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HEMOLYSIS AND HIGH GLUCOSE CONCENTRATION HAVE AN EFFECT ON ROUTINE CHEMISTRY ANALYTES AND THYROID ASSAY.

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INTRODUCTION:

Hemolytic specimens are common in laboratory practise, accounting for approximately 3% of all samples referred to a clinical lab .It can have an impact on the results of analytes like AST, LDH, potassium, and sodium. High glucose levels have been shown to interfere with Jaffe creatinine assays in peritoneal dialysate, as well as creatinine, calcium, albumin, chloride, and FT4 levels in serum . Diabetes, a rapidly growing chronic disease characterised by hyperglycemia, has a 9.8percent prevalence in our state. As a result, the likelihood of glucose acting as an interference factor is relatively high..

MATERIALS AND METHODS:

Baseline pools with analytes in the normal reference range were created from samples received at the medical college's 24 hour central biochemistry laboratory,Kurnool.Pools were divided into three and five aliquots for hemolysis and glucose interference, respectively, and spiked with varying concentrations of free haemoglobin and glucose assay, respectively. The routine analytes and thyroid assay were performed on the samples are analysed.

RESULTS:

Hemolysis increased AST and potassium levels, whereas sodium levels decreased in all three groups.Totalprotein ,albumin levels were lower in group 3 when haemoglobin (468mg/dL) was added. Creatinine levels increased after groups were spiked with glucose greater than 294 mg/dL. Calcium levels increased in groups 3, 4, and 5 (glucose >294 mg/dL), and albumin levels were significantly affected in group 5 (598 mg/dL). Even at 468.5 mg/dL, hemolysis had no effect on glucose, TSH, ft4, creatinine, or urea, whereas glucose at 598 mg/dL had no effect on urea, sodium, potassium, TSH, free t4, or triglycerides.

CONCLUSION:

Routine determination of plasma or serum free haemoglobin concentrations is important in this study to avoid preanalytical visual inspection for hemolysis detection, improper sample rejection, and/or rerun due to hemolysis. New samples must be requested for the analytes that interfered with hemolysis.

Key words :Hemolysis ,high Glucose Concentration ,Thyroid Assays , Hemoglobin.

INTRODUCTION:

According to the ISO 15189 accreditation standard, medical laboratories are responsible for the quality and management of the pre-examination phase [1]. It is the laboratory person's responsibility to ensure that the correct sample is taken from the correct patient at the correct time, and that the correct test results are provided to the requesting physician in a timely manner. If the specimen's quality is harmed to the point where the expected effect exceeds the allowable error, resulting in clinically significant bias, the sample should be rejected for analysis. As responsible laboratory personnel, our rationale should be "no result is always better than a wrong result," and patient benefit should always take precedence [2]. Hemolytic specimens are common in laboratory practise, accounting for 3 percent of all routine samples referred to a clinical laboratory and accounting for 39 percent to 69 percent of all unsuitable specimens received in such laboratories [3]. Some analytes, such as AST, LDH, potassium, and sodium, can be affected by mild or almost undetectable hemolysis (-0.6 g/L of serum haemoglobin). There are no absolute indications of the degree of lysis responsible for such interference, nor are there definitive strategies for reporting and handling results on hemolyzed specimens [4]. High levels of glucose have been shown to interfere with creatinine assays, particularly the Jaffe method in peritoneal dialysate[5]. High serum concentrations also interfere with creatinine, calcium, albumin, chloride, and FT4 concentrations[6]. Diabetes is a rapidly growing chronic disease characterised by hyperglycemia in this day and age. Diabetes is prevalent in 9.8 percent of Tamil Nadu residents. As a result, the likelihood of glucose acting as an interference factor is relatively high. As a result, it is critical to investigate common interfering factors such as hemolysis,and high glucose levels on routine analytes to learn about their potential effects on thecorrect interpretation of results .A developing country like India lacks both knowledge and data about these interferences, as well as the ability to identify them .As a result, we set out to assess the effects of hemolysis and highGlucose interferes with routine biochemistry analytes and thyroid assays.

