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## COMPARATIVE STUDY OF GLUCOMETER AND LABORATORY GLUCOSE OXIDASE METHOD FOR THE ESTIMATION OF BLOOD GLUCOSE LEVELS IN NEONATES

Harish J[1], Srinivas H. A[2], Soumya A[3]

**ABSTRACT: INTRODUCTION:** Hypoglycemia is one of the most common metabolic problems encountered in neonates. Hypoglycemia in neonates can be symptomatic and asymptomatic. Hypoglycemia is known to be associated with brain dysfunction and neuromotor developmental retardation. The glucose oxidase method used in the laboratory for determining the blood glucose concentration is precise and specific for glucose. As it is usually performed in the main laboratory, the results are not available quickly enough for timely appropriate management. The glucometers are often used for blood glucose estimation in NICU. Many studies have shown that their results co-relate well with the laboratory measured glucose levels in the normoglycemic and hyperglycemic range but are not satisfactory in the lower range. **OBJECTIVES:** This is a prospective study done to determine the efficacy of glucometer in estimation of blood glucose levels in neonates in comparison with the laboratory values. **METHODS:** 250 neonates admitted in NICU, KIMS hospital, Bangalore with varied symptomatology were enrolled in this study. Blood glucose estimation was done by glucometer and laboratory method using the same venous sample at the time of admission. For the last 50 cases glucometer estimation of capillary blood was also done. Statistical analysis was done by using Pearson correlation. Hypoglycemia was defined as blood glucose level <45mg%. Laboratory value was taken as gold standard. **RESULTS:** The study enrolled 250 neonates of which 63.2% were males and 36.8% were females.103 cases[41.2%] were found to be hypoglycemic by lab values while 52 [20.8%] were found to be hypoglycemic by glucometer. The overall incidence of hypoglycemia in our study was 41.2%. 49.7% of the LBW babies were found to be hypoglycemic while only 12% of normal birth weight babies were hypoglycemic. 31.3% of term babies were hypoglycemic while 55.3% of preterms were hypoglycemic. Hypoglycemia was common in neonates with risk factors like prematurity, meconium aspiration, septicemia, birth asphyxia etc. Glucometer had a specificity of 93.88%, sensitivity of 41.75%, positive predictive value of 82.69%, negative predictive value of 69.70% and accuracy of 72.4%.The correlation between the glucometer and laboratory values is good when the gold standard value i.e. lab value is >45mg%[0.756] and when the value is <45mg%, there is just a moderate correlation[0.417]. Though the pick-up rate of hypoglycemia by venous blood glucometer [28%] was slightly better compared to the capillary blood glucometer value [22%], the overall pick-up rate of hypoglycemia by glucometer is very low compared to the laboratory method [56%]. **CONCLUSION:** The glucometer as a sole measuring device to screen neonatal hypoglycemia is not satisfactory and confirmation with the laboratory measurements of plasma glucose is still of up most importance.
**KEYWORDS:** Hypoglycemis, neonates, glucometer, glucose oxidase method.

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**INTRODUCTION:** Hypoglycemia is one of the most common metabolic problems encountered in the newborns. The term "hypoglycemia" refers to a reduction in the glucose concentration of the circulating blood. It is almost 100 years since hypoglycemia was first described in children and over 50 years since it was recognized in newborns and infants.[1]

Variable incidence has been reported by various authors in different weight and gestational age groups.[2] The overall incidence of hypoglycemia in neonates varies from 0.2 to 11.4%.[3,4] However in the presence of certain risk factors i.e. small for date, large for date, infants of diabetic mothers, prematurity etc., the probability of hypoglycemia increases many folds.[3]

Hypoglycemia in neonates can be symptomatic and asymptomatic.[5,6,7] The most common symptoms such as jitteriness, convulsions, apathy, hypotonia, coma, refusal to feed, cyanosis, high pitched cry, hypothermia are very nonspecific and especially in small sick infants, these symptoms may be easily missed. Therefore hypoglycemia must always be confirmed biochemically and by response to treatment. Hypoglycemia is known to be associated with brain dysfunction and neuromotor developmental retardation in both symptomatic and asymptomatic cases.[1,3,8]

