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate was washed off and 100 µl of substrate was added. The plate was then incubated to allow the hydrolysis of the substrate by the enzyme. The optical density at 450 nm was read using ELISA reader.

### Platelet counts

Platelet counts were performed on whole blood of those individuals who were found seropositive for dengue infection. Platelet counts were performed using Sysmex XE-5000 Automated Hematology Analyzer.

### Statistical analysis

The data was entered and analyzed by using IBM SPSS Statistics version 20 (IBM Corp, Armonk, NY). Frequency and percentages were calculated for qualitative variables like IgM positivity, age group, platelets counts groups and bleeding manifestation were expressed. Pearson Chi square and Fisher's exact tests were used to observe the association between qualitative variables. A p-value ≤ 0.05 was considered as statistical significant.

## RESULTS

In this study, 750 individuals were screened for dengue infection and 250 of them were found to be seropositive. Amongst 250 seropositive patients, 155 were males and 95 were females. They were divided into 3 age groups. Age group of less than 15 years contained 101 patients, whereas 92 of them were between 15 and 50 years and 57 were above 50 years of age. The data obtained from the study shows that dengue is more prevalent in children (p-value 0.002) (Figure 1).

In this study, out of 250 seropositive cases, 82 patients show platelet counts within normal range. In 5 patients, platelet count was found to be severely low, that is, <25,000/µl. In 106 patients, platelet count was found to be moderately low, that is, 25,000/µl to 100,000/µl. 57 cases were borderline, that is, having platelet counts of 100,000/µl to 150,000/µl (Table1). There was no statistically significant association found between gender and platelet group (p-value 0.875), or age and platelet group (p-value 0.960).

Bleeding manifestations were equally common in both genders. There was no predilection for any age group among the patients who developed bleeding manifestations. Bleeding was significantly related to thrombocytopenia (Table2).

## DISCUSSION

Dengue fever outbreaks have become a cyclical nightmare

## Age groups of seropositive dengue patients
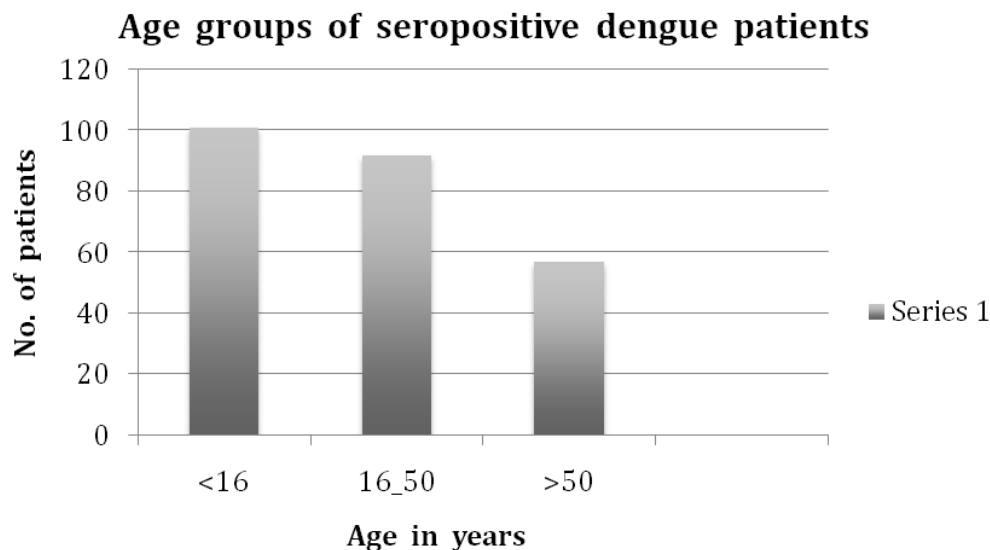


**Figure 1.** Age groups of patients.

**Table 1.** Association of gender and age groups with platelet counts.

| Characteristic | Group | Platelet count | | | | Total | p-value |
|---|---|---|---|---|---|---|---|
| | | Normal (%) | Border line (%) | Moderately low (%) | Severely low (%) | | |
| Gender | Male | 53 (34.2) | 33 (21.3) | 66 (42.6) | 3 (1.9) | 155 | 0.875 |
| | Female | 29 (30.5) | 24 (25.3) | 40 (42.1) | 2 (2.1) | 95 | |
| Age | ≤15 | 37 (36.6) | 22(21.8) | 40 (39.6) | 2 (2.0) | 101 | |
| | 16-50 | 29 (31.9) | 21(23.1) | 39 (42.9) | 2 (2.2) | 91 | 0.960 |
| | >50 | 16 (27.6) | 14(24.1) | 27 (46.6) | 1 (1.7) | 58 | |
| Total | n (%) | 82 (32.8) | 57 (22.8) | 106(42.4) | 5 (2.0) | 250 | |

**Table 2.** Association of bleeding manifestation with gender, age and platelet counts.

| Characteristic | Group | Bleeding manifestation | | p-value |
|---|---|---|---|---|
| | | Present (%) | Absent (%) | |
| Gender | Male | 18 (11.6) | 137 (88.4) | 0.597 |
| | Female | 9 (9.5) | 86 (90.5) | |
| Age | ≤15 | 14 (13.9) | 87 (86.1) | |
| | 16-50 | 5 (5.5) | 86 (94.5) | 0.124 |
| | >50 | 8 (13.8) | 50 (86.2) | |
| Platelet counts | Normal | 0 | 82 (100) | |
| | Borderline | 0 | 57 (100) | < 0.001 |
| | Moderately low | 83 (78.3) | 23 (21.7) | |
| | Severely low | 4 (80) | 1 (20) | |

in Pakistan for the last several years. This study showed that the majority of dengue positive patients were children, that is, in accordance with a study conducted in Indonesia (Chairulfatah et al., 2003) and another study conducted in India (Narayanan et al., 2003).

Thrombocytopenia is common in dengue infection. Severe

bleeding is related to severe thrombocytopenia. Out of 250 dengue patients in this study, 32.8% had normal platelet counts, whereas 2% had severe thrombocytopenia and the remaining 65.2% were found to have mild to moderate thrombocytopenia. These results are similar to the findings of Narayanan et al. (2003), who reported that in the serologically-confirmed dengue cases, the prevalence of thrombocytopenia was 58% on admission and 83% during hospitalization. The results of this study also correlate with the findings of Sumarmo (1983).

Bleeding was significantly related to the severity of thrombocytopenia as 80% of patients having platelet count lower than 25000/µl showed bleeding manifestations and 23% of patients showing moderately low platelets also manifested bleeding. There was no predilection for any age group or gender for thrombocytopenia or bleeding among the dengue patients. These results are in accordance with a study conducted in India by Narayanan et al. (2003).

During an epidemic, people of effected community should be screened serologically for dengue infection. If positive, people may keep themselves within a safe zone of platelet count by proper care, therapy and management since all serologically positive cases do not develop thrombocytopenia as seen in current study as well.

