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(RESEARCH ARTICLE)

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Comparison of platelet count by automated hematology analyzer and peripheral blood smear in thrombocytopenic patients

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Abstract

Platelet count estimation is one of the common as well as important laboratory investigations to diagnose many diseases like dengue, malaria etc. Different methods for platelet estimations are automated haematology analyzer, peripheral blood smear examination method etc. Most common causes of inaccurate platelet count by automated analyser are the presence of giant platelets, platelet clumps etc. leading to erroneous result like false low platelet count etc. We aimed to compare platelet count estimation by automated haematology analyzer and the peripheral blood smear examination in thrombocytopenic patients. A comparative cross-sectional study was conducted on 100 blood samples of patients which came thrombocytopenic on automated haematology analyzer. Each case was also analyzed by peripheral blood smear examination and compared with its corresponding automated haematology platelet count value. Our study included 63% males and 37% females, with the mean age of 38.8 years. Most of the patients belonged to the age group of 30-40 years (34%). The mean platelet count on automated analyzers was 85.46 ± 38.81 x 10^3/uL whereas on peripheral smear was 92.13 ± 38.30×10^3/uL with a significant difference between the two groups (p-value <0.0001).Pseudo-thrombocytopenia was observed in 10% of patients, with giant platelets observed in 29% of the cases on blood smear. In view of false low platelet count by automated analyser, we concluded that manual examination by peripheral blood smear examination should always be considered whose platelet count is low by automation.

Keywords: Thrombocytopenia; Pseudo-thrombocytopenia; Automated Hematology analyzer; Peripheral blood smear; Giant platelets.

1. Introduction

Platelets (thrombocytes) play an important role in homeostasis and thrombosis in the body. They are one of the formed blood elements measuring 2-3µm. [1] They are anucleated cells synthesized by cytoplasmic fragmentation of one of the hematopoietic stem cells megakaryocytes, with their cytoplasm filled with granules. [2] Typically, a platelet has a lifespan of 7-12 days after which it will be destroyed by macrophages in the spleen. [3] Platelet count normally ranges from 150 to 450×10^3 /ul. [1] Platelet estimation is one of the critical parameters in diagnosis, treatment and the patient care. Thrombocytopenia i.e. low platelet count can be seen in many disorders like dengue fever, malaria, malignancy etc.. [4] For the management of thrombocytopenia in clinical care, a timely and exact platelet count is essential. [5]

In a hematology laboratory, various methods are available for counting platelets i.e. by an automated or semi-automated hematology analyzer, manual method by examining peripheral blood smears (PBS) under microscope or through a Neubauer chamber. [6] Although hematology analyzers normally estimate an accurate platelet count, their accuracy has been brought into question while enumerating low platelet counts, platelet abnormalities, or platelet-like fragment interference. [7] Mostly platelet count is estimated by an automated analyzer but has its own drawbacks, especially while analyzing decreased platelet counts. The accuracy of platelet count by automated cell counter is compromised

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while processing blood samples with giant platelet (Image 1), platelet clumps, platelet satellitism, small RBCs or presence of RBC or WBC fragments etc. In such cases, platelet count verification by manual methods is of utmost importance for critical care as well as thrombocytopenic patients evaluation which can lead to life-threatening bleeds. [8]

Peripheral blood smear examination is highly useful in the diagnosis of unexplained thrombocytopenia i.e. false low platelet count (pseudo-thrombocytopenia) and also in monitoring the therapeutic responses. [9] Our aim is to study and compare platelet count estimation performed by the automated cell counter method and the PBS examination method in thrombocytopenic patients.

2. Method and materials

The present study was carried out in the Department of Pathology at a diagnostic centre, Akshaya Health Centre in urban Bangalore, over the period of 6 months with 100 patients (October 2023 – March, 2024). Inclusion criteria were all the samples of the patients having thrombocytopenia with platelet count less than 150,000/uL on automated blood cell counter. The inadequate samples, haemolysed samples, clotted samples and sample showing platelet clumps on blood smear were excluded from the study. Venous blood samples were collected for all the patients in ethylenediaminetetraacetic acid (EDTA) vacutainers tube and were stored at room temperature until analyzed within two hours.