The definition of clinically significant hypoglycemia remains one of the most confused and contentious issues in contemporary neonatology.[9] It is not possible to define a blood glucose level that requires intervention in every newborn infant because there is uncertainty over the level and duration of hypoglycemia that cause brain damage and little is known of the vulnerability of the brain of infants at different gestational ages for such damage. In symptomatic infant, plasma glucose concentration should be measured and if the value is <45mg% [2.5m mol/l], clinical interventions aimed at increasing blood glucose concentrations are indicated.[9]

The methods for determining blood/plasma glucose concentration include reductiometric method, glucose oxidase method and hexokinase method.[1,10] The glucose oxidase method used in the laboratory for determining the blood glucose concentration is precise and specific for glucose.[1] As it is usually performed in the main laboratory, the results are not available quickly enough for timely appropriate management. The development of reagent strip blood glucose tests in the 1970s facilitated the practice of screening for hypoglycemia in newborns.[1] Currently used glucometers were initially developed for glucose monitoring in adult diabetics. These glucometers are often used for blood glucose estimation in NICU. Many studies have shown that their results co-relate well with the laboratory measured glucose levels in the normoglycemic and hyperglycemic range but are not satisfactory in the lower range.[11-18]

**OBJECTIVES:** 1] To determine the efficacy of glucometer in the estimation of blood glucose levels in newborns, in comparison with the glucose oxidase method used in the laboratory.

**REVIEW OF LITERATURE: HISTORICAL BACKGROUND:** It is almost a century sine hypoglycemia was first described in children and over 50 years since it was first described in newborns. Given the numerous advances which have since occurred in the care of the newborns it is surprising that so much controversy still surrounds the definition, significance, detection and management of neonatal hypoglycemia. Paradoxically, technological developments in the form of bedside glucose monitoring have exacerbated rather than eased the problem by facilitating screening for an ill-characterized clinical entity.

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**METHODS FOR MEASURING BLOOD/PLASMA GLUCOSE CONCENTRATION:**

1. **REDUCTIOMETRIC METHODS:** Traditional methods for measurement of blood glucose depend on the reducing property of glucose. An example is the ferricyanide method. These methods measure total reducing sugar concentrations.[1] Enzymatic methods have largely superseded reductiometric methods in clinical practice.

2. **GLUCOSE OXIDASE METHOD:** Glucose oxidase catalyses the oxidation of glucose to yield glucoronic acid and hydrogen peroxide. The concentration of hydrogen peroxide liberated is measured using a peroxidase step coupled to a colored oxygen acceptor or an electrode. These reactions form the basis of both reagent strips and bench top glucose electrode methods.[1]

3. **HEXOKINASE METHOD:** Hexokinase catalyses the phosphorylation of glucose by ATP. Glucose-6-phosphate is then reduced by glucose dehydrogense yielding NADPH/ H+ which can be measured using a suitable spectrophotometric indicator system. This method is precise and highly specific for glucose but the main drawback is its cost.[10]

4. **REAGENT STRIPS:** These were initially developed for monitoring blood glucose concentration in diabetics and not intended for detection of hypoglycemia. Care must be taken to avoid contamination by alcohol skin-cleansers, to cover the whole surface of the test pad and to time the reaction precisely before wiping the strip. Even when all these precautions are taken, they tend to under-estimate systematically the mean of a series of measurements in the range of glucose concentrations relevant to the diagnosis of neonatal hypoglycaemia.[1]

Several commercially available systems are available and have been evaluated for neonatal use. Reagent strip methods are prone to many errors when used to screen for neonatal hypoglycemia. Confirmation with laboratory measured plasma glucose is still of up most importance.[11-18]

Several commercially available systems are available and have been evaluated for neonatal use including:

Dextrostix, BM-test-glycemie, Chemstrip bG, Glucostix.

Care must be taken to avoid contamination by alcohol skin-cleansers, to cover the whole surface of the test-pad, and to time the reaction precisely before wiping the strip. Reagent strip methods are prone to many errors when used to screen for neonatal hypoglycemia.

Reagent strips are subject to false positive and false negative results.

**FALSE POSITIVE:**
- Low hematocrit values (<35%).
- Contamination with iso propoyl alcohol.

**FALSE NEGATIVE:**
- High hematocrit values (>55%).
- Glucose values > 200mg/dl.
- Hyperglycemic- hyperosmolar states with or without ketosis.
- Delay in lab analysis.