Patients with significantly low levels of platelets bleed and may need platelet transfusion. Since neither cure nor vaccine exists for dengue fever, prevention is the only option to control the human and economic cost of the epidemic.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

### REFERENCES

Centers for Disease Control and Prevention (2000). Imported dengue—United States, 1997 and 1998. MMWR Morb. Mortal Wkly Rep. 248-53

Chairulfatah A, Setiabudi D, Agoes R, Colebunders R (2003). Thrombocytopenia and Platelet Transfusions in Dengue Haemorrhagic Fever and Dengue Shock Syndrome. Dengue Bull. 27:138-143

Cines DB, McMillan R (2005). Management of adult idiopathic thrombocytopenic purpura. Annu. Rev. Med. 56:425-42

Dacie and Lewis (2006). Practical haematology, 10th ed, Churchill Livingstone, Philadelphia

Gubler DJ (2002). Epidemic dengue/ dengue hemorrhagic fever as a public health, social and economic problems in the 21st century. Trends Microbiol. 10:100–103.

Halstead SB (2007). Dengue. Lancet 370:1644-52.

Shakoor MT, Ayub S, Ayub Z (2012). Dengue fever: Pakistan's worst nightmare. WHO South-East Asia J. Public Health 229:231-229

Narayanan M,Aravind MA, Ambikapathy P, Prema R, Jeyapaul MP (2003). Dengue Fever – Clinical and Laboratory Parameters Associated with Complications. Dengue Bull. 27:108-115

Sumarmo P (1983).Dengue haemorrhagic fever (thesis). University of Indonesia Press.

World Health Organization (WHO) (2013). Dengue and severe dengue. Fact sheet 117. Retrieved on 14 October. Available from: www.who.int/mediacentre/factsheets/fs117/en/

*Full Length Research Paper*

# A Cd36 polymorphism associated with eight-times increased susceptibility to cerebral malaria in Central Sudan

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**Malaria is one of the biggest known health threats in Africa. Erythrocytes infected with falciparum malaria adhere to a variety of host receptors, including CD36. Cerebral malaria (CM) is a major life-threatening complication of *Plasmodium falciparum* infection. The human protein CD36 is a major receptor for *P. falciparum*-infected erythrocytes and contributes to the pathology of *P. falciparum* malaria. The aim of the present study was to determine the role of the adhesion molecule CD36 in children with CM at Central Sudan. A case-control study included 70 children with cerebral malaria (CM) and 84 controls were enrolled in this study. The method was a mutational analysis for the polymorphism in the CD36-188 T > G using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) where the distribution of CD36 to 188 T > G genotypes differed significantly between CM patients and controls and children carrying the mutant G allele were associated with eight-times increased relative risk for susceptibility to cerebral malaria (*P-value* = 0.005; odds ratio = 7.962; 95% CI = 1.571 to 29.903).**

**Key words:** *Plasmodium falciparum*, cerebral malaria, erythrocytes, Central Sudan.

## INTRODUCTION

Malaria remains a major public health threat to more than 600 million Africans. In sub-Saharan Africa, the disease distribution is closely linked with seasonal patterns of the climate and local environment (Grover-Kopec et al., 2006). According to World Health Organization (WHO) classification of malaria endemic countries, Sudan is categorized in group 4. Sudan represents more than 50% of the total estimated malaria cases in the group (WHO, 2002). In two camps in Khartoum state, Sudan, most of the recorded malaria cases were among children. Risk of malaria attack was significantly associated with tribe,

language, education, water supply and food expenditure (Saeed and Ahmed, 2003).

Falciparum malaria is characterized by cytoadherence of host erythrocytes containing mature asexual-stage parasites and the sequestration of these forms in tissue microvasculature (Montgomery et al., 2006). Erythrocytes infected with falciparum malaria adhere to a variety of host receptors, including CD36, which are widely expressed on endothelium, platelets and leucocytes (Combes et al., 2006). The human protein CD36 is a major receptor for *Plasmodium falciparum*-infected

erythrocytes and contributes to the pathology of *P. falciparum* malaria (Omi et al., 2003). Cerebral malaria (CM) is a major life-threatening complication of *P. falciparum* infection. CM in humans is defined as the presence of unarousable coma with exclusion of other encephalopathies and confirmation of *P. falciparum* infection (Hunt and Grau, 2003). CM is estimated to affect more than 785,000 children who are younger than 9 years in sub-Saharan Africa every year, with 15 to 30% case fatality rate (Murphy and Breman, 2001).

In Gedarif Hospital, Eastern Sudan, the reported CM cases of during three malaria seasons showed that the number of CM patients has increased 6 folds in the year 2003 compared to the year 2001 (Giha et al., 2008). The aim of the present study was to determine the role of the adhesion molecule CD36 in children with CM at Central Sudan, to evaluate the role of CD36 in susceptibility to CM cases and to determine the genetic variations in the CD36 polymorphisms (-188) in CM cases.

## MATERIALS AND METHODS

### Study area

The study was conducted in three cities: Wad Medani; at the Wad Medani Paediatrics Teaching Hospital, Sinnar; at the Sinnar Teaching Hospital, and Singa; at the Singa Hospital. These cities lie along the bank of the Blue Nile River and are characterized by a prolonged raining period that provides a suitable environment for the breeding of the malaria vector, *Anopheles* mosquitoes. These areas are endemic for *P. falciparum* malaria especially during the autumn season.

### Selection of cases and controls

Children admitted to the three hospitals and suspected for cerebral malaria were registered. The mean age of the study subjects was (6.8 ± 3.2) years; the minimum age was 8 months old and the maximum age was 14 years old. Seventy cases were confirmed as having CM by a Pediatrician, according to the criteria of CM (Molyneux, 1990). Children with blood films negative for asexual stages or those with other diseases that may contribute to the coma were excluded from the study (Molyneux, 1990). Eighty-four children were selected as a control group from pupils in schools from the three cities and from other children admitted to the three hospitals with diseases other than CM, and were matched in age, sex and ethnic group.

### Collection of blood samples

Samples of 3 ml venous blood were collected in heparinized tubes from the patients and controls. The blood samples were kept frozen at -70°C for later DNA extraction and genetic analyses. DNA was extracted using the salting-out method and was purified by phenol-chloroform method (Sambrook et al., 1989). The quantity of the obtained DNA was measured using an ultra violet (UV) spectrophotometer.

### Mutation analysis

PCR-restriction fragment length polymorphism (PCR-RFLP) method

was used in the present study. Genotypes for the mutation CD36 – 188 T > G was identified from the restriction enzyme (*Nde*1) digested fragments of PCR amplified products of the exon 10 of CD36 gene with the primer pairs: 5' CTATGCTGTATTTGAATCCGACG 3' and 5' ATGGACTGTGCTACTGAGGTTAT 3', respectively.

PCR reactions were performed in a reaction volume of 30 µl containing 3 µl from 10 × PCR buffer, 3 µl of 200 µM dNTPs, 1.5 µl MgCl$_2$, 200 ng genomic DNA, 2 µl of 10 picomoles from each primer, 0.2 µl Taq DNA polymerase and completed to the final volume with distilled water. The denaturation temperature was 90°C for 1 min, annealing temperature 55°C for 2 min and prolongation temperature 72°C for 1 min. This reaction was run for 40 cycles. In 2% agarose gel, the PCR product was stained in 1 µg/ml Ethidium bromide for 10 to 15 min and visualized under Ultra Violet (UV) light in a Gel Documentation System (GDS) to check for the presence or absence of the DNA bands. The mutation of thymine for guanine at position (-188) appolishes a restriction site for *Nde*1. Digestion with this enzyme was used for typing this polymorphism. In a total volume of 15 µl, 2 µl PCR product was digested overnight at 65°C with 2.5 U *Nde*1.1.5 µl 10× NE Buffer 1 [100 mM Bis Tris Propane-HCl, 100 mM magnesium chloride and 10 mM dithiothreitol (pH 7.0)] and deionized water. Digestion reactions were loaded on 8% non-denaturaning polyacrylamide gel (30% Protogel (Acrylamide/Bis-acrylamide), 37.5:1) (FMC BioProducts) and electrophoresed at 140 V for one hour. The gel was stained in 1 µg/ml ethidium bromide solution for 10 to 15 min and visualized under UV light in a Gel Documentation System (GDS).

