IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
September 2018 Vol.:10, Issue:3

Estimation of Fall in Haemoglobin and Its Application in Validating the Current Criteria of Assessing It's Clinical Significance in Bioequivalence Studies

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Suhas S. Khandave[1*], Vaishnavi S. Sawant[1], Trupti V. Deshmukh[1], Urmila Khandave[1], Shivali S. Bendre[1]

[1]Accutest Research Laboratories (I) Pvt Ltd, A-77, Khairne MIDC, TTC Industrial Area, Khairne, Navi Mumbai, 400 709, Maharashtra, India.

Submission: 22 August 2018
Accepted: 30 August 2018
Published: 30 September 2018

Keywords: Bioequivalence, Hemoglobin, Blood loss, Baseline

ABSTRACT

The healthcare cost has been reduced significantly due to introduction of generics equivalents of brand name drugs. Determination of bioequivalence constitutes the most critical step during the development of generic drug products. Safety assessment of the subjects is equally important while assessing the bioequivalence of the test product with comparator product. Assessment of hemoglobin level during post study evaluation has remained a matter of subjective difference and inconsistency while concluding it's clinical significance in bioequivalence studies. The current approach of evaluating clinical significance of hemoglobin change from baseline is based on the information derived from acute blood loss conditions. Since the blood loss in bioequivalence studies is sub-acute in nature, the current criteria may not be appropriate to assess the clinical significance of hemoglobin fall. In the present study, the trend of hemoglobin fall from baseline was studied in 300 bioequivalence studies in male as well as female subjects. The studies were grouped in three different categories based on the total blood loss. The fall in hemoglobin was proportionate to the blood loss and marginally more in female subjects than male subjects. However, the highest fall from baseline observed in our study was less than 0.5 gm/dL which was too low from the conventionally followed limit of 1 gm/dL. The current criteria to assess the clinical significance of fall in hemoglobin is vulnerable to miss identification of drug's potential to induce fall in hemoglobin and hence needs to be optimized.

INTRODUCTION:

New drug delivery as well as availability of generic drugs has resulted in increase in life expectancy of human beings during the last few decades across the world. Considerable attention has been given to the reduction in overall cost of healthcare which is rising due to increase in the cost of medication. Introduction of generic equivalents of brand-name drugs has remained a major strategy for lowering the cost of medication thereby reducing the total health care costs. [1] The total prescription cost has been reduced significantly without compromising quality due to this strategy. [2]

Multisource drug products are products marketed by more than one manufacturer that contain the same active pharmaceutical ingredient (API) or drug substance in the same dosage form, the same strength and are intended to be given by the same route of administration. The trend of manufacturing multisource drug products for both domestic and international markets by the generic pharmaceutical industry is increasing in recent days. [3, 4]

The generic products are considered interchangeable with the innovator products on the basis of their therapeutic equivalence. Various test methods deemed suitable by regulatory authorities are followed for demonstration of therapeutic equivalence. The therapeutic equivalence of the multisource pharmaceutical products is demonstrated by that both, pharmaceutically equivalence and bioequivalence. Determination of bioequivalence is thought to be the most difficult and important step during generic drug product development. [5]

In every in-vivo bioequivalence study, the safety assessment of the study participants or subjects is of wider importance. For the safety concern, the subjects undergo various clinical as well as laboratory assessment tests before as well as after the study participation. Every subject to get enrolled into the bioequivalence study needs to pass through all the required clinical and laboratory parameters. Clinical parameters generally involves breath alcohol test, demographic data, physical examination including vital sign measurement, ECG recording, chest X-ray etc. Laboratory parameters involve a number of hematological, biochemical, serological and other investigations. Among all laboratory parameters, hemoglobin assessment is the most vital and critical as the blood loss involved in any BE study directly affects the hemoglobin level. The fall in hemoglobin level is unavoidable and inherent phenomenon associated with any bioequivalence study. Thus, if the subject has less than

optimum hemoglobin level during screening, his participation can further complicate the hematological parameters due to confirmed blood loss in the study irrespective of the drug or its pharmacodynamic properties.