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**VARIATIONS AND ERRORS IN MEASUREMENTS:**

**Properties of the sample and sources of error:** Arterial blood has a slightly higher glucose concentration than venous. The magnitude of this difference varies with tissue glucose demands and will be greatest under anaerobic conditions.

Capillary sampling is unreliable if peripheral blood flow is reduced. Samples must be always be free-flowing as squeezing the heel causes haemolysis which interferes with the assay unless deproteinisation is performed. The sample should either be analyzed immediately or deproteinised (for example using perchloric acid) and chilled. Glycolysis otherwise continues. Commercially available sodium fluoride coated tubes do not always ensure a fluoride concentration sufficient to inhibit glycolysis

Contamination by alcohol used for skin preparation leads to erroneously high values.

One of the problems with neonatal samples is that haematocrit may vary from <40 to>70%. Red cells contain less water than an equivalent volume of plasma though the glucose concentration in red cell water is the same as that in the plasma. Plasma glucose concentration is therefore higher than that of whole blood, on average by about 18%.

All methods employing paper reagent strips are subject to an intrinsic haematocrit bias; the higher the haematocrit value, the lower the result. Possible reasons include discoloration of the test-pad and resistance to wiping or washing before reading. Also the higher sample viscosity impedes diffusion of plasma into the test-pad of the strip.

Bilirubin also interferes with glucose oxidase-peroxidase based strip methods. Bilirubin inhibits both steps of the assay leading to falsely low values.

Haemolysis also produces falsely low values. This may be attributable to presence of hemoglobin or to release of reduced glutathione which competes with the chromogen for hydrogen peroxide released in the assay.

5. **GLUCOSE ELECTRODE SYSTEMS:** Based on the glucose oxidase method. The device measures plasma glucose concentration on a whole blood uncentrifuged 25microlitre sample.[1]

6. **OTHER BEDSIDE SYSTEMS:** The Hemo Cue beta-glucose photometer is an optical method measuring whole blood glucose on small [5microlitre] samples utilizing disposable cuvettes. Blood is hemolysed in the cuvette and the NADH formed by enzymatic glucose oxidation reduces methylthiazolyldiphenyl tetrazolium to produce a formazan dye, the concentration of which is determined spectrophotometrically. Only one study has evaluated its application to neonatal samples.

**MATERIALS AND METHODS:**

1. **SOURCE OF DATA [SAMPLE]:** 250 neonates getting admitted as inpatients in NICU in KIMS Hospital, Bangalore during the study period of december2013 to november 2014 were taken as the source of data for the study.

2. **METHOD OF COLLECTION OF DATA:** 250 admitted neonates satisfying the inclusion criteria were included in the study. Collection of data was by relevant investigations i.e. glucose estimation by glucometer and laboratory glucose oxidase method by using the same venous

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sample. For 50 of these cases, glucose estimation of both venous and capillary blood was done by using the glucometer along with the routine laboratory venous blood glucose estimation. Blood glucose estimation was done at the time of admission.

3. **INCLUSION CRITERIA:** 250 Neonates admitted in NICU, KIMS Hospital,Bangalore for varied symptomatology during the study period.

4. **EXCLUSION CRITERIA:** Neonates admitted in postnatal ward who were healthy, term and breast-fed.

Preterm and SGA newborns with birth weight more than 1.8kgs, active and healthy with no risk factors were shifted to mother side and were not included in the study.

5. **METHODOLOGY:**
**Our study had 2 parts:** First, estimation of venous blood level by glucometer and by laboratory glucose oxidase method. Laboratory glucose oxidase method was taken as the gold standard for the glucose estimation. For the last 50 cases, estimation of the capillary blood glucose level by glucometer was also done.

Second part of the study was to compare the glucometer values with laboratory values. For the last 50 cases, capillary and venous blood glucometer values were compared with laboratory values as gold standard.

Statistical analysis was done by using 'Pearson Correlation'.

1. **GLUCOMETER:** Used is ACCU-CHEK Advantage/ Sensor glucometer, manufactured by Roche Diagnostics, 2003.