### Statistical analysis

Statistical analysis was done using statistical package for the social sciences (SPSS) programme and Chi-square test.

## RESULTS

70 cerebral malaria (CM) cases and 84 controls were included in the study. 39 (55.7%) were males and 31 (44.3%) were females. The highest incidence of the disease was among the age group 4 < 6 years. There were 11 different tribal stocks included in the study subjects. Juhaina Arab group had the higher incidence of CM among the study subjects (22.9%). The mutant allele remains uncut with *Nde*1, it is a characteristic of the homozygous mutant type (-188 GG) appears as single 213 base pair band. Complete cleavage into the 148 and 65 bp fragments is a characteristic of the homozygous wild type (-188 TT) which appears as two bands. Incomplete cleavage into 213, 148 and 65 bp is a characteristic of the heterozygous (–188 TG) and appears as three bands. In this study the allele frequency for CD36-188 T > G in CM cases was 88% wild type T allele and 12% mutant type G allele. The allele frequency in controls was 99% T allele and 1% G allele. The distribution of CD36-188 T > G genotypes differs significantly between CM patients and controls and those carrying the mutant G allele were associated with eight times increased relative risk for susceptibility to CM (P-value = 0.003; odds ratio = 7.636; 95% CI = 1.048 to 13.630) (Table 1).

**Table 1.** The frequency of CD36 -188 T>G alleles in CM patients and controls.

| Study subjects | Genotype | |
|---|---|---|
| | T T | GG+TG |
| CM Patients | 59 (84.3%) | 11 (15.7%) |
| Controls | 82 (97.6%) | 2 (2.4%) |
| (P-value = 0.003; odds ratio = 7.636; 95% CI = 1.048 – 13.630) | | |
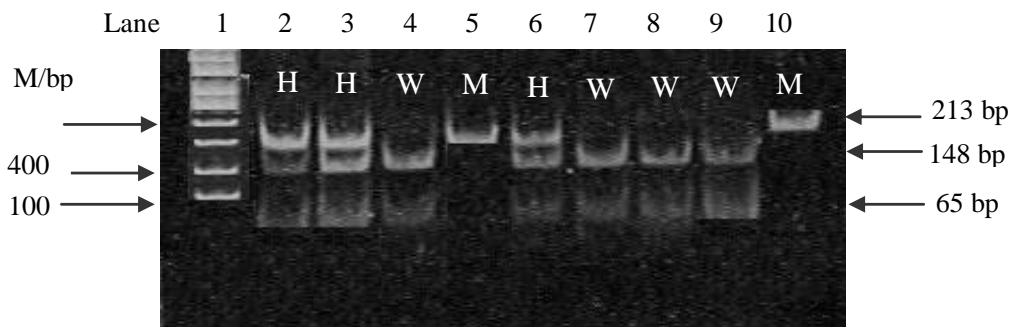


H: heterozygous type          W: homozygous wild type          M: homozygous mutant type

**Figure 1.** Pattern of the CD36-188 T>G alleles in the CM cases using PCR-RFLP. Lane 1: 100 bp DNA marker, lanes 2, 3 and 6 are heterozygous type (TG) for CD36 -188, lane 4, 7, 8 and 9 are homozygous wild type (TT), lane 5 and 10 are homozygous mutant type (GG).
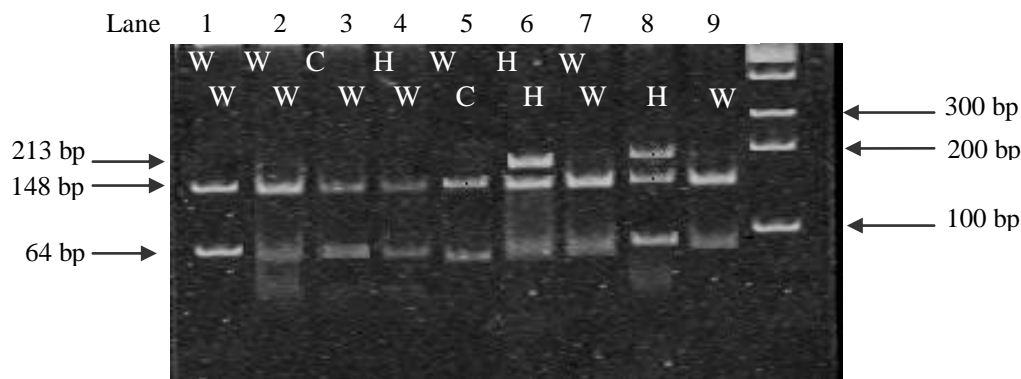


W: homozygous wild type, H: heterozygous type

**Figure 2.** Pattern of CD36 –188 T>G alleles in the controls using PCR-RFLP. Lanes 1, 2, 3, 4, 5, 7 and 9 were homozygous wild type (TT), lane 6 and 8 were heterozygous mutant for CD36-188.

**Genetic analysis**

*CD36-188 T > G genotypes in CM and controls*

CD36 -188 T > G polymorphism was screened using the PCR-RFLP method both for CM cases and controls (Figures 1 and 2). The gene frequency for CD36-188 T > G in CM cases was 84.3% homozygous wild type (TT), 7.1% homozygous mutant type (GG) and 8.6% heterozygous mutant type (TG). The gene frequency in controls was 97.6% homozygous wild type (TT), 2.4% homozygous mutant type (GG) and 0.0% heterozygous mutant type (TG). The distribution of CD36-188 T > G genotypes differs significantly between CM patients and

**Table 2.** The allele frequency of CD36 -188T>G in CM patients and controls.

| CD36 -188T>G | Genotypes | | | Alleles | |
|---|---|---|---|---|---|
| Group | T T | G G | T G | T | G |
| CM patients | 59 (84.3%) | 5 (7.1%) | 6 (8.6%) | 0.88 | 0.12 |
| Control | 82 (97.6%) | 2 (2.4%) | 0 (0.0%) | 0.99 | 0.01 |

($\chi^2$ = 9.846, df = 2, *P-value* = 0.007).

controls ($P$ = 0.007) (Table 2).

