

# Differences in Platelet Glycoprotein Expression During Menstruation Cycle and Ovulatory Phase

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## Abstract

**Introduction:** The variations in the physiological properties of female platelets during menstrual cycle phases, pregnancy and postmenopausal women to interactions with platelet CD49b, CD42b, CD41a and CD61 expression glycoprotein receptors were not well understood. Therefore, the aim of the present study was to evaluate the expression of these glycoprotein receptors during menstruation and ovulatory phase of menstrual cycle in reproductive age women.

**Methods:** It is across section study including 44 healthy young non-hormonal contraceptives taking women aged between 19-44 years to determine the effect of estrogen on the expression level of platelet glycoprotein receptors (GPIb, GPIIa, GPIIb and GPIIIa) in its resting state in women in reproductive age.

**Results:** No significant difference in the expression of CD42b, CD41a and CD61 between menstruation and ovulatory phases in resting platelets in all subjects. However, this study showed a significant difference in CD49b expression in none-Arab ethnic subjects compared to Arab women.

**Conclusion and Recommendations:** This study suggested CD49b glycoprotein receptor used to be the commonly expression on the surface of platelet at some stage in menstruation and ovulatory segment of menstrual cycle in reproductive age women in turn extended platelet activity. Further studies including large number of subjects, platelet integrin gene polymorphisms and progesterone factors changes in platelet clotting associated to menstrual cycle should be conducted.

**Keywords:** hemostasis, platelets, glycoprotein, estrogen, menstruation, ovulatory phase

## 1. Introduction

Platelet plasma membrane supports more than 50 categories of receptors. These heterodimeric receptors support platelet adhesion and aggregation in the process of hemostasis. In case of blood vessel wall injury, platelets are recruited to the site of vascular injury where the action of glycoprotein (GP) receptors take place due to exposure of subendothelial matrix which contains ligands for these receptors that includes collagen (Kiefel et al., 1995). Platelet aggregation activity is higher in females compared with males. The association between the phase of the menstrual cycle and platelet activity appears to vary with the type of agonist, but platelet aggregation is consistently lowest in the mid-luteal phase irrespective of the agonist used (Nir et al., 2010). Several physiologic properties of platelets from women have been shown to vary with the phase of the menstrual cycle. We have found that platelets from women bound more fibrinogen during the luteal phase (defined as 14 or fewer days from the onset of the next menstrual cycle, or the second half of the menstrual cycle) than during the follicular, suggesting a hormonal regulation of glycoprotein (GP) IIb-IIIa activation (Faraday et al., 1997). The number of  $\alpha$ 2-adrenergic receptors has been shown to peak at the onset of menses and to drop to 74% to 79% of that value during the middle of the cycle. Studies (Jones et al., 1983; Tarantino et al., 1994) suggest estrogen and/or progesterone regulate platelet activation and function. Estrogen- and androgen-responsive genes (Chen & Mehta, 1996; Gopal et al., 2000). There are controversies between the differences in platelets receptors expression between menstrual cycle phases during pregnancy or with hormone replacement therapy (HRT), but the exact effects are still not well understood. In the present study; we aimed to evaluate the expression of these CD Markers: CD42b; GPIb alpha,

CD41 and CD61; GP IIb/IIIa and CD49b; GP Ia/IIa receptors during menstruation and ovulatory phase of menstrual cycle in women at reproductive age.

## 2. Patients and Methods

### 2.1 Study Design & Subjects

This is a cross-sectional study conducted at Imam Abdulrahman Bin Faisal University and King Fahad Hospital University /Saudi Arabia from May 2014 to January 2016; to determine the effect of estrogen on the expression level of platelet glycoprotein receptors (GPIb, GPIIa, GPIIb and GPIIIa) in its resting state in women in reproductive age. The study population consisted of 44 healthy young non-hormonal contraceptives taking women aged between 19–44 years old subdivided into two groups; Arab and non-Arab ethnic women.

### 2.2 Data Collection

The study was approved by the institutional review board (IRB) of the university (IRB-2014-04-282). Survey questionnaires were designed and distributed in Arabic and English. The survey included questions about demographic information, menstrual cycle status, physical and health status that could interfere with platelet reactivity or affect estrogen level. All subjects who fulfill the criteria of the study requirements gave their written informed consent to participate in the study. An informed consent was obtained from the patients, this informed consent procedure was conducted after the patient were interviewed and it was held about their participation, the purpose, and the risks related to the study.