**TEST PRINCIPLE:** Bioamperometry-glucose dehydrogenase in the strip converts the glucose in the blood sample to gluconolactone. This reaction creates a harmless electrical current that the glucometer interprets for that blood glucose.

**PROCEDURE:** Touch the drop of the blood collected, to the curve at the edge of the test strip. No part of the yellow color on the strip should be visible after applying the initial drop of blood. Blood will be drawn into the strip automatically. Do not place the blood drop on the top of the strip. Test result will appear within 30 seconds.

2. **LABORATORY METHOD:** Enzymatic calorimetric test, GOD-POD i.e. glucose oxidase-peroxidase method.

**PRINCIPLE:**
- Glucose + $O_2$----------Gluconic acid + H2O2
- $H_2O_2$ + 4-Aminoantipyrine + Phenol--------------Chinomine + $4H_2O_2$

The first step is catalyzed by glucose oxidase and the second by peroxidase enzyme. The reagents and standard are ready to use.

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**PROCEDURE:**

- Monoreagent--------1000microlitre.
- Sample ---------10micolitre.
- Mix well; incubate for 5 minutes at $37^0$c.
- Read the absorbance ΔA against reagent blank.

$$\text{Calculation -------glucose mg/dl} = \frac{\Delta A\ SAMPLE}{\Delta A\ Standard} \times 100$$

**RESULTS:**

**STUDY DESIGN:** A Prospective correlation study consisting of 250 cases was undertaken to study the correlation of Glucometer and laboratory glucose oxidase method for estimating blood glucose levels in newborns.

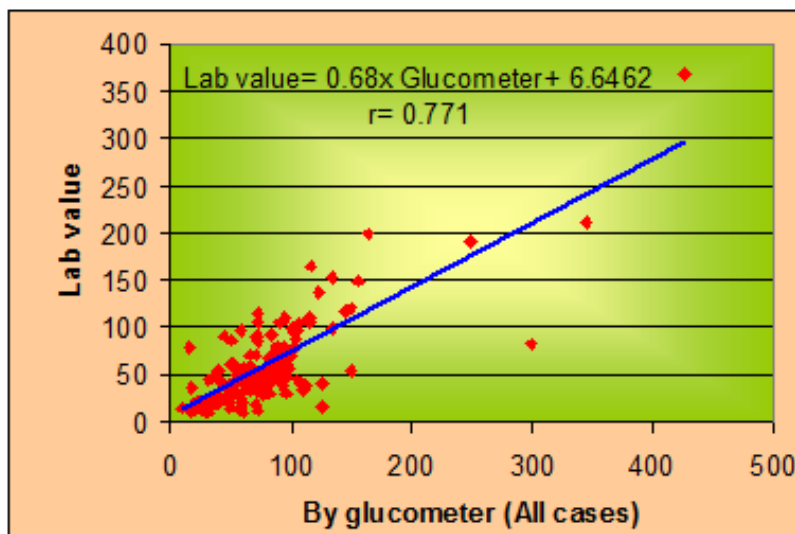| Value | Percentage |
|---|---|
| True Positive | 43 |
| True Negative | 138 |
| False Positive | 9 |
| False Negative | 60 |
| Sensitivity | 41.75 |
| Specificity | 93.88 |
| PPV | 82.69 |
| NPV | 69.70 |
| Table 1: Diagnostic value of Glucometer in relation to Lab value in detecting the hypoglycemia | |



**Fig. 1: Pearson correlation of Glucometer and Lab value in Different GA (all cases)**

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Every increase of one unit of Glucometer value there is an increase of 0.68 units in Lab value (with 62.5% accurately).