Each blood sample was mixed properly for 10 minutes with automated mixer. The platelet count estimation done by processing blood samples in an automated hematology analyzer UNITRON BIO-MEDICALS (UBM) Fx-19T automated cell counter. The hematology analyzer calibration, quality control as well as the maintenance were done as recommended by the manufacturer. The blood samples with low platelet count on hemato-analyzer were used to prepare air dried blood smear and was stained manually with Leishman's stain as per standard procedure. The PBS was then examined under light microscopy with x100 oil immersion lens. In a Leishman's stained peripheral blood preparation, platelet can be identified as small purple coloured bodies with irregular borders (Figure 1). The platelets were counted in ideal zone of peripheral smear where RBC's border were just touching each other in monolayer with fairly even distribution of platelets and WBC's. Platelets were counted in 10 ideal zones. The average number of platelets was calculated and was multiplied by fifteen thousand. In an ideal zone of blood peripheral film, each platelet on an average 100x oil immersion field represents 15,000 platelets / µl, estimating final platelet count. [10]

Qualitative variables were described as frequency, and quantitative variables were measured as mean and standard deviation and keeping the 95% confidence interval and p-value of <0.05. Paired T-test was applied to compare the mean platelet count obtained by both methods.

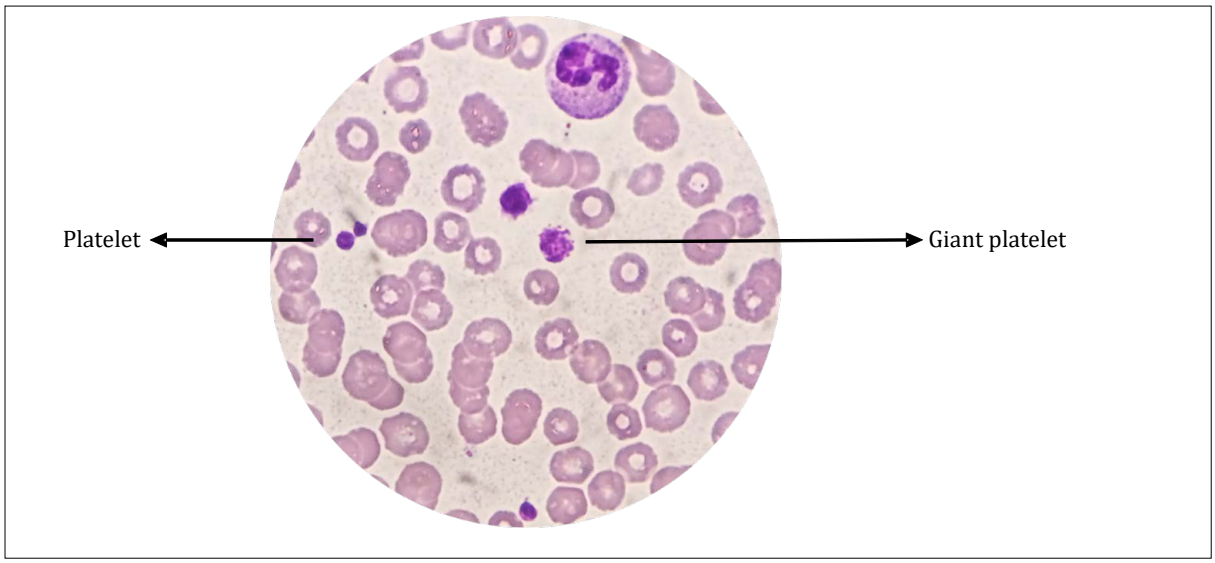


Figure 1 Peripheral blood smear (PBS) under 100x oil immersion showing platelets including giant platelets

3. Result and discussion

We studied 100 patients with thrombocytopenia i.e. < 150 x 10^3/uL platelet count on automated cell counter. We observed 63% of the patients were male and rest 37% were female with male: female of 1.7:1 (Figure 2). Most of the patients belonged to the age group of 30-40 years i.e. 34% followed by the age group of 20-30 years i.e. 23% (Figure 3).The mean age was found to be 38.8 years. The mean platelet count on automated analyzers was 85.46 ± 38.81 x 10^3/uL whereas the mean platelet count verified on peripheral smear was 92.13 ± 38.30×10^3/uL with a significant difference between the two groups (p-value <0.0001) (Table 1, Figure 4).