### 2.3 The Inclusion Criteria

All women with a regular ovarian cycle, normal platelet function, without any intake of medications or other drugs that interfere, non-smoking, non-pregnant.

### 2.4 Exclusion Criteria

The exclusion criteria were all women with a family history of thromboembolic or bleeding disorders, high blood pressure, abnormal lipid profile, liver or renal disease, endocrinal and gynecological disorders, chronic diseases, hemoglobinopathies (sickle cell anemia disease, thalassemia or other), allergic conditions, presence of infection at the time of sampling, subjects with diabetes or performing regular strenuous exercise (Feuring et al., 2002; Rosin et al., 2006; Malipatil & Patil, 2013).

### 2.5 Measurements

Quantitative analysis of Estrogen was done using citrated blood samples; the level was assessed by ARCHITECT *I*-optical system. The principle of quantitative determination was based on Chemiluminescent Microparticle Immunoassay (CMIA).

Flow cytometer was used for assessment of platelet glycoprotein receptors; Platelet Rich Plasma (PRP) was separated from citrated whole blood for determination of platelet glycoprotein receptors expression. Resting platelets were stained directly with different conjugated monoclonal antibodies: fluorescein isothiocyanate (FITC) Mouse Anti-Human CD49b, FITC Mouse Anti-Human CD42b, phycoerythrin (PE) Mouse Anti-Human CD41a and PE Mouse Anti-Human CD61. Appropriate isotype controls (FITC Mouse IgG1<sub>k</sub> Isotype Control and PE Mouse IgG1<sub>k</sub> Isotype Control) were used to confirm the specificity of primary antibody binding and to roll out non-specific Fc receptor binding or other cellular protein interactions. After incubation, EDTA-PBS buffer was added to all tubes to remove unbound antibodies. The supernatant was decanted, and the platelets were suspended in 1% BD CellFIX™ then analyzed by FACS Caliber.

Statistical analysis was carried out using SPSS version 20 and Microsoft Office Excel 2010. 10000 events were acquired for each sample in evaluation of platelets glycoprotein receptors. Dot plots and quadrant were used to calculate the median fluorescent intensity (MFI) of CD markers. The variation in glycoprotein receptors expression during menstrual cycle phases was determined as mean and standard deviation (SD). The *p*-values were determined for any difference in the expression of glycoprotein receptors, where a *p*-value ≤ 0.05 was considered statistically significant. Calculations were performed using the SPSS version 16. Paired *t*-tests were applied to evaluate the result in menstruation and ovulatory phase. Mean ± standard error (SE) of the mean were used to plot the data.

## 3. Results

Forty-four (44) healthy females were recruited for evaluation of platelet glycoprotein receptors expression in resting state during different menstrual cycle phases.

### 3.1 CD49b Expression

Variation in the CD49b expression during menstrual cycle phases was determined. A lack of significant difference of CD49b was exhibited between menstruation and ovulatory phase in resting platelets (mean ± SD = 40.65 ± 8.83 and 40.96 ± 8.28, respectively,  $p = 0.79$ ). Additionally, there was an absence of a significant difference in CD49b among Arab females (mean ± SD = 40.87 ± 9.01 and 40.18 ± 7.96, during menstruation and ovulatory phase; respectively,  $p = 0.65$ ). There was, however, significant difference in the expression of CD49b in none-Arab ethnic female (mean ± SD during menstruation and ovulatory phase = 40.08 ± 8.63 and 43.06 ± 9.08 respectively,  $p = 0.03$ ) (Figure 1).