Accurate assessment of anemia or fall in hemoglobin level although clinically insignificant due to blood loss in bioequivalence studies is a problem for clinical investigators. The post study drop in hemoglobin is not assessed uniformly by the clinical investigators to decide its clinical significance (occurrence of anemia) and treat it with proper hematinics. Differentiation between "blood loss induced anemia" and "drug induced anemia" has remained a big challenge for investigators across the globe. There exists inconsistency in assessing the clinical significance of hemoglobin fall in BE studies across the globe. Few investigators look for clinical symptoms of anemia while others look for a percent change in hemoglobin level from the baseline (screening value) to label the hemoglobin fall as significant event. The most commonly followed approach in the field of clinical research industry across the globe includes the fall in hemoglobin arbitrarily by 1 gm/dL from the baseline when the total blood loss in the study is about or less than 500 ml. [6] The biggest limitation of this approach is that not all BE studies include blood loss of 500 ml. For studies having a total blood loss below 350 ml, such approach can underestimate the effect of drugs in producing fall in hemoglobin. To avoid this underestimation, fall in hemoglobin level should directly be correlated with the total blood loss while determining the significance rather than adhering to a general rule. In bioequivalence studies, the blood loss happens gradually in bits and pieces over a period of 1-2 days in parallel design, 2 weeks in crossover designs and 4-6 weeks in case of replicate studies. This nature of blood loss complicates the picture further. Thus, fall in hemoglobin with blood loss alone does not remain adequate but it has to be correlated with duration of blood loss as well and accordingly, the criteria for determining the clinical significance for any fall in hemoglobin should be decided. So far, no study or report to assess the clinical significance of fall in hemoglobin in bioequivalence studies with due emphasis on total blood loss and/or duration of blood loss is made available. The present study (meta-analysis) is aimed to identify the extent of drop in hemoglobin level proportionate to the actual blood loss observed in bioequivalence studies. Moreover, this study is expected to redefine the criteria for evaluating clinical significance of hemoglobin fall from baseline in bioequivalence studies.

MATERIALS AND METHODS:

This research involved the meta-analysis of laboratory reports for hemoglobin levels in recently conducted BA/BE studies at Accutest Research Laboratories Ltd Navi Mumbai, Maharashtra. All the studies included in this meta-analysis were similar in general outline. They were conducted with two-period crossover approach and had a washout period ranging from 7 to 10 days. Thus, the influence of study duration on fall in hemoglobin levels was eliminated. The number of subjects participating in each study varied from 24 to 72. All the studies were conducted after getting the approval from the Ethics Committees in accordance with the ICG-GCP guidelines.[7] The study design, sample size (number of subjects), PK sampling points, matrix, analytics etc were based on the guidelines given by the Health Authority of the United States, USFDA and European Union, EMEA. [8, 9]

All the studies conducted were having following major inclusion and exclusion criteria to select the most appropriate subjects for the study. The few of exclusion and inclusion criteria were:

Inclusion Criteria:

1. Male human subjects, age in the range of 18 – 45 years both inclusive

2. Body Mass Index between 18.5-30 Kg / m^2 ,extremes included

3. Subjects with clinically acceptable findings as determined by haemogram, biochemistry, urinalysis, 12 lead ECG and chest X-ray

Exclusion criteria:

1. Requiring medication for any ailment having enzyme-modifying activity in the previous 28 days, prior to dosing day

2. Subjects who have taken prescription medications or over-the-counter products (including vitamins, minerals and herbal medicines) within 14 days prior to administration of study drugs

3. Any medical or surgical conditions, which might significantly interfere with the functioning of gastrointestinal tract, blood–forming organs etc

4. History of cardiovascular, renal, hepatic, ophthalmic, pulmonary, neurological, metabolic, haematological, gastrointestinal, endocrine, immunological or psychiatric diseases

A total of 30 studies were selected for the present meta-analysis. On the basis of total blood loss, these studies were classified into 3 groups. Each group had 10 studies.

Group A – The total blood loss was in the range of 166 ml -265 ml.

Group B - The total blood loss was in the range of 266 ml - 365 ml.

Group C - The total blood loss was in the range of 366 ml -465 ml.