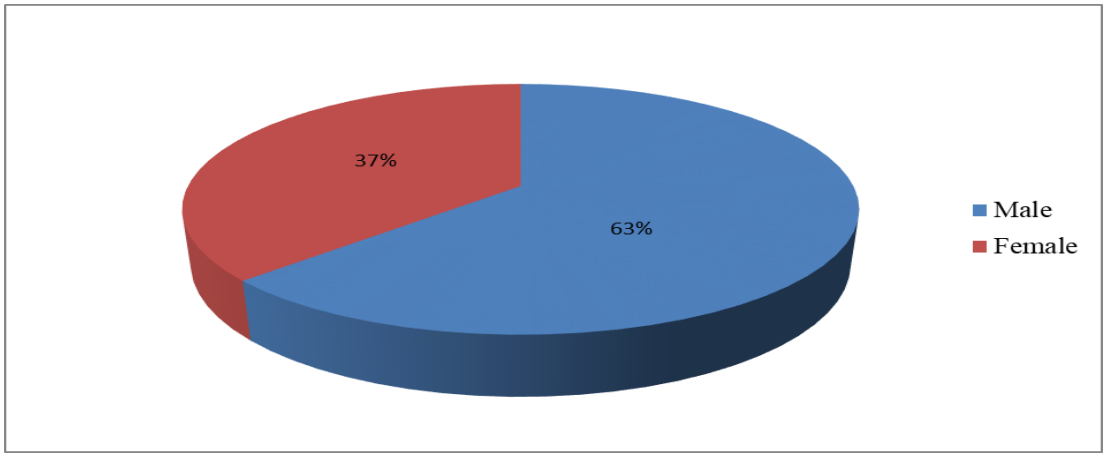


Figure 2 Gender wise distribution of the patients

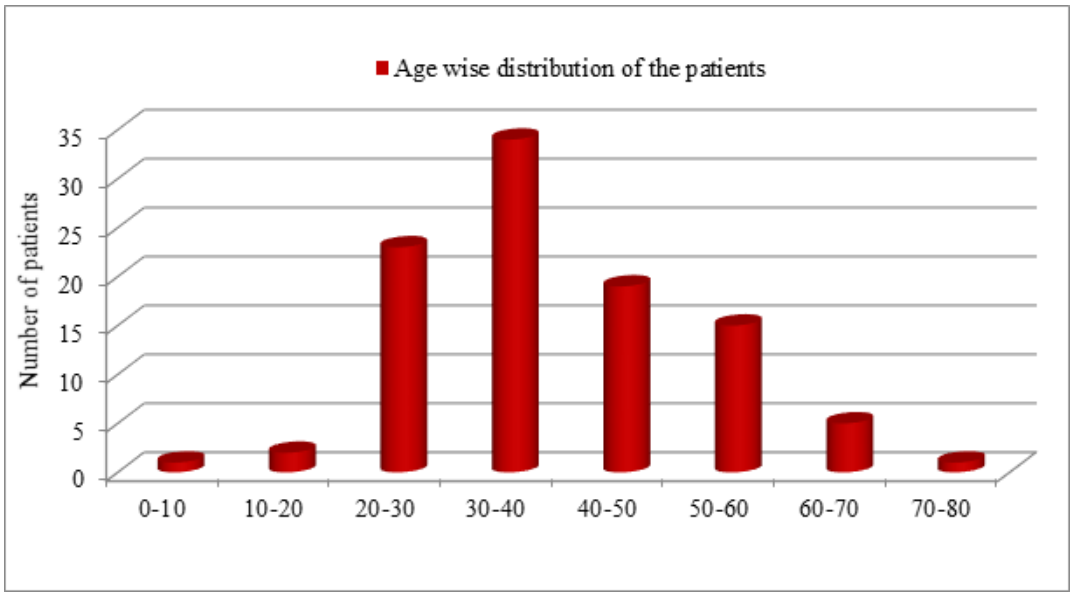


Figure 3 Age wise distribution of the patients

Table 1 Mean and standard deviation values of platelet estimation by manual peripheral blood smear examination and automated cell counter