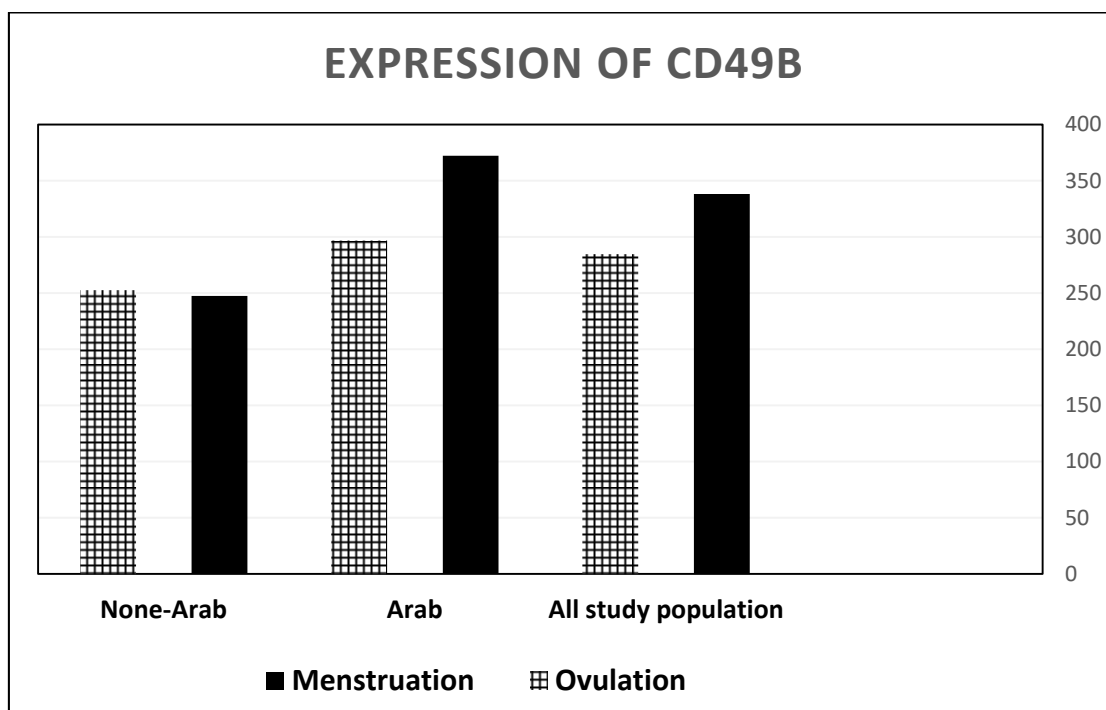


Figure 1. Expression of platelet CD49b during menstruation and ovulation; in all study population ( $p = 0.79$ ), Arab ( $p = 0.65$ ) and none-Arab females ( $p = 0.03$ ). The MFI values presented as mean+S.E.

### 3.2 CD42b Expression

This study showed that there were no significant differences in platelet CD42b expression in resting state during menstrual cycle phases. The results were represented as mean ± SD of MFI during menstruation and ovulatory phase (152.71 ± 31.54 and 146.02 ± 44.75; respectively,  $p = 0.37$ ). Additionally, there were no differences in CD42b expression during menstruation and ovulatory phase neither in Arab (mean ± SD = 159.09 ± 31.80 and 149.25 ± 49.77,  $p = 0.32$ ) nor none-Arab females (mean ± SD = 135.69 ± 24.60 and 137.40 ± 27.09,  $p = 0.85$ ) (Figure 2).