## DISCUSSION

Clustering of CM in certain tribes such as Juhaina Arab stocks may be due to more representation in the population in the study area, and some families and tribes may be genetically susceptible to CM. This result shows that those carrying the mutant G allele were more likely to produce the adhesion molecule CD36 in the endothelial microvaculature in response to malaria infection. The production of the adhesion molecule CD36 leads to massive sequestration of the infected red blood corpuscles (RBCs) leading to the known complications of CM. The association of CD36-188 mutant G allele; with increased susceptibility to CM; showed that there is clustering of certain alleles, not only in Sudanese populations, but also in African populations as shown by Pain et al. (2001) in a study in Kilifi, Kenya. This study was consistent with a study done by Aitman et al. (2000) in children from East and West Africa, that showed a significant association of CD36 mutations with susceptibility to severe malaria in general, and to CM in particular. *In vitro* studies indicate that sequestration of parasitized red blood corpuscles (PRBCs) in the microvessels is mediated by the attachment of knobs on PRBCs to receptors on the endothelial cell surface such as CD36, TSP and ICAM-1 (Aikawa et al., 1992). In mice infected with malaria, erythrocytes infected with malaria parasite adhere *in vitro* to purified CD36, a critical endothelium receptor for binding *P. falciparum*-infected erythrocytes (Mota et al., 2000).

## Conclusion

The G allele in the mutation T188G is associated with eight-times increased relative risk for susceptibility to CM in children in the Central region of Sudan. This study recommend the establishment of studies exploring the genetic factors related to CM in children in Central Sudan where clustering of CM in certain tribes and families points to the possibility of involvement of a genetic factor in the control of susceptibility to CM. Other mutations in CD36 gene should be studied.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## REFERENCES

Aikawa M, Brown AE, Smith CD, Tegoshi T, Howard RJ, Hasler TH, Ito Y, Collins WE, Webster HK (1992). "A primate model for human cerebral malaria: Plasmodium coatneyi-infected rhesus monkeys." Am. J. Trop. Med. Hyg. 46(4):391-7.

Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, Curtis BR, McKeigue PM, Kwiatkowski D, Greenwood BM, Snow RW, Hill AV, Scott J (2000). "Malaria susceptibility and CD36 mutation." Nature 405:1015–16.

Combes V, Coltel N, Faille D, Wassmer SC, Grau GE (2006). "Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier." Int. J. Parasitol. 36(5):541-6.

Giha HA, Elbashir MI, A-Elbasit IE, A-Gadir TME, ELGhazali G (2008). "Bimodal transmission of cerebral malaria and severe malarial anemia and reciprocal co-existence of sexual and asexual parasitemia in an area of seasonal malaria transmission." Parasitol. Res. 103(1):81-85.

Grover-Kopec EK, Blumenthal MB, Ceccato P, Dinku T, Omumbo JA, Connor SJ, Malar J (2006). "Web-based climate information resources for malaria control in Africa." Malar J. 5(1):38.

Hunt NH, Grau GE (2003). "Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria." Trends Immunol. (24,9):491-9.

Molyneux ME(1990). "The clinical features of cerebral malaria in children." Med trop(50): 65-67.

Montgomery J, Milner DAJr, Tse MA, Njobvu A, Kayira K, Dzamalala CP, Taylor TE, Rogerson SJ, Craig AG, Molyneux ME (2006). "Genetic analysis of circulating and sequestered populations of Plasmodium falciparum in fatal pediatric malaria." J. Infect. Dis. 194(1):115-22.

Mota MM, Jarra W, Hirst E, Patnaik PK, Holder AA (2000). "Plasmodium chabaudi-infected erythrocytes adhere to CD36 and bind to microvascular endothelial cells in an organ-specific way." Infect. Immun. 68(7):4135-44.

Murphy SC, Breman JG (2001). "Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy." Am J Trop Med Hyg 64(1-2 Suppl):57-67.

Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, Looareesuwan S, Tokunaga K (2003). "CD36 polymorphism is associated with protection from cerebral malaria." Am. J. Hum. Genet. 72(2):364-74.

Pain A, Urban BC, Kai O, Casals-Pascual C, Shafi J, Marsh K, Roberts DJ (2001). "A non-sense mutation in Cd36 gene is associated with protection from severe malaria." Lancet 357(9267):1502-3.

Saeed IE, Ahmed ES (2003). "Determinants of malaria mortality among displaced people in Khartoum state, Sudan." East Mediterr Health J 9(4): 593-9.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A laboratory Manual (2nd Edition). Cold Spring Harbor Laboratory, New York.

WHO (2002). "Current epidemiological situation." TDR Roll Back Malaria,: Report.

*Full Length Research Paper*

# Standardization of the method to obtain therapeutic-quality platelet-rich plasma

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**Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability inorder to implement and standardize the ideal methodology in centrifugation strength and time for obtaining therapeutic-quality PRP, allowing its application to provide better and rapid recovery of muscular injuries, tendinitis, bone and ligament lesions. To evaluate PRP therapy, 150 patients with muscular lesions, tendinitis, shoulder, knee, ankle, hand and elbow injuries were treated. On application of PRP, we obtained 100% clinically significant symptomatic improvement in all 150 patients treated, who had musculoskeletal and ligament injuries, with a marked reduction of pain and inflammation. We concluded that the ideal concentration for obtaining PRP is at 1000 rpm with a time of 5 min; in addition, under these conditions the plasma lacks leukocytes and erythrocytes. The results were reproducible because the experiment was repeated at two institutions under the same conditions and similar results were obtained. The regeneration obtained in the affected patients is due to the fact that growth factors were released from the activated platelets; these initiate and modulate cicatrization in the tissues, which is a recent innovation to promote cicatrization, accelerating the power of tissue regeneration, with a platelet concentrate suspended in plasma.**

**Key words:** Growth factors, platelet activation, application, tissue regeneration, therapeutic quality.

## INTRODUCTION

Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability. In short term clinical practice, it is utilized to concentrate growth factor-rich plasma (GFRP) by up to 388% above values found in normal plasma, for later application in tissues, in a search to enhance the osteo-induction biological cascade. The pharmaceutical way in which PRP is utilized clinically is obtained by means of its gelling on adding thrombin and $CaCl_2$ to it. PRP gel is a compound of fibrinogen and activated platelets (by the addition of

thrombin), which determines the release of a cascade of the growth factors (GF) of platelet granules within a fibrin mesh (Camargo et al., 2012).

PRP is an innocuous, 100%-compatible material with minimal possibility of rejection. It acts as a bio-stimulator and as a bio-mediator against cellular aging; it restores and repairs tissue with properties indistinguishable from the original tissue and is easy to apply through minimally invasive therapeutic methods. Additionally, it allows the conservation of the harmony and physiology of the patient's own tissue structure that was unable to be improved by synthetic substances (De Boulle, 2007). Given its unsubstitutable source of benefits for humans, its use has been extended beyond curative medicine for further use in esthetic, cosmetic and sports medicine etc., in which the donor and the recipient is the patient him/herself, thus constituting an autologous material (Rosenthal et al., 2011). PRP is a new technology that is centered on improving the curative response after an injury. PRP is obtained from the peripheral blood of patients and is centrifuged to obtain a sample that is highly concentrated in platelets; the blood sample is submitted to degranulation to release GF with curative properties (Lopez-Vidriero et al., 2010).

Platelet activation in response to tissue and vascular damage causes the formation of a platelet tamponade and a blood clot whose functions are the consequence of hemostatis and the secretion of biologically active proteins involved in the tissue regeneration process. These proteins, the GF proteins, are secreted basically by the platelet structure, but not exclusively, and are also able to be produced by fibroblasts. PRP contains a small number of leukocyte cells that also contribute to cell defense by means of synthesis of interleukins that intervene in the unspecific immune response, contains cytokines, thrombin and other GF that are implicated in the cicatrization of wounds, possess biological properties and inherent adhesives (Rodriguez-Flores et al., 2012).