INFLUENCING AND INTERFERENCE FACTORS:

The effects on laboratory results of biological origin that most commonly occur in vivo but can also be derived from the sample in vitro during transport and storage are referred to as influencing factors. Changes in the amount of the analyte to be measured in a defined matrix are caused by biological influence factors. They affect the concentration of the measured (affected) analyte in an independent manner. These factors, such as circadian rhythms, are either present in healthy people or appear as side effects of disease and treatment. Influencing factors can be modifiable, such as diet, time of day, or season, or they can't, like genetic background, ethnicity, gender, and so on .[7]

Interferences are mechanisms and factors that cause lab test results for a specific analyte to be falsely increased or decreased.[8] Interference factors and their mechanisms vary depending on the analyte and analytical method used, and they have the potential to change the result of a sample constituent after the specimen has been collected.

They differ from the analyte being measured and obstruct the analytical procedure. As a result, their effect is method dependent and can be reduced or eliminated by using a more specific method to measure the analyte.

Interferents that could be present include the following:

1.Sample biological constituents (for example, acetoacetate, which interferes with creatinine by the Jaffe method).

2. The presence of exogenous molecules in the sample (drug interferences)

3. Exogenous molecules introduced into the sample during or after the sampling procedure (for example, anticoagulants, tube additives, intravenous infusions, and so on).[9]

In clinical laboratory measurements, interfering substances are a significant source of error. In some cases, such mistakes endanger the patient. As a result, clinical laboratories should consider the effects of potentially interfering substances in their risk analyses during the design stage [10]

So we aimed to study the influence of hemolysis and high glucose concentrations on routine chemistry analytes and thyroid assay.

AIM: The goal of this study is to see how hemolysis and high glucose concentrations affect routine chemistry analytes and thyroid assays.

OBJECTIVES:

1. To investigate the effect of hemolysis on routine chemistry analytes and thyroid assays at various concentrations of free haemoglobin.
2. To determine the impact of high glucose concentrations at different levels on routine chemistry analytes and thyroid assays.

MATERIALS AND METHODS: This study was approved by Institutional Ethics Committee,Kurnool Medical college Kurnool.

STUDY DESIGN: Descriptive study.

STUDY DURATION: 10 months (June 2021-March 2022)

INCLUSION CRITERIA: Samples of patients in the age group- 18 to 65 years.

Samples with analyte concentrations in normal reference range.

EXCLUSION CRITERIA: Visible hemolysis of serum -free hemoglobin< 50 mg/dL. Visible lipemia of serum sample – Triglycerides < 300 mg/dL.Visible Icteric sera –bilirubin concentration < 1.98 mg/dL.

SAMPLE COLLECTION: Serum samples with routine chemistry analytes in the normal reference range received at 24 hours central laboratory remaining after analysis were pooled for the study purpose.

HEMOGLOBIN INTERFERENCE:

PREPARATION OF HEMOGLOBIN STOCK SOLUTION[10]:

1.5 mL heparinized blood was collected and centrifuged for 10 minutes.

2. Discarded plasma and replaced with 10 mL isotonic saline.

3. Inverted tube slowly for ten times. Centrifuged for ten minutes and discarded saline wash.

4. Washed with saline twice again. Diluted the cells with an equal volume of distilled water. Inverted tube and mixed well for ten times.

5. Froze overnight.

6. Thawed cells and brought to room temperature.

7. Centrifuged 30 minutes and stroma was removed.

8.Saved supernatant (hemolysate).

9. Discarded red cell debris in pellet.

10. Analysed haemoglobin concentration in the hemolysate by cyanhemoglobin method using Drabkin's reagent.

Serum sample pools were divided into 4 aliquots- on Serum sample pools were divided into 4 aliquots- one baseline pool and three aliquots were spiked with different hemoglobin concentrations and analyte interference were studied, for the following analytes – glucose, urea, creatinine, AST, sodium, potassium, albumin, total protein, TSH and ft4.