The blood loss in all three groups was not acute in nature but happened over a period of approximately 2 weeks. Around 15-30 PK samples, each consisting of 4-7 ml were withdrawn over a period of 1-2 days in each study period. Approximately 50 ml blood was withdrawn for the safety analysis. This included 20-25 ml blood loss for screening examination (baseline evaluation) and similar blood loss during post study evaluation. The baseline and post study hemoglobin measurement was performed by same hematology analyzer with pre-defined standard procedure. Also, no subject had intake of drug or drug products except for the study drug during the period of study execution. The demographic features of all subjects (gender, age, height, weight, BMI) for all the studies in each of the three groups was recorded and tabulated. The pre study (baseline) and post study hemoglobin values of all the subjects included in the study for each mentioned group was recorded and tabulated. On the basis of the pre study and post study values, the percent fall in the hemoglobin was calculated and tabulated.

RESULTS AND DISCUSSION:

A total of 300 studies comprising 12100 subjects were included in this analysis. A total of 1190 subjects were dropped out or withdrawn due to adverse events during the clinical phase of the study and hence 10910 subjects' data (3830, 4030 and 4240 for group A, B and C respectively) was considered for the post study hemoglobin assessment.

All the subjects were omnivorous and Asian in origin. They were free from any ongoing diseases as evident from their clinical history, clinical examination and laboratory parameters.

The subjects had their BMI within the range of 18 – 25 for male as well as female subjects.

The gender wise distribution and demographic features of these subjects were comparable among the groups.

The demographic features of the subjects are presented in Table 1.

Table 1: Summary of demographic features for all the study participants

PARAMETER	GROUP A	GROUP B	GROUP C
No of studies	100	100	100
Male subjects	3120	3640	3470
Female subjects	710	390	770
Mean Height +/-SD (Male)	166 +/-9.4 cm	164 +/-7.8 cm	165 +/-5.4 cm
Mean Height +/-SD (Female)	154 +/-8.3 cm	153 +/-4.8 cm	152 +/-6.4 cm
Mean Weight + -SD (Male)	62 +/-6.2 kg	61 +/-7.3 kg	60 +/-6.6 kg
Mean Weight + -SD (Female)	49 +/-3.7 kg	50 +/-3.2 kg	49 +/-3.6 kg
Mean BMI + -SD (Male)	22.49 +/-1.9	22.67 +/-1.7	22.03 +/-2.1
Mean BMI + -SD (Female)	20.66 +/-1.4	21.35 +/-1.6	21.20 +/-1.5

The distribution of the studies was uniformly spread across the pre-defined range of blood loss for every group. Thus, the mean blood loss per study was close to the respective mid-point of the range of blood loss for all the groups. The group specific blood losses are mentioned in Table 2.

Table 2: Blood loss pattern for each study group

Group	Min blood loss	Max blood loss	Avg. blood loss
A (166-265 ml)	168 ml	262 ml	218 ml
B (266-365 ml)	272 ml	359 ml	312 ml
C (366-465 ml)	363 ml	464 ml	408 ml

The fall in hemoglobin was far less than expected in each group. Here, the expected or desired fall in hemoglobin is considered by 1 gm/dL from the baseline which is conventionally considered as clinically significant. In general, this fall constitutes approximately 8 % of the baseline value assuming baseline hemoglobin value of 12 gm/dL.

As expected, the fall in hemoglobin was directly proportional to the blood loss. The pattern of hemoglobin change from baseline is presented in Figure 1.