The concentrated preparation (the concentrate) is at present, injected into the patients at the site of injury. This can be effected intralesionally, intra-articularly or surrounding the involved tissue (Lopez-Vidriero et al., 2010). This technique provides novel concepts because it was thought that platelets only acted in tissue hemostatic and currently it is known that platelets can also exert an influence on wound repair, on vascular ulcer implantology, with good clinical evolution and promising results in the fields of sports medicine and arthroscopy (Jovani, 2009). Reconstruction of the anterior cruciate ligament in particular has demonstrated improved maturation of the autograft, diminution of donor-zone mortality and the pain threshold, in addition to improved allograft incorporation. Due to acceleration of the biological integration of the graft through the use of PRP, patients can initiate more intensive rehabilitation programs and can return to sports faster. Due to its autologous origin, its

its easy preparation and excellent safety profile, PRP has opened up another therapeutic portal (Freymiller and Aghabo, 2004). The hematological products contribute to saving millions of lives annually, spectacularly improving the life quality and expectancy with potentially fatal disorders and making it possible to carry out complex medical and surgical procedures. Numerous clinical applications have been described for PRP, for ulcers, maxillofacial surgery and spinal surgery (Rodriguez-Flores et al., 2012). Applications in ortho-pedics include treatment for tendon and bone injuries, joint replacements, fractures in patients with diabetes, wound cicatrization etc.

PRP allows for better and more rapid recovery from muscle, tendon, bone and ligament injuries because it has GFα and GFβ, which impede the proliferation, migration, differentiation and regeneration of the damaged tissue, favoring the activation of molecular processes in diana cells and resulting in angiogenesis, mitogenesis, chemotaxis and the synthesis of collagen and the extracellular matrix (ECM). The ruptures of ligaments involve serious injuries that affect different population groups, such as persons engaging in sports; thus, the rupture of ligaments is more frequent in young adults. Sports that entertain a greater risk of this injury type are those that imply change of direction and of rhythm (acceleration), such as skiing, basketball, or tennis. Other risk factors are muscular weakness, discoordination or lack of joint flexibility.

According to the World Health Organization (WHO, 2009), hematology services worldwide face the immense challenge of obtaining supplies of blood products sufficient for attending to the needs of patients and at the same time guaranteeing their quality and safety in the face of old and new threats to public health. Only if great attention is paid to the availability, safety and quality of blood products can the health-related objectives of the development of the millennium be reached. Therefore, the WHO has cited the demand to guarantee the availability, safety and quality of all blood-derived products; thus, developed countries have put into practice policies, strategies and procedures that have made possible widespread access to a complete gamma of safe blood products.

Consequently, the need arises to implement in the clinical laboratory an efficient methodology for obtaining PRP because the strength of centrifugation as well as the time of same exert an influence on the variability in the platelet concentrate and consequently on clinical efficacy, in that these factors vary in the different centrifuges utilized in the different laboratories on applying mechanical force incorrectly, with which the attributes of the platelets can be harmed. We in Mexico comply with the demand of the WHO for regimented surveillance of the quality of blood products. Our aim was to implement and standardize the optimal method in centrifugation strength and

No.    23
Date        21/05/201
Time            10:45
Mode    WB

WBC      !    0.0×10³/μL
RBC      !    0.01×10⁶/μL
HGB      !    0.0g/dL
HCT      !    0.1%
MCV           ---.-fL
MCH           ---.-pg
MCHC          ---.-g/dL
PLT    PL*    33×10³/μL

                    WBC

LYM%          ---.-%
MXD%          ---.-%
NEUT%         ---.-%
LYM#          ---.-×10³/μL
MXD#          ---.-×10³/μL
NEUT#         ---.-×10³/μL

                    RBC

RDW_SD        ---.-fL
RDW_CV        ---.-%

                    PLT

MPU    PL*    5.7fL

**Figure 1.** Hemogram at 1800 rpm for 10 min, the results show total absence of leukocytes, erythrocytes and 33,000 platelets/μL, therefore, this speed and time parameters are inconvenient for the preparation of a PRP.

time for obtaining therapeutic-quality PRP, allowing with its application better and more rapid recovery of muscle, tendon, bone and ligament injuries.

## METHODOLOGY

To evaluate therapy with PRP, we treated 150 patients with muscular and tendon lesions in shoulder, knee, hand and elbow, to which PRP were applied as part of the protocol utilized in surgery, as well as part of the patients' treatment. We proceeded to carry out asepsis in the antecubital region near the puncture site with ethyl alcohol at 70%; this is mainly to avoid any type of contamination at the time of sample-taking. We took 6 to 8 vacuum tubes of peripheral blood with sodium citrate 0.109 M. Later, the tubes were placed in automated Sismex KX-21 Hematology analyzer (ROCHE®) equipment and we centrifuged these at 3200, 3000, 2800, 1800 and 1000 rpm for 5 and 10 min. We performed the preparation of PRP for its application at the injury site, mixing 0.5 ml of calcium gluconate at 10% with 1.0 ml de PRP for each $cm^3$ of the lesion's surface. For example, in the meniscus region, infiltration is carried out on the medial meniscus in the medial portal region and in the mid-collateral and meniscus capsule zone, with a 22 × 32 and a 10 to 20 ml needle (black needle) syringe. Infiltration with local anesthesia, ropivacaine, is performed in the area of the injury; with the calcium gluconate-PRP mixture applied in the previously anesthetized region. Informed consent was obtained from the 150 patients.

## RESULTS

The experiment was repeated five times at different speeds and at times of 5 and 10 min. From the respective repetitions, the mean was obtained, the result of which was that at 10 min of centrifugation at 3200, 3000, 2800, 1800, and 1000 rpm, concentrates of 0, 0, 0, 2 and 33 platelets/$10^3$, respectively were obtained. At a time of 5 min at 3200, 3000, 2800, 1800 and 1000 rpm, concentrates of 20, 50, 135, 186 and 223 platelets/$10^3$ were obtained, respectively (Figures 1 and 2).

It was determined that at 1000 rpm with a time of 5 min, an ideal concentrate was obtained for PRP; the results showed the total absence of leukocytes, erythrocytes and 223.000 platelets/$mm^3$; thus, speed and time are adequate parameters for obtaining PRP. The results were reproducible because the experiment was repeated at two institutions under the same conditions and similar results were obtained. Later, once the plasma fraction was separated under aseptic conditions, we proceeded to application of the PRP, within a lapse of 5 to 10 min. On evaluating patients after application of the PRP, 100% significant symptomatic clinical improvement was obtained in the 150 treated patients with musculoskeletal and ligament injuries, the patients referring with less pain and observing tissue regeneration, as well as little or null inflammation.