1.Group I (0-50 mg/dL) non hemolysed – Baseline pool.

2.Group II (51-100 mg/dL) mildly hemolysed.

3.Group III (101-250 mg/dL) moderately hemolysed.

4.Group IV (251-500 mg/dL) severely hemolysed. The results were analysed to determine if mild, moderate and severe hemolysis affected the analyte concentrations. Number of replicates n= 10 All

analytes were measured with Beckman coulter AU480 chemistry analyser, Roche Electrolyte analyser ,immunoassay analyser. The effects of the hemolysis were evaluated according to the total allowable error recommendations of Clinical Laboratory Improvement Amendments (CLIA'88). CLIA'88 regulations have established fixed limits for assessing method and laboratory performance for Specific Regulated analytes. In practice, The Total Allowable Error for a given analytical method must be less than the respective CLIA fixed Limits for the analyte in question.[11]

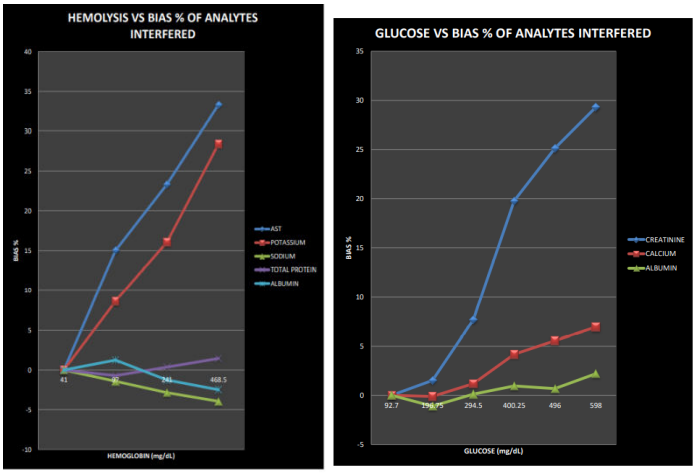
GLUCOSE INTERFERENCE Prepared a 20x stock solution of the glucose concentration to be spiked:

1. 2% stock – 100 mg/dL of glucose.
2. 4% stock – 200 mg/dL of glucose.
3. 6% stock – 300 mg/dL of glucose.
4. 8% stock – 400 mg/dL of glucose.
5. 10% stock – 500 mg/dL of glucose.

0.25 mL of 20x stock solution added to a 5-ml measuring cylinder and was made up to volume with baseline pool. Mixed well. Number of replicates n= 10 Serum concentrations of urea, creatinine, calcium, albumin, triglycerides, Sodium, potassium, free t4, TSH, were analysed in all groups respectively. Analysed the test (T) and control (C) samples in alternating order (e.g., C1T1C2T2C3T3....CnTn). All analytes were measured with Beckman coulter AU480 chemistry analyser, Roche Electrolyte analyser , immunoassay analyser. Results were recorded for data analysis. The effects of the high glucose concentrations were evaluated according to the total allowable error recommendations of Clinical Laboratory Improvement Amendments (CLIA'88). CLIA'88 regulations have established fixed limits for assessing method and laboratory performance for Specific Regulated analytes. In practice, the Total Allowable Error For a given analytical method must be less than the respective CLIA fixed Limits for the analyte in question.

STATISTICAL ANALYSIS

1. Statistical analyses were performed using Graph pad software.
2. Results were expressed as a median (min-max) as the number replicates (n=10) were relatively small for each group.
3. Interference effects were checked by Wilcoxon signed rank test for paired data when concentrations were compared with baseline.
4. The clinical significance was calculated as bias % with p value < 0.05. 5. Bias %= [(CX-C1) / C1)] × 100; where C1 represents the median of the baseline sample and CX represents the median of the experimented sample. The criterion for the presence of a bias specific for that analyte was defined according to the desirable bias recommended by CLIA'88 guidelines.