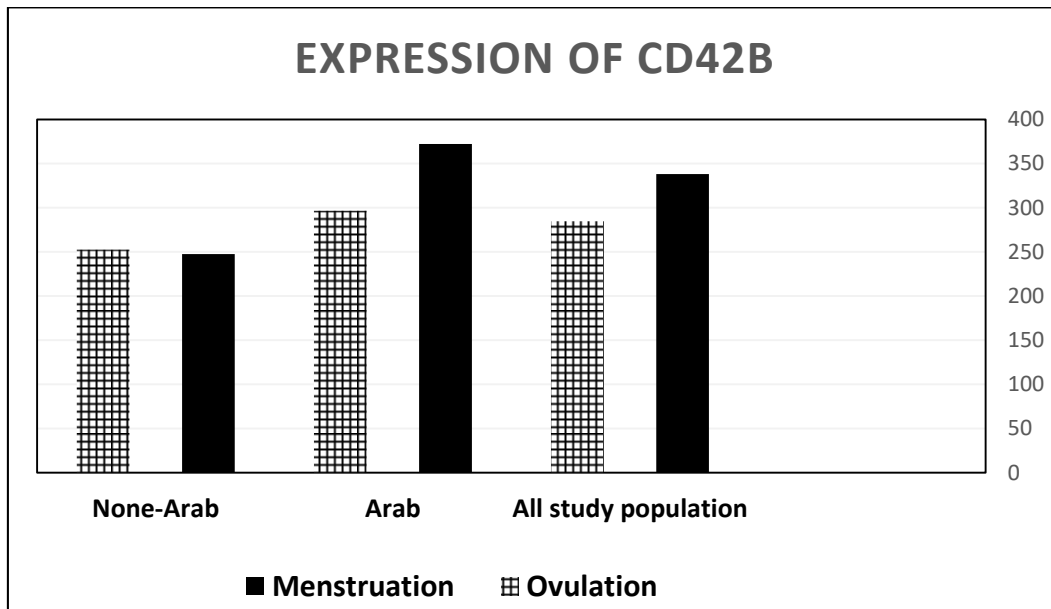


Figure 2. Expression of platelet CD42b during menstruation and ovulatory phase; in all study population ( $p=0.37$ ), Arab ( $p=0.32$ ) and none-Arab females ( $p=0.85$ ). The MFI values presented as mean+S.E.

### 3.3 CD41a Expression

The expression of platelet CD41a was assessed for study subjects during menstruation and ovulatory phase in resting state. The MFI values with mean  $\pm$  SD =  $64.97 \pm 61.79$  and  $47.35 \pm 6.76$ ,  $p = 0.06$ . There were no significant differences in CD41a expression between menstruation and ovulatory phase. The absence of significant differences was also seen among Arab (mean  $\pm$  SD =  $72.32 \pm 71.27$  and  $48.46 \pm 6.79$ ,  $p = 0.07$ ) and none-Arab females (mean  $\pm$  SD =  $45.36 \pm 5.94$  and  $44.39 \pm 5.97$ ,  $p=0.65$ ) (Figure 3).

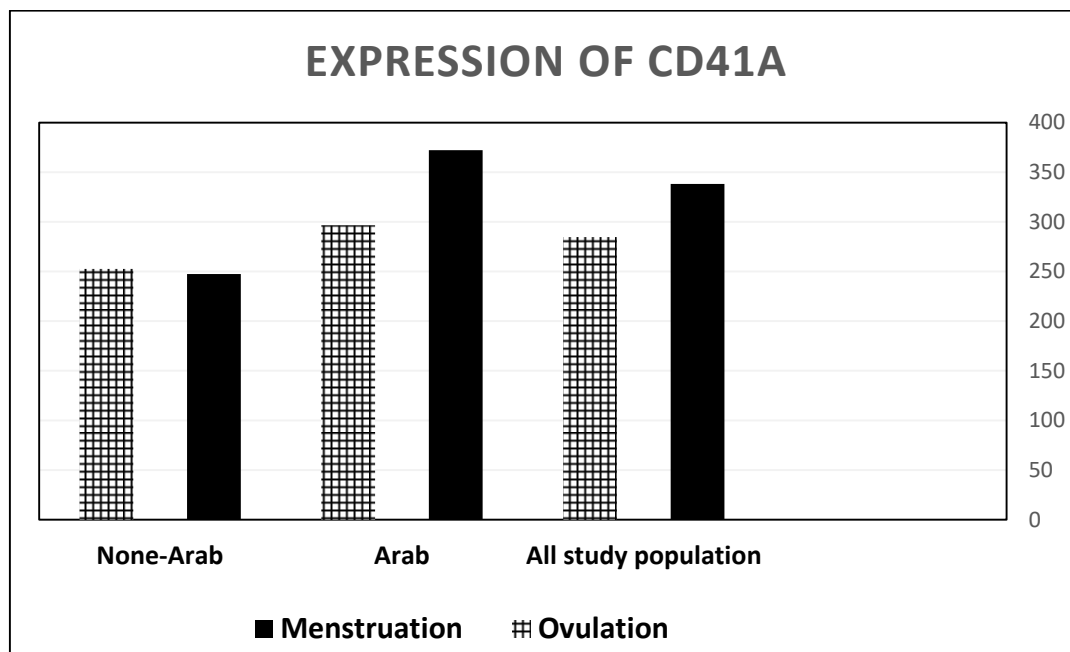


Figure 3. Expression of platelet CD41a during menstruation and ovulation; in all study population ( $p = 0.06$ ), Arab ( $p = 0.07$ ) and none-Arab females ( $p = 0.65$ ). The MFI values presented as mean+S.E.