## DISCUSSION

Administration of PRP is always safe because patients

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No.    29
Date          21/05/2011
Time                11:12
Mode   WB

WBC        !   0.0×10³/μL
RBC        !   0.01×10⁶/μL
HGB        !    0.0g/dL
HCT        !    0.1%
MCV            ---.-fL
MCH            ---.-pg
MCHC           ---.-g/dL
PLT            223×10³/μL

                         WBC




LYM%           ---.-%
MXD%           ---.-%
NEUT%          ---.-%
LYM#           ---.-×10³/μL
MXD#           ---.-×10³/μL
NEUT#          ---.-×10³/μL

                         RBC




RDW_SD         ---.-fL
RDW_CV         ---.-%

                         PLT




MPV       -    6.9fL
```
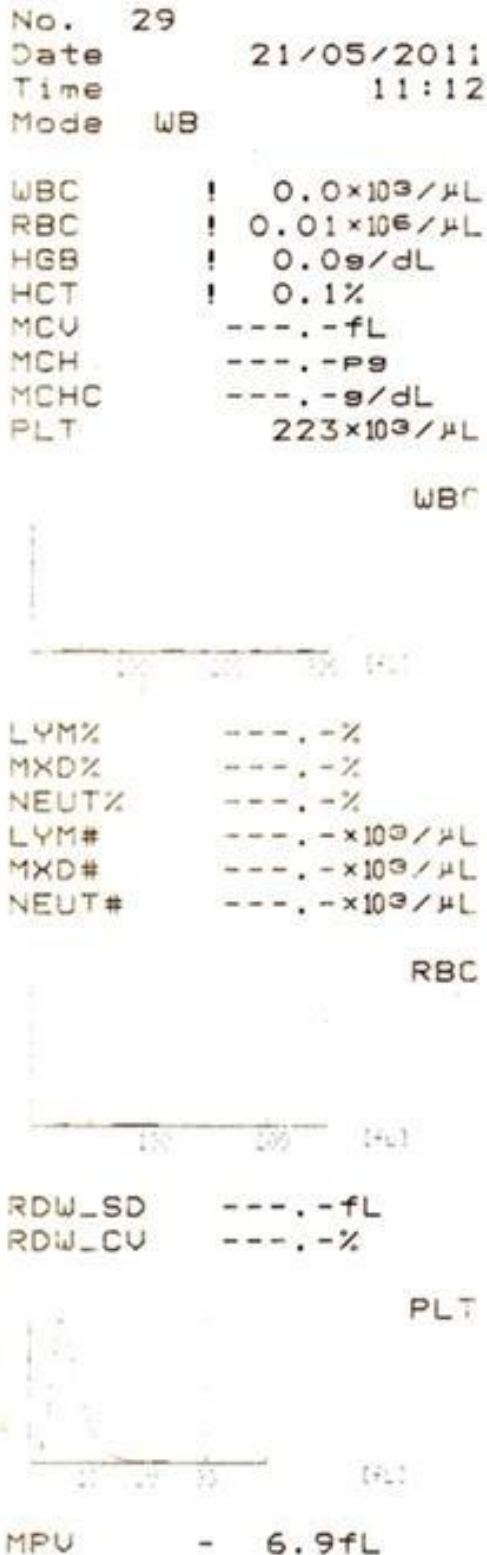
**Figure 2.** Hemogram at 1000 rpm for 5 min. The results show total absence of leukocytes, erythrocytes and 223,000 platelets/μL. Therefore, this speed and time parameters are suitable for the preparation of a PRP.

are not at risk of the transmission of diseases such as human immunodeficiency virus (HIV), hepatitis, among others; similarly, it can be employed in persons with resistance to being transfused without any problem (Rivera-Tocancipa and Rivera-Ortiz, 2011). In this respect, it is reported that PRP does not cause any negative effect on patients because these are autologous preparations. The procedure consists of processing the patient's own blood to obtain the PRP. However, there are counter indications in patients who suffer from coagulation disorders (thrombocytopenia, hypofibrino-genia, or who are receiving anticoagulant therapy). This technique will reduce recovery time by one half in patients with muscle lesions, tendon lesions or bone fractures (Schwartz et al, 2011; Garcia-Gimenez and Gonzalez-Nicolas, 2005).

With reference to the latter, the use of platelet preparations is subjected to numerous variables, which will be indicative of their clinical efficiency. Some of these variables are: platelet concentrate, technique for obtaining the platelets, concentration of secreted protein, manipulation and clinical application. In order to obtain optimal results requires concentrating the platelets between 3 and 5 times above the baseline value. Bearing in mind that a normal individual has in the area of 200,000 platelets/μl, an optimal centrifuge would be 1,000,000 platelets/μl; in our results, we were able to obtain 223,000 platelets/mm³ of plasma, maintaining stability. In this respect, it has been demonstrated that higher concentrations do not increase the effect with regard to wound cicatrization (Schwartz et al., 2011). Five percent of PRP is sufficient in a clot to promote the cicatrization (Hernandez and Rossani, 2007).

In the majority of studies carried out to date with PRP, selected protocols under empirical bases have been utilized and for this, PRP has been obtained by the procedures described but with great variables, for example, centrifugal strength ranges from 1000 to 6000 rpm and time of centrifugation from 2 to 15 and 20 min. But in practice, the number of leukocytes has not been controlled in the preparations, which impedes discerning between the usefulness of one or another preparation. In this investigation, we opted for utilizing 1000, 1800, 2800 and 3000 rpm and solely times of 5 and 10 min.

Controversially, it is mentioned that PRP contamination by leukocytes exerts an influence on the final number of GF and that greater risk contamination is obtained in pre-parations obtained by means of conventional centrifuges. However, in the present research, we were able to prove that it is indeed possible to utilize conven-tional centri-fuges to obtain PRP, although the process requires pulchritude in the manipulation. There has been commentary on the fact that collecting PRP with a pipette renders the technique very imprecise because nearly non-intentionally, red blood cells and leukocytes are included (Jovani, 2009); this datum is in agreement with

another study, although it is difficult to maintain sterility, it is not impossible, thus the unavoidability of protocolizing the procedure and being careful to procure correct obtaining of PRP (Rodriguez-Flores et al., 2012). In this sense, it is reported that the platelets can be damaged, thus becoming activated above all if the same sample is centrifuged various times (Jovani, 2009).

It was possible to prove the latter with the results of this project, in which it was determined that speed and time directly influence the platelet concentration in citrated plasma, because at a greater time and speed, the concentration of platelets diminishes. There are other methods, such as electrophoresis, but these require more time and adequate equipment. In this respect, analysis was performed in which regional anticoagulation with trisodium citrate proved to be safe and effective strategy that helps in reducing costs in patients requiring continuous kidney replacement therapy due to renal injury in pediatric intensive care (Fernandez et al., 2012).

It has been determined that the participants' gender and age do not exert an influence on platelet count, because no statistically significant difference was observed (Jovani, 2009), with which we are in agreement, because we did not find differences in terms of the patients and the final platelet count number.

In Hematology, the term PC is employed to denominate a concentration $>1$ ml/µl and it is synonymous with PRP (Lopez-Vidriero et al., 2010). It has been indicated that when anticoagulated blood is centrifuged, it forms three layers according to the density. The middle layer is formed of white globules and the upper layer, by the plasma. The plasma layer is divided into upper with platelet-poor plasma, middle with platelets and lower with PRP (Rodriguez-Flores et al., 2012), which can be proven on centrifuging the samples. In our research, we chose to use sodium citrate, as did Jovani (2009), who cites that sodium citrate is a salt that uptakes the calcium ions found in the blood and that neutralizes these, forming a chemical compound called a chelate, thus impeding coagulation of the blood. In addition, sodium citrate does not alter the platelet membrane receptors and will permit reversibility of the process on adding calcium in the form of $CaCl_2$. Sodium citrate has been used as an anticoagulant by diverse authors (Freymiller and Aghabo, 2004; Schwartz et al., 2011; Hernandez and Rossani, 2007; Martinez-Gonzalez et al., 2002; Perez-Sierra, 2010; Sanchez-Perez and Diez-Quijano, 2008; Reyes et al., 2002; Restituto, 2010; Chen et al., 2009; Ruiz-Macarilla et al., 2012; Fierro-Sierna et al., 2011).