RESULTS :

The median hemoglobin concentration for groups -baseline, 1, 2, and 3 were measured as 41mg/dL, 97 mg/dL, 241 mg/dL, and 468.5 mg/dL respectively. 2. Aspartate aminotransferase, potassium, sodium levels were found to be affected significantly in all three groups with free hemoglobin concentration > 97 mg/dL. 3. The bias % for AST in the groups 1, 2, 3 was calculated as 15 %, 23.33 %, and 33.33 % with p value 0.002 in all three groups respectively. 4. The bias % for potassium in group 1, 2, 3 was calculated as 8.64 %, 16.04 %, and 28.39 % with p value 0.002 in all three groups respectively. 5. Sodium had significant negative interference in all three groups with bias % in group 1, 2, 3 were calculated as -1.43 %, -2.86 %, -3.94 % with p value 0.002 in all three groups respectively. 6. Total protein had significant positive interference in group 3 with free hemoglobin concentration > 468.5 mg/dL and the bias % was 1.45 % with p value 0.007. 7. Albumin has significant difference in group 3 with free hemoglobin concentration > 468.5 mg/dL, bias % -2.5 % and p value 0.003

GLUCOSE INTERFERENCE: 1. The median glucose concentrations for group- base line, 1, 2, 3, 4, and 5 were measured as 92.7 mg/dL, 196.75 mg/dL, 294.5 mg/dL, 400.25 mg/dL, 496 mg/dL and 598 mg/dL respectively. 2. Creatinine was found to be most affected from glucose values > 294.5 mg/dL onwards and the bias % in group 2,3,4,5 were calculated as 7.69 %, 19.48 %, 25.12 %, 29.23 % with p value 0.002 in all four groups respectively. 3. Calcium was affected with glucose concentration > 400.25 mg/dL and the bias % in group 3,4,5 were calculated as 4.18%, 5.56%, 6.94% with p value 0.002 in all three groups respectively. 4. Albumin had significant interference in group 5 with glucose concentration > 598 mg/dL, bias % 2.19 and p value 0.054

DISCUSSION:

We conducted this study to better understand the effects of hemolysis and elevated glucose levels on routine biochemistry analytes performed in our hospital lab. Hemolysis is an important preanalytical variable that frequently occurs after specimen collection and can alter the results of the analytical process [12] According to the College of American Pathologists (CAP), the most common reason for sample rejection is hemolysis[13], which causes inconvenience and delays in clinical decision making, with a significant impact on patient care [14]. Hemolysis is also more likely during sample storage and transportation. This should be considered when performing laboratory measurements. Its impact should not be overlooked because analytical results were influenced by haemoglobin as an interference factor. Values were often false, either under or overestimated or giving unexpected pathological findings and this was particularly important for measurements of analytes such as potassium, LDH, AST, acid phosphatase, and neurone-specific enolase[15]. In vitro hemolysis may interfere with laboratory testing due to: I leakage of haemoglobin and other intracellular components into the surrounding fluid, which causes false increases in levels of some analytes or dilution effects; or (ii) dilution effects. (ii) Hemoglobin in the sample can disrupt chemical reactions by changing the molar extinction coefficient of the substrate or reaction product being measured. iii)In addition to haemoglobin, RBCs contain a variety of structural proteins, enzymes, lipids, and carbohydrates, and many of these may interact or compete with the assay reagents [14] (iv) spectrophotometric interference caused by an increase in optical absorbance or a change in the blank value for measurements at 415, 540, and 570 nm, where haemoglobin absorbs strongly. [16]

In our study, AST, potassium, and sodium levels were significantly affected, with % bias exceeding the CLIA '88 regulations in all three groups spiked with varying concentrations of haemoglobin > 97 mg/dL.

This finding was consistent with the findings of Lippie et al, who found that adding blood Hemolysate generated a consistent and dose dependent trend toward overestimation of AST and

potassium. Thus, an interference mechanism has been previously classified, and it is due to large differences in intracellular and extracellular concentrations for these two analytes.