### 3.4 CD61 Expression

This study data showed that there were no significant differences in platelet CD61 expression in resting state during menstruation and ovulatory phase. The results were represented as mean  $\pm$  SD of MFI during menstruation and ovulatory phase =  $338.19 \pm 196.93$  and  $284.62 \pm 71.10$ , respectively,  $p = 0.09$ ). Furthermore, absence of significant differences was also seen during menstruation and ovulatory phase in Arab (mean  $\pm$  SD =  $372.21 \pm 215.72$  and  $296.64 \pm 72.57$ , respectively,  $p = 0.07$ ) and none-Arab females (mean  $\pm$  SD =  $247.49 \pm 90.05$  and  $252.58 \pm 62.31$  respectively,  $p = 0.88$ ) (Figure 4).

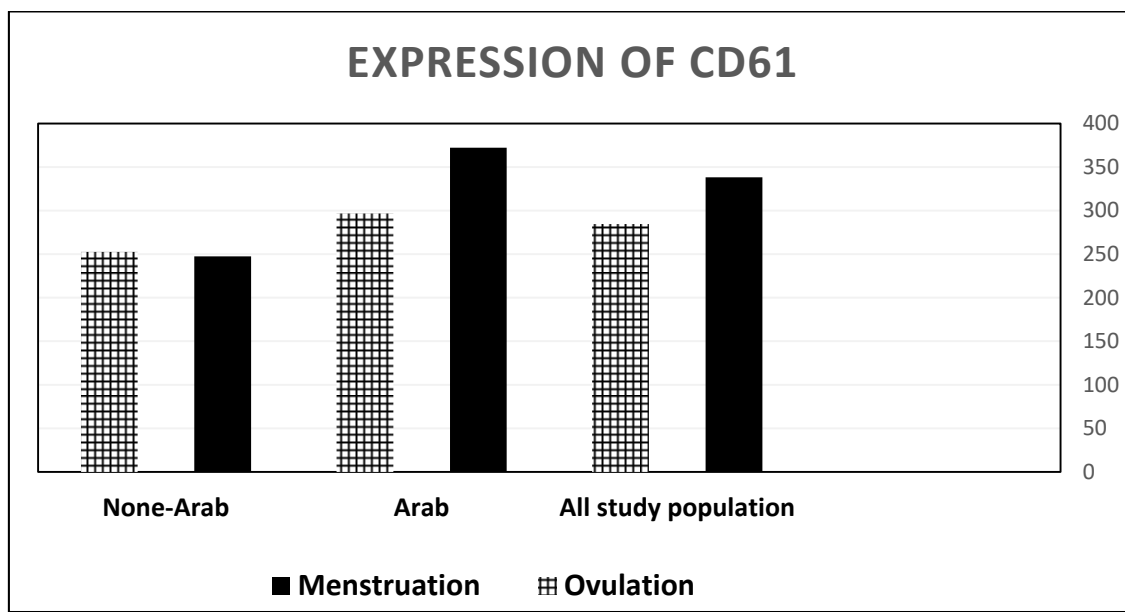


Figure 4. Expression of platelet CD61 during menstruation and ovulatory phase; in all study population ( $p = 0.09$ ), Arab ( $p = 0.07$ ) and none-Arab females ( $p = 0.88$ ). The MFI values presented as mean+S.E.