However, additionally citric acid and dextrose (CAD) has been employed as an anticoagulant; this is the oldest and most utilized anticoagulant, but it diminishes the pH of plasmas and delays and interferes with the processes of aggregation and platelet secretion (Jovani, 2009; Alanis-Blancas et al., 2010; BIOMET, 2011). In another study, no anticoagulant was utilized due to the urgency of

its use in the operating room (Roby, 2011). It is noteworthy that although the anticoagulant is very important in obtaining PRP, there are some authors who fail to mention it (Garcia-Gimenez and Gonzalez-Nicolas, 2005; Perez-Sierra, 2010; Saenz-Torres et al., 2007; Gonzalez-Ossa and Ortiz-Orrego, 2004; Orozco-Delclos, 2007).

On performing an analysis on the rpm utilized in each research, great variability was found as follows: 1,200 rpm (Rosenthal et al., 2011); 1,300 rpm (González-Ossa and Ortiz-Orrego, 2004); 1,400 rpm (Martinez-Gonzalez et al., 2002; Acosta and Potdevin, 2011; Beca et al., 2007); 1,500 rpm (Roby, 2011; Torres, 2006); 1,800 rpm (Freymiller and Aghabo, 2004; Reyes et al., 2002; Bedon-Rodriguez and Villota-Gonzalez, 2012); 2,400 to 3,600 rpm (Hernandez and Rossani, 2007); 3,000 rpm (Orozco-Delclos, 2007); 3,200 rpm (Beca et al., 2007; BIOMET, 2011); 3,650 rpm (Martinez-Gonzalez et al., 2002); 5,400 to 5,600 rpm (Hernandez and Rossani, 2007; Reyes et al., 2009), and 6000 rpm (Chen et al., 2009). With respect to time of centrifugation, the review was carried out on the time utilized as follows: 2 min (Perez-Sierra, 2010); 3 min (Hernandez and Rossani, 2007); 7 min (Jovani, 2009; Perez-Sierra, 2010; Sanchez-Perez and Diez-Quijano, 2008; Acosta and Potdevin, 2011; Beca et al., 2007; Torres, 2006); 8 min (Freymiller and Aghabo, 2004; Schwartz et al., 2011; Reyes et al., 2002; Bedon-Rodriguez and Villota-Gonzalez, 2012); 12 min (Ruiz-Macarilla et al., 2012); 15 min (BIOMET, 2011), and 20 min (Gonzalez-Ossa and Ortiz-Orrego, 2004). In previously performed works, this research coincides with the time utilized of 5 min (Chen et al., 2009; Roby, 2011) and 10 min (Jovani, 2009; Saenz-Torres et al., 2007). The technique employed in this research was of only a sole centrifugation, which coincides with that applied by other authors (Jovani, 2009; Freymiller and Aghabo, 2004; Garcia-Gimenez and Gonzalez-Nicolas, 2005; Sanchez-Perez and Diez-Quijano, 2008; Reyes et al., 2002; Restituto, 2010; Chen et al., 2009; Fierro-Serna et al., 2011; Alanis-Blancas et al., 2010; BIOMET, 2011; Gonzalez-Ossa and Ortiz-Orrego, 2004; Orozco-Delclos, 2007). In addition, the double-centrifugation technique was utilized (Schwartz et al., 2011; Hernandez and Rossani, 2007; Martinez-Gonzalez et al., 2002; Perez-Sierra, 2010; Reyes et al., 2009; Acosta and Potdevin, 2011; Saenz-Torres et al., 2007; Beca et al., 2007). In this regard, the blood was centrifuged for 3 min at 2,400 to 3,600 rpm in two cycles and it was observed that in the first cycle, PRP is extracted and in the second, "platelet-exquisite" PRP is obtained (Hernandez and Rossani, 2007).

On the other hand, on performing application of PRP, there was a satisfactory response in patients in 3 to10 days; this fact is in agreement with Fierro-Serna and collaborators (2011), who found out that the use of growth factor-rich platelets (GFRP) can benefit the post-

operatory period of patients after the surgical removal of the lower third molars, with soft tissue regeneration and less pain and inflammation. In another experiment, cicatrization and gum retraction was observed 39 days after PRP treatment (Chen et al., 2009). In this work, PRP was applied within a lapse of 5 to 10 min, a lesser amount of time than that employed in other procedures, in which the transfer was 12 to 14 min, achieving overcorrection with PRP-enriched lipografts. As can be observed, in each research analyzed, this coincides with some authors in centrifugation time or speed, but none coincide in terms of the parameters standardized in the present investigation. It is timely to point out that a study was performed in which it is considered that the intrinsic factors such as race, gender and age influence the final concentration of cells and GF in pure PRP in horses (Giraldo et al., 2013).

Finally, the benefit was observed of GLRP in the closing of recurrent palatine fistula in the treatment of cleft lip and palate (Bedon-Rodriguez and Villota-Gonzalez, 2012). Also recently, the antibacterial effect was evaluated *in vitro* of pure PRP against micro-organisms that colonize the oral cavity, finding activity in the following: *Enterococcus faecalis*; *Candida albicans*, *Streptococcus agalactiae* and *Streptococcus oralis* (Drago et al., 2013), in which it is suggested that platelet concentrates can be used against postoperatory infections and that they would represent the missing link between osteoinducive and antimicrobial activity. Likewise, Sánchez and collaborators affirm that certain molecules implied in tissue repair, such as GF, stimulate cell receptors; thus, the biological response depends not only on the amount of GF, but also on the cell receptors of each patient (Sanchez, 2010), because GF are autogenous endomodulators of PRP that naturally mold the treated zone as a "zone memory code" (Hernandez and Rossani, 2007). With the present research, it is proposed that for obtaining PRP, the following should be considered: the type of anticoagulant utilized for preparing the PRP; the technique to employ and the time and speed of centrifugation; in addition, it is probable that PRP obtained with these results aids in improving therapeutic behavior in tissue regeneration.

## Conclusion

Centrifugation speed and time exert an influence directly on the platelet concentration in citrated plasma; the best parameters for obtaining PRP in our experiment were at 1,000 rpm for 5 min. This procedure, which is standardized in research can be carried out in every clinical laboratory to obtain therapeutic-quality PRP. The experiment was conducted in different laboratories and with different equipment and no significant differences was found, but it is necessary for each laboratory to determine the most adequate parameters for obtaining good quality PRP. On administering PRP, a better and rapid recovery was achieved for muscle, tendon, bone and ligament injuries because PRP contains GF which are tissue and tendon regenerators for application in Traumatology.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