Variables	Number Of Samples(n)	Mean (x1000/ µl)	Standard Deviation	t value(Paired t-test)	p value
Automated Method	100	85.46	38.81	13.055	<0.0001
Manual Method	100	92.13	38.30		

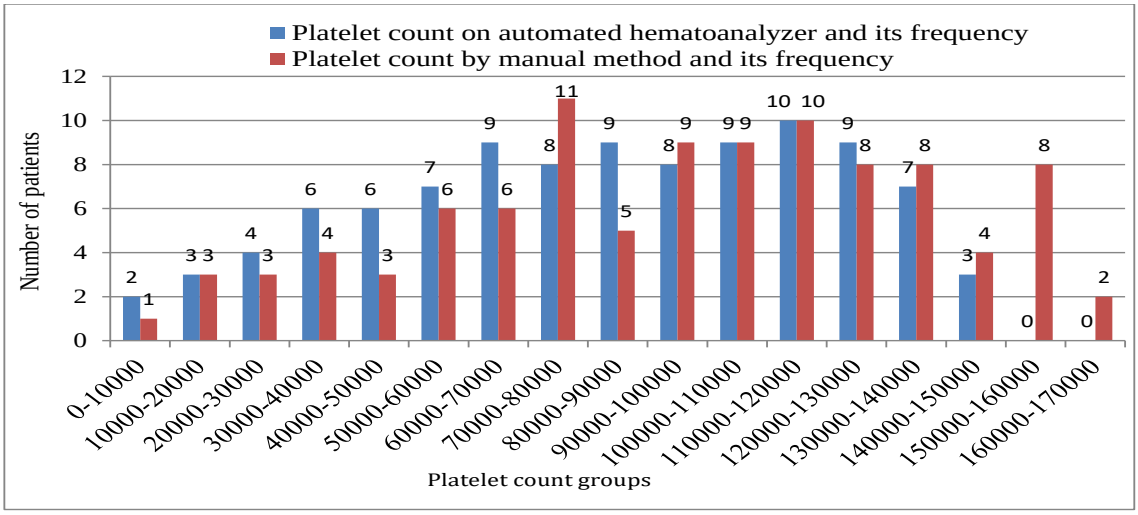


Figure 4 Comparison of platelet counts on automated hematology analyzer and manual method.

On manual examination, we observed that 10% of the patients which were previously diagnosed as thrombocytopenic on automation were found to be adequate in manual method i.e. pseudo-thrombocytopenia cases (Figure 5). We also evidenced that when examined on peripheral smear the actual platelet count was actually higher than automated count for most of the thrombocytopenic cases.

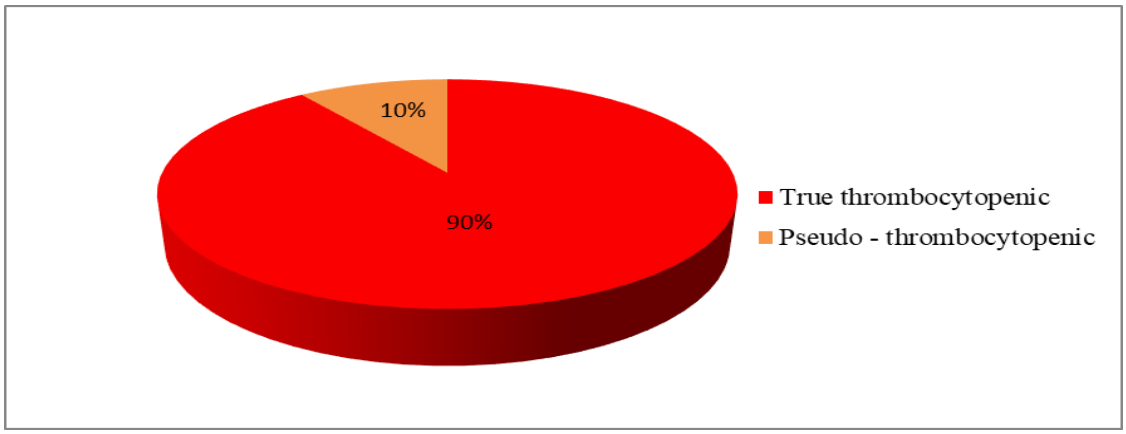


Figure 5 True and pseudo-thrombocytopenic patients by manual method.