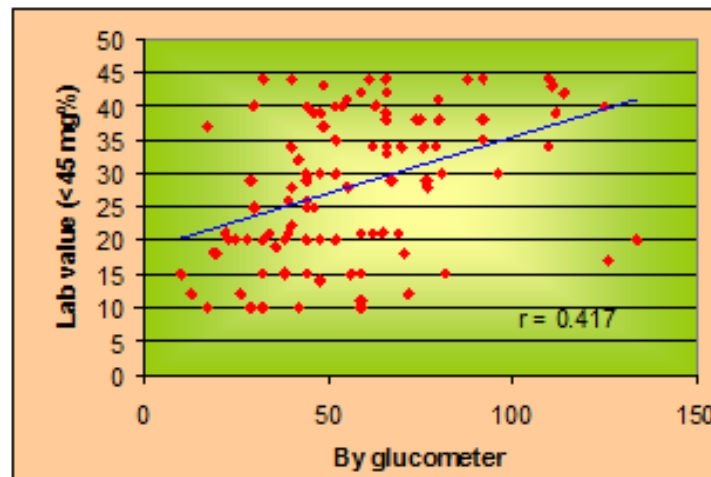| | Number | Pearson correlation | P value |
|---|---|---|---|
| Glucometer vs. Lab (<45 mg %) | 103 | 0.417 | P<0.001** |
| Glucometer vs. (>45 mg %) | 147 | 0.758 | P<0.001** |
| Table 2: Pearson correlation between Glucometer and Lab value with lab value >45mg% and <45mg% groups | | | |



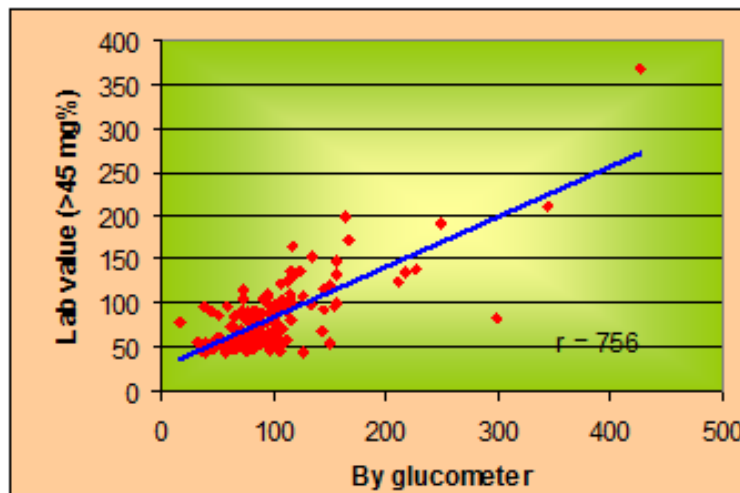**Fig. 2a: Pearson correlation between Glucometer and Lab value [lab value <45mg%]**



**Fig. 2b: Pearson correlation between Glucometer and Lab value [lab value>45mg%]**

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Table 2 and figure 2a & 2b, shows that there is a large correlation [0.756] between the glucometer and lab values when the blood glucose values, as determined by the gold standard is >45mg%. But when the lab values [gold standard] are <45mg% then there is just moderate correlation [0.417] between the two.

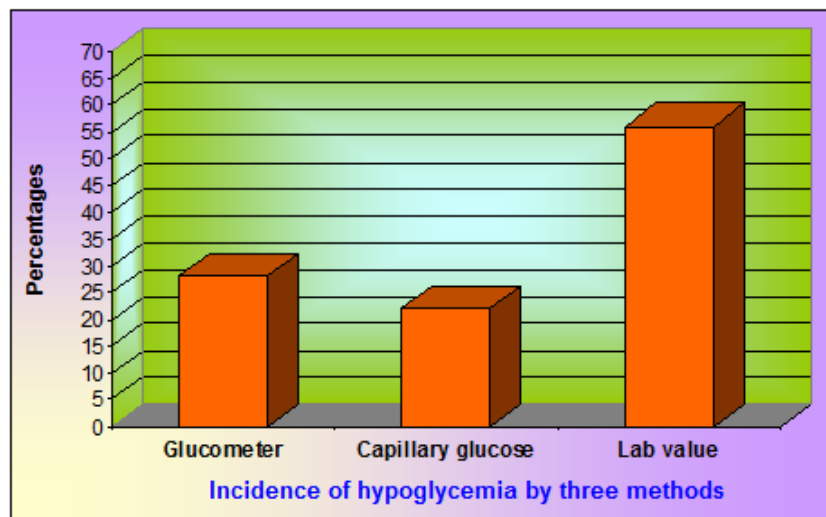| Methods | Number | Pick-up rate of hypoglycemia |
|---|---|---|
| Venous blood-glucometer | 50 | 14 (28.0%) |
| Capillary blood—glucometer | 50 | 11 (22.0%) |
| Lab value | 50 | 28 (56.0%) |
| Table 3: Pick-up rate of venous blood [Glucometer], capillary blood [Glucometer] and Lab value | | |



**Fig. 3: Pick-up rate of Glucometer, capillary Glucose and Lab value**

Table 3 and figure 3 shows the pick-up rate of hypoglycemia by glucometer using venous blood and capillary blood in relation to the gold standard-lab value.