## 4. Discussion

In this study we have addressed whether the expression of glycoprotein receptors of resting platelets was affected by estrogen levels during different phases of the menstrual cycle. Our data showed that no variation occurred in CD42b, CD41a, and CD61 expression among all study population. However, increase the surface expression level of CD49b during ovulatory phase was noted in none-Arab females. These findings suggest a role of estrogen in modulation of CD49b. Generally, Normal menstruation starts with markedly decrease in progesterone and estrogen hormones where the cessation of menstrual bleeding is achieved by endometrial hemostasis through platelet aggregation, deposition of fibrin and thrombus formation (Davies & Kadir, 2012). Estrogen was described to be strongly affecting platelet activity by activation of GPIIb/IIIa and platelet aggregation when induced by thrombin (Moro et al., 2005). The level of P-selectin reached the peak on day 14 of menstruation where the estrogen level is high during menstrual cycle phases. Platelet GPIIb/IIIa activity was reported to be increased in premenopausal women compared to men, which attributed to variation in estrogen and/or progestins levels (Faraday et al., 1997). Roshan, et al., 2005 stated that the expression of CD62P was increased in postmenopausal women, while the activation status of platelet was decreased in postmenopausal women assessed by a decrease level of P-selectin and GPIIb/IIIa expression (Aldrighi et al., 2005). Modulation of platelet function was determined by a decrease secretion of P-selectin without alteration of GPIIb/IIIa expression after estrogen replacement therapy (ERT) in postmenopausal women (Nakano et al., 2002). It was reported that ERT regulate GPVI expression but did not affect the level of GPIb or GPIIb/IIIa (Leng et al., 2005). Moreover, Williams, et al., 2005 stated that long-term ERT did not cause alteration in platelet activation that assessed by determination of the activation and aggregation markers P-selectin. Platelets were described to have a critical role in thrombosis in postmenopausal women (Aldrighi et al., 2005). The estrogen replacement therapy (ERT) modulates platelet function by decreasing  $Ca^{2+}$  influx and increasing production of *c*-AMP and reducing P-selectin level (Leng et al., 2005). The same report demonstrated that there was no significant change in expression level of GPIIb/IIIa after short term ERT. The level of GPIIb/IIIa in activated platelets by thrombin showed no differences between pregnant

and non-pregnant women (Williams et al., 2005). The increased expression of resting platelet CD63 and activated platelet CD61, CD62P and CD63 in preeclampsia and normotensive pregnant women when compared to non-pregnant women (Sheu et al., 2002). The effect of ERT on platelet function was found to be dependent on the agonist hormones formulation and mode of delivery (Williams et al., 2005). Furthermore, the platelet physiological activity was affected by many factors including gender, race, diet and test system used (Holthe et al., 2004). The presence of significant difference in GP49b in the current study may have several explanations such as; A previous literature has not reliably found differences in platelet receptor expression between pregnant and nonpregnant or in response to short term estrogen and progesterone, But some studies confirmed that estrogen secreted by the growing ovarian follicles may causes prolonged vasoconstriction enabling the formation of a clot over the denuded endometrial vessels (Edman C. D., 1983; Reed & Carr, 2018) besides the variation in the expression levels of GPIa/IIb between healthy individuals (Miller et al., 2014) and the density of GPIa/IIb vary 3- to 4-fold and the differences in the activity and antigenicity of platelet GPIa/IIb may result from polymorphism(s) in  $\alpha 2\beta 1$  gene as described in previous studies (Curtis & McFarland et al., 2014; Kunicki et al., 1993; Best et al., 2003). Therefore, integrin gene polymorphism, numbers of subjects and the short time scales relative to platelet turnover should be taken into consideration for suggested future studies to determine of the exact effects of this expression of platelet glycoprotein receptor for protection against hemorrhage, minimizing the risk of thromboembolism and recurrent spontaneous abortion in pregnant women.

## 5. Conclusion

In conclusion, The CD49b glycoprotein receptor was the commonly expression on the surface of platelet during menstruation and ovulatory phase of menstrual cycle in none-Arab compared to Arab reproductive age women in turn extended platelet activity in conjunction with interaction to the vascular wall consequences in elevated thrombus formation. The estrogen hormone determination and the appropriate time for sample collection for diagnosis of bleeding disorders due to platelet dysfunction might help in the designing a safe strategy of HRT. The conclusions derived from this study are limited. The need of greater understanding of the complex physiology and pharmacogenetic effects of sex hormones and their receptors on hemostasis is important for prevention of bleeding and thrombosis, for the management of abnormal uterine bleeding and to determine the benefit and which patient at risk of using hormone therapy. This can be achieved by conducting a larger controlled interventional study to investigate the effect of other sex hormones (progesterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) on platelet function and platelet glycoprotein receptors expression gene polymorphisms to cyclic versus non-cyclic shifts in expression for future large subject studies.

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## Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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