We observed 29% of the total cases were showing giant platelets on peripheral smear examination (Figure 6).

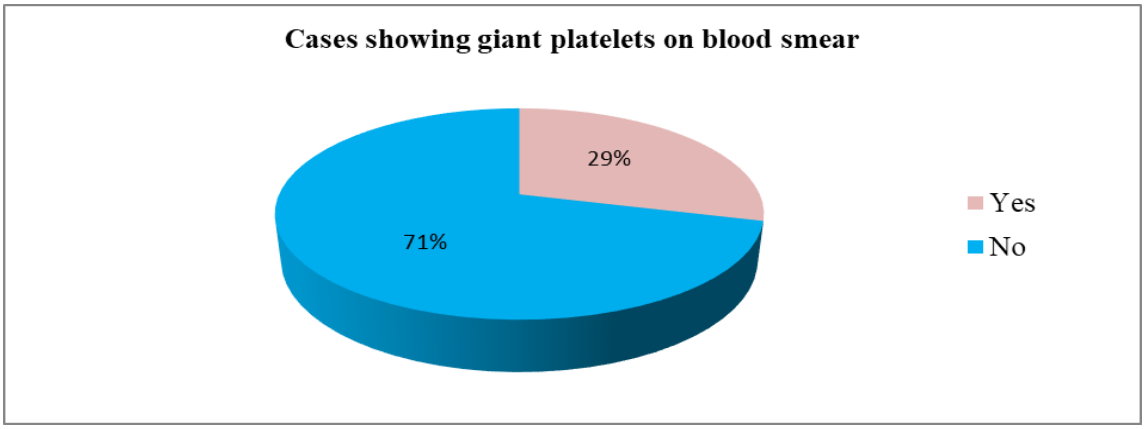
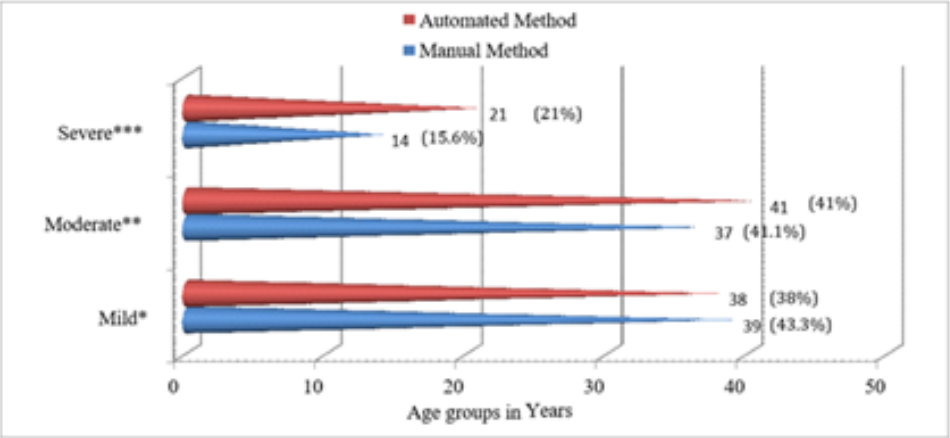


Figure 6 Percentage of the cases showing giant platelets by manual method.

We have divided the thrombocytopenic cases according to severity i.e. mild, moderate and severe thrombocytopenia. On automation, out of 100 thrombocytopenic cases 38 (38%) mild, 41(41%) moderate and 21 (21%) severe thrombocytopenic cases were there whereas by manual method only 90 cases out of 100 were thrombocytopenic with 39 (43.3%) mild, 37 (41.1%) moderate and 14 (15.6%) severe thrombocytopenic cases (Figure 7).



*Platelet count = 0<50 x1000/ µl, **Platelet count = 51-100 x1000/ µl, *** Platelet count = > 100 x1000/ µl

Figure 7 Comparison of grades of thrombocytopenia in automated and manual method.