The activity of AST in erythrocytes is 40 times higher than in plasma. In patients with AST activities in the reference interval ,hemolysis with hemoglobin values of 150 mg/dL causes an elevated AST activity [12]. In our study the % bias increase from baseline values for AST was 15 %, 23.33 %, and 33.33 % for each group respectively.[17]

The potassium content of red blood cells is approximately 25 times that of plasma. Even if in vitro hemolysis is not visible, potassium concentrations rise, and potassium is released from erythrocytes into plasma or serum during whole blood storage, as described by Sonntag et al. As a result, appropriate measures for the rapid transport and separation of plasma or serum from cellular blood constituents must be established.

In our study, the percent bias increase from baseline values for potassium was 8.64 percent, 16.04 percent, and 28.39 percent for each group, respectively.

In our study, mean sodium values were significantly lower, and the percent bias increase from baseline values was 1.43 percent, -2.86 percent, and -3.94 percent for each group, when compared to baseline specimens containing no hemolysate. This was explained in terms of dilutional effects, which corresponds to a study conducted by G. Lippi et al.[13]

Total protein had significant positive interference with a percent bias of 1.45 from baseline exceeding CLIA '88 limits in group 3 with free haemoglobin concentrations greater than 468.5 mg/dL, which is consistent with the findings of Koseogluet al.[18] Visible hemolysis caused by increased endogenous absorbance at 540 nm interfered with protein estimation using the biuret method (Kaplan).

Albumin was found to have significant negative interference with percent bias -2.5 from baseline exceeding CLIA '88 limits in group 3 with free haemoglobin concentration > 468.5 mg/dL, severe hemolysis, and it followed the pattern observed in Giuseppe et al studies

In our study, the analytes that were free of significant haemoglobin interference included glucose, urea, creatinine, TSH, and free t4.

In groups 2,3,4,5 spiked with glucose concentrations > 294.5 mg/dL, creatinine levels were significantly elevated in a proportionate manner, with percent bias 7.69%, 19.48%, 25.12%, and 29.23% from baseline exceeding the CLIA '88 regulations, which is similar to the results obtained by Cuhadar et al [6]. This can be explained by slow-reacting substances such as protein, glucose, and ascorbic acid producing falsely high creatinine values by reducing alkaline picrate to picramate[19], which has an absorbance maximum of 485 nm, resulting in an overestimation of creatinine (Kaplan).

Calcium levels were significantly affected with percent bias exceeding the CLIA '88 regulations in groups 3, 4, 5 with glucose concentrations > 400.25 mg/dL, which contradicts the findings of Cuhadar et al., 2016.[6]

Albumin levels were significantly affected with percent bias exceeding the CLIA '88 regulations in group 5 spiked with glucose concentration > 598 mg/dL, which contradicted the findings of Cuhadar et al., 2016.[6]

TSH, free t4, sodium, potassium, urea, and triglycerides were among the analytes in our study that were not significantly influenced by high glucose levels. Based on our findings, we conclude that glucose and haemoglobin can act as potential interfering substances, potentially affecting patient treatment and management. Hemoglobin interferes with potassium, AST, sodium, protein, and albumin estimation. Glucose has a significant impact on the estimation of creatinine, calcium, and albumin.

Because hemolysis can compromise specimen integrity and the dependability of laboratory results, standardised reporting of the occurrence of hemolysis may help to improve the quality and efficiency of the preanalytical laboratory process.

CONCLUSION

Based on our findings, we conclude that glucose and haemoglobin can act as potential interfering substances, potentially influencing patient treatment and management. Hemoglobin interferes with the estimation of potassium, AST, sodium, protein, and albumin. Glucose causes significant interferences in the estimation of creatinine, calcium, and albumin.

Because hemolysis can compromise specimen integrity and the dependability of laboratory results, standardised reporting of the occurrence of hemolysis may aid in the improvement of the quality and efficiency of the preanalytical laboratory process.

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