### REFERENCES

Acosta I, Potdevin D (2011). Rejuvenecimiento cutáneo facial con materiales autólogos. Másters Thesis, Universidad Autónoma de Barcelona, España.

Alanís-Blancas LM, Zamora-Muñoz P, Cruz-Miranda A (2010). Uso del plasma rico en plaquetas en el tratamiento de las lesiones meniscales. Reporte de dos casos. Anales Med. México 55(3):147-153.

Beca T, Hernández G, Morales S, Bascones A (2007). Plasma rico en plaquetas. Una revisión bibliográfica. Av. Periodon. Implantol. 19(1):39-52.

Bedón-Rodríguez M, Villota-González LG (2012). Labio y paladar hendido: tendencias actuales en el manejo exitoso. Arch. Med. 12(1):107-119.

BIOMET Biologics, Inc. GPS III Platelet Separation System (2011). Warsaw, IN, USA. Available at: http://www.biomet.com.mx/biomet2011/pdf/BIOLOGICS/GPS%

Camargo J, Vanegas N, Vélez JD, López JC, Herrera JM (2012). Plasma rico en plaquetas como sistema de entrega local de antibióticos en infecciones óseas: estudio in vitro. Rev. Col. Or. Tra. 26(1):39-49.

Chen PRY, Salza I, Trobo JI, Gutiérrez DV (2009). Regeneración de tejidos periodontales con plasma rico en plaquetas (PRP): metodología de obtención y aplicación. V Congreso Nacional de la Sociedad Española de Odontología Veterinaria (SEOVE). Facultad de Veterinaria. Universidad Compultense, España.

De-Boulle K (2007). Management of complications after implantation of fillers. J. Cosmet. Dermatol. 3:2-15.

Drago L, Bortolin M, Vassena C, Taschieri S, Del Fabbro M (2013). Antimicrobial activity of pure platelet-rich plasma against microorganisms isolated from oral cavity. BMC Microbiol. 13(47):1-5.

Fernández J, Oliveros C, González L (2012). Guía de manejo anticoagulación de citrato de sodio para terapia de reemplazo renal en niños. Acta Colombiana de Cuidado Intensivo. 12(3):179-84.

Fierro-Serna VM, Martínez-Rider R, Hidalgo-Hurtado JA (2011). Colocación de plasma rico en factores de crecimiento postextracción de terceros molares inferiores: reporte de un caso. Rev. Odontol. Mex. 15(2): 109-114.

Freymiller EG, Aghabo TL (2004). Platelet-rich plasma; ready or not? J. Oral Maxillo Fac. Surg. 62:484-488.

García-Giménez JV, González-Nicolás-Albandea JA (2005). Tratamiento del envejecimiento cutáneo mediante bioestimulación con factores de crecimiento autógenos. Int. J. Cosmet. Med. Surg. 7(2):8-14.

Giraldo CE, López C, Álvarez ME, Samudio IJ, Prades M, Carmona JV (2013). Effects of the breed, sex and age on cellular content and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. BMC Vet. Res. 9(29):1-10.

González-Ossa S, Ortiz-Orrego GE (2004). Plasma rico en plaquetas: una alternativa para acelerar el proceso de cicatrización ósea. CES Odontol. 17(1):71-74.

Hernández I, Rossani G (2007). Lipoinjerto enriquecido con plasma rico en plaquetas. Int. J. Cosmet. Med. Surg. 9(2):8-10.

Jovani MM (2009). El plasma rico en plaquetas en la regeneración

ósea post-exodoncia. Estudio radiográfico. Tesis doctoral. Departamento de Estomatología. Universidad de Valencia.

López-Vidriero E, Goulding KA, Simón DA (2010). The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy 26(2):269-278.

Martínez-González JM, Cano-Sánchez J, Gonzalo-Lafuente JC, Campo-Trapero J, Esparza-Gómez G, Seoane-Lestón M (2002). Do ambulatory-use Platelet Rich Plasma (PRP) concentrates present risks? Med. Oral. 7(5):375-390.

Orozco-Delclós L (2007). Nuevas aplicaciones clínicas del plasma rico en plaquetas en patologías muscoloesqueléticas. Conferencia. Facultad de Medicina. Universidad Nacional Autónoma de México (UNAM). Symposium: Células Madre: del laboratorio a la clínica.

Pérez-Sierra AL (2010). Estudio de microscopía electrónica y cuantificación de los factores de crecimiento mediante un nuevo procedimiento de obtención de plasma rico en plaquetas. Tesis doctoral. Universidad Complutense de Madrid Facultad de Medicina.

Reyes M, Montero S, Cifuentes J, Zarzar E (2002). Actualización de la técnica de obtención y uso del plasma rico en factores de crecimiento (PRGF). Rev. Dental Chile 93(2):25-28.

Restituto P (2010). Estudio de la Díada CD40/CD40L y su modulación por la adiponectina en el síndrome metabólico. Depósito Académico Digital Universidad de Navarra.

Rivera-Tocancipá D, Rivera-Ortiz CA (2011). Sangrado mayor en paciente adulto, Testigo de Jehová. Rev. Fac. Salud. 3(1):55-67.

Roby M (2011). Uso intraoperatorio de plasma rico en plaquetas (PRP) en deportistas. Rev. Assoc. Argent Ortop. Traumatol. Deporte 18(2):71-75.

Rodríguez-Flores J, Palomar-Gallego MA, García-Denche JT (2012). Plasma Rico en Plaquetas. Fundamentos biológicos y aplicaciones en cirugía maxilofacial y estética facial. Rev. Esp. Cir. Oral Maxilofac. 34(1):8-17.

Rosenthal J, Woolfry AE, Pawlowska A (2011). Hematopoietic cell transportation with autologous cord blood in patients with severe aplastic anemia: an opportunity to revisit the controversy regarding cord blood banking for private use. Pediatr. Blood Cancer 56(7):1009-1012.

Ruiz-Macarilla L, Hernández-Hermoso JA, Maculé-Beneyto F (2012). Efecto del plasma rico en plaquetas en la incorporación biológica de una plastia tendinosa en un túnel óseo. Tesis Doctoral. Departamento de Cirugía y Especialidades Quirúrgicas. Universidad de Barcelona, España.

Sáenz-Torres BC, Calvo-Benito J, Gayá-Puig A (2007). Calidad del plasma rico en plaquetas: estudio de la activación plaquetaria. Rev. Esp. Cir. Oral Maxilofac. 29(4):240-248.

Sánchez M (2010). Un plasma rico en plaquetas: ¿una moda o una realidad? Archiv. Med. Deporte XXVII(138):252-254.

Sánchez-Pérez N, Díez-Quijano C (2008). Estudio piloto sobre la regeneración ósea obtenida mediante la aplicación de cemento de Brushita y plasma rico en plaquetas en un modelo experimental: estudio histomorfométrico. Odontología UCM.

Schwartz A, Martínez-Sánchez G, Re L (2011). Factores de crecimiento derivados de plaquetas y sus aplicaciones en medicina regenerativa. Potencialidades del uso del ozono como activador. Rev. Española Ozonoterapia 1(1):54-73.

Torres J (2006). Influencia del Plasma Rico en Plaquetas en la regeneración ósea: estudio densitométrico y morfométrico en calota de conejas osteoporóticas. Tesis Doctoral. Universidad Rey Juan Carlos, España.

World Health Organization (2009). Availability, safety and quality of blood products.EB125/5. Available at: http://apps.who.int/iris/bitstream/10665/2269/1/B125_5-en.pdf?ua=1

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