Over the last many years, significant improvements have been made in automated haematology analyzers used for both analytical purposes as well as the whole blood cells description. Because of it, manual procedures have been gradually losing their importance in haematology. [6] Although hematology analyzers often produce precise platelet counts especially in healthy individuals, their precision is doubtful while estimating low platelet counts, in the context of giant platelet, platelet clumps, fragmented RBCs, small RBCs and platelet satellitism. [11] When an automated platelet count is low or flagged, platelet count estimation from the manual method by examining blood smears should be the gold standard, since no machine, no matter how costly or effective, can completely replace human judgment. [12]

We observed that out of 100 patients, 63% were male and rest 37% were female with male: female of 1.7:1. Tariq et al., (2023) [13] studied 60 adult patients including 31 females and 29 males with an approximate male-female ratio of 1:1. Castromayor et al., (2019) [12] also observed the 384 adult patients with thrombocytopenia, with an approximately 1:1 ratio based on sex. [12] Another Indian study from Assam, Gogoi et al., (2018) [14], observed 797 thrombocytopenic patients with 71% male and 29% female (male: female = 2.44:1).

Most of the patients in the present study belonged to the age group of 30-40 years (34%) followed by the age group of 20-30 years (23%) with mean age of 38.8 years. Gogoi et al., (2018) [14] showed similar observation with mostly (22.5%) belonged to the age group of 30–40 years followed by 20–30 years age group i.e. 19%. Tariq et al., (2023) [13] evidenced the mean age of 43.7 years. However, Castromayor et al., (2019) [12] stated that thrombocytopenia was most common between 6th to 8th decades of life affecting 36% of patients.

The mean platelet count on automated analyzers was 85.46 ± 38.81 x 10^3/uL whereas on peripheral smear was 92.13 ± 38.30×10^3/uL with a significant difference between the two groups (p-value <0.0001). It's mostly because of presence of giants platelets in the samples. Tariq et al., (2023) [13] observed the mean platelet count on automated analyzers was 58 ± 28×109 / L whereas the platelet count verified on peripheral smear was 117±13×109 /L with a significant difference (p-value of <0.001).However, they also included the samples in their study showing platelet clumps apart from giant platelets. Castromayor et al., (2019) [12] evidenced that the mean of the automated platelet levels was approximately 76 ± 45 x 109 /L while the manual platelet count results was 170 ± 99 x10 9 /L with a significant difference with p value < 0.05.

We noted 10% pseudo-thrombocytopenia cases. Gogoi et al., (2018) [14] also documented similar 9.8% pseudo-thrombocytopenic cases in their study. However, Tariq et al., (2023) [13] showed 42% pseudo-thrombocytopenic patients owning to the inclusion of the cases with platelet clumps apart from giant platelets.

29% of the cases showed giant platelets in the present study whereas Tariq et al., (2023) [13] Gogoi et al., (2018) [14] reported giant platelets in 39% and 11.5% of the cases respectively. However, Tariq et al., (2023) [13] also reported platelet clumps in their study.

Gogoi et al., (2018) [14] observed 797 thrombocytopenic cases (55.8% mild, 32.2% moderate and 12% severe thrombocytopenic) cases on automation and only 423 cases (56.6% mild, 30% moderate and 13.4% severe thrombocytopenia) on peripheral smear in comparison to 38%, 41% and 21% on automation and 43.3%, 41.1% and 15.6% on peripheral smear in the present study respectively. It showed the shift in grades of thrombocytopenia cases on peripheral smear examination with detecting the cases with adequate platelet count which were previously thrombocytopenic on automation.

Limitation of the present study was the less sample size and exclusion of the cases showing platelet clumps on peripheral smear as it's also one of the main contributors to pseudo-thrombocytopenic cases. [8]

4. Conclusion

Thrombocytopenia affects both male and female sex almost equally mostly middle aged people. In thrombocytopenia, it is crucial to confirm automated hematoanalyzer platelet count by examining peripheral smear to confirm the platelet count, especially in samples with abnormal platelets morphology like giant platelets before treatment. It may prevent patients from unnecessary further investigations and treatment. Thus, the peripheral smear examination remains the gold standard method for accurate platelet count estimation.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

No conflict of interest to declare.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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