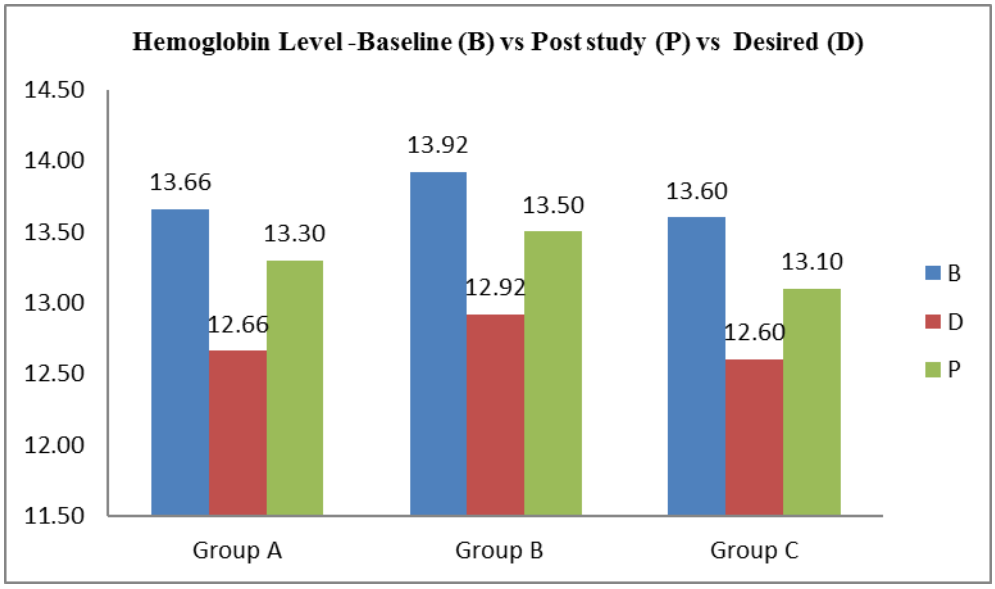

Figure 1: The change in hemoglobin levels from the baseline values (gm/dL)

The percentage fall in mean hemoglobin from the baseline values is presented in figure 2. It is observed that the fall in hemoglobin values was very low and within a range of 2.64 to 3.68 % from the baseline as against the expected fall of 8 %.

We further sub analyzed the percentage fall in hemoglobin from baseline gender wise. In general, the fall in hemoglobin from baseline was proportional to the blood loss in male as well as female subjects but it was relatively more in female subjects. However, the difference was not significant to pay further attention for identifying any gender specific trend. Table 3 and Figure 2 represent these facts and findings.

Table 3: Gender wise and overall fall in Hb (gm/dL) from baseline

	Male			Female			Both (male and female)		
	Pre-study	Post-study	% Fall	Pre-study	Post-study	% Fall	Pre-study	Post-study	% Fall
Group A	13.64	13.36	2.052	13.41	13.02	2.908	13.66	13.30	2.64
Group B	13.94	13.53	2.941	13.69	13.17	3.798	13.92	13.50	3.02
Group C	13.63	13.15	3.521	13.44	12.86	4.315	13.60	13.10	3.68

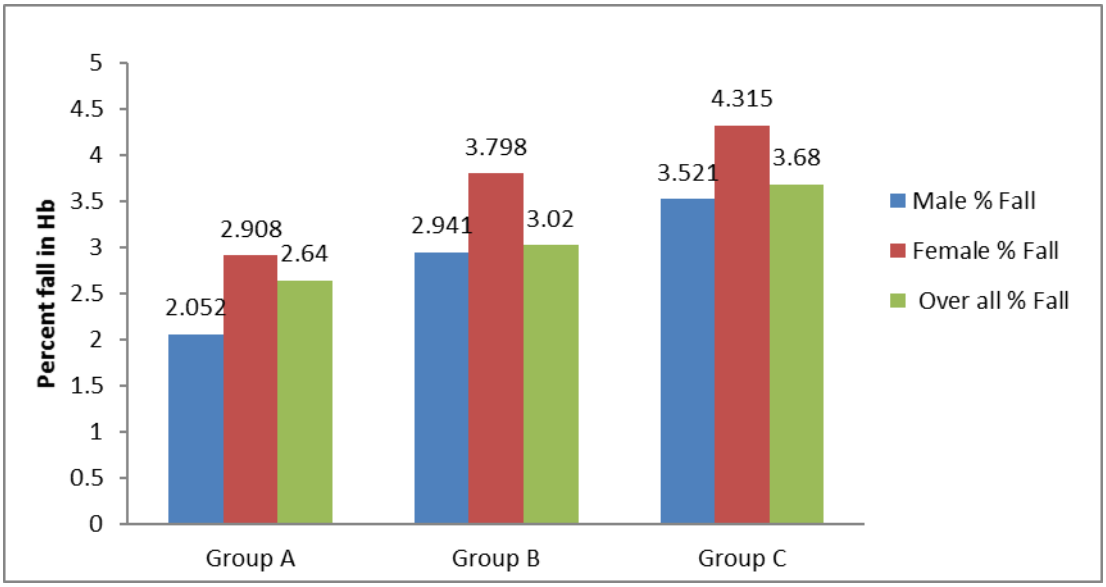

Figure 2: Group wise and gender wise percent fall in Hb (gm/dL) from baseline

The bioequivalence studies typically represent the scenario of sub-acute blood loss. In literature, referencing is done to different scenarios wherein fall in hemoglobin was observed post to acute blood loss. Whether or not the results obtained in the present study are in accordance with the previously concluded results by other researchers cannot be commented due to this difference. We have attempted to correlate and discuss our results in light of the available literature and knowledge with this limitation.

Immediately after acute blood loss, the laboratory parameters related to red blood cells such as hemoglobin, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration are all normal. This is because during acute blood loss, red cells but also the plasma is lost. So the blood remaining in the patient is totally normal. After a few hours, the blood starts to become more dilute as the fluid from tissues is pulled into vessels. The hemoglobin (and the RBC) at this point, both now appears decreased.[10] This fall continues till the time formation of new red blood cells and hemoglobin is observed or whole blood transfusion is done in severe cases. In acute blood loss, the fall in hemoglobin is documented differently by different researchers. Few researchers have noticed and documented the fall in hemoglobin as 0.7g/dL, 1 gm/dL, 1.5 g/dL and 1.8 gm/dL with an acute blood loss of 213 ml, 295 ml, 424 ml and 496 ml respectively in healthy adult male and female subjects. [11, 12] The fall in hemoglobin observed in the present study is far less than this fall. The difference naturally can be attributed to the type or nature of blood loss (acute vs sub-acute blood loss).

In our study, we observed the fall in blood hemoglobin levels only by approximately 0.5 gm/dL when the blood loss was approximately 400 ml. This fall is far low when compared with the expected fall as documented in the literature or followed by the medical personnel conventionally. Christopher et al has proposed a formula to calculate the change in hemoglobin on the basis of blood loss and plasma expanders but it is suitable for situations where the blood loss is acute in nature like surgery or accidental blood loss. [13] This formula provides a fall in hemoglobin higher than the one observed in the present study. Since the blood loss is not acute in nature in bioequivalence studies, the haemostatic mechanisms getting activated over a period of time help to overcome or compensate the blood loss induced fall in hemoglobin partially.

The fall in hemoglobin in bioequivalence studies is expected due to two reasons, blood loss and the effect of study drug. From the present study data, the fall in hemoglobin up to 0.5 gm% from baseline can be attributed only to the blood loss rather than the effect of study drugs. The current criteria of labeling the fall in hemoglobin as clinically insignificant when it is < 1 gm % from the baseline may result in missing the "drug induced hemoglobin lowering potential." If the hemoglobin drop is between 0.5 gm% and 1 gm% and observed in majority of the subjects, it should be treated as evidence or at least as indicator of "drug induced hemoglobin lowering potential" whether or not it is clinically insignificant.

CONCLUSION:

Thus, we would like to conclude that the present criteria of labeling the fall in hemoglobin as clinically significant when it is more than 1 gm/dL from the baseline needs to be optimized. Any fall up to 0.5 gm/dL from baseline can be attributed to blood loss involved in the bioequivalence studies. Any fall in hemoglobin ranging from 0.5 to 1 gm/dL can be drug induced adverse event if consistently observed in majority of the subjects. Fall in hemoglobin above 1 gm/dL from the baseline should be labeled as a drug induced adverse event rather than merely attributing it to the blood loss.

The results obtained in the present study can act a guiding tool for the clinical investigators to assess the clinical significance of fall in hemoglobin levels in bioequivalence studies. Additional studies to understand correlation of hemoglobin fall with the duration of blood loss can help in further redefining of the current criteria to determine it's clinical significance.

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