Pick-up rate of hypoglycemia by glucometer using capillary blood was the least [22%] followed by glucometer estimation using venous blood [28%].

**DISCUSSION:** In this study we evaluated the efficacy of glucometers in estimating the blood glucose levels in newborns, in comparison with laboratory glucose oxidase method. Main stress was laid on detection of hypoglycemia by both the methods and to know if glucometer is a good screening tool to detect hypoglycemia. Laboratory glucose oxidase method of blood glucose estimation was taken as gold standard.

In this prospective correlation study, 250 neonates who were admitted in NICU with varied symptomatology were enrolled. Of these majority [87.6%] were admitted in the first 7 days of life while the rest 12.4% admitted were more than 7 days old. This is due to the fact that majority of the

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clinical conditions associated with hypoglycemia such as septicemia[early onset], birth asphyxia, prematurity, respiratory distress etc. manifest in the early neonatal period and seek admission.

Hypoglycemia was defined as blood glucose level less than 45mg% as defined by the study by Cornblath et al.[9] 103 [41.2%] were found to be hypoglycemic by laboratory glucose oxidase method whereas by glucometer screening 52[20.8%] were found to be hypoglycemic. Thus the incidence of hypoglycemia as detected by the gold standard method i.e. laboratory glucose oxidase method in our study is 41.2%.

| STUDIES | INCIDENCE |
|---|---|
| PK SINGHAL et al[3] | 4.8% |
| PK MISHRA & BINA SHARMA[2] | 9.7% |
| OUR STUDY | 41.2% |
| Comparison of overall incidence of hypoglycemia with other studies | |

This wide variation in the incidence of hypoglycemia could be attributed to the lack of uniform definition of hypoglycemia, varied sample size and risk factors. In the PK Singhal et al study hypoglycemia was defined as blood glucose level <30 mg%[3] while in the PK Mishra and Bina Sharma study it was taken as 20mg%[2]. In our study the cut-off value for hypoglycemia was taken as 45mg%. Also our study group consisted of sick/ septic/preterm/lbw newborns referred from other hospitals/ nursing homes without I.V. fluids or feeds during transportation and the duration of transport was usually >8hours.This has probably given rise to a higher incidence of hypoglycemia.

**HYPOGLYCEMIA AND GLUCOMETERS:** The results depict the poor pick-up rate of hypoglycemia by glucometer in comparison with the laboratory values. It shows that nearly half of the cases of proven hypoglycemia [by lab method] were missed by glucometer, indicating that glucometers are not good screening tool for detecting hypoglycemia in newborns.

| STUDIES | SENSITIVITY | SPECIFICITY |
|---|---|---|
| 1. Dahlberg et al[14] | 100% | 84% |
| 2. Mehta et al[15] | 86% | 89% |
| 3. H O HT et al[16] | 92.3% | |
| 4. M Ellis et al[17] | 83% | 62% |
| 5. Hamid MH et al[18] | 98% | 93% |
| 6. Our Study | 41.75% | 93.88% |

Our study shows that glucometers have a specificity of 93.88% to detect hypoglycemia which is in correlation with few of the other studies mentioned. But in contrast with the other studies, our study showed that glucometers had a very poor sensitivity of 41.75% to detect hypoglycemia. This may be due to the glucometer brand used.

Our study also showed that glucometers had a positive predictive value of 82.69% and a negative predictive value of 69.7%, which in contrast to the results obtained by H O HT et al[16] and Dahlberg et al[14], is low. The overall accuracy of glucometers to detect hypoglycemia in newborns in comparison with the gold standard i.e. laboratory value is 72.40%.

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**CORRELATION OF GLUCOMETER AND LAB VALUE [OVER ALL BLOOD GLUCOSE LEVELS]:**

| | |
|---|---|
| Hamid et al[18] | 0.976 |
| Our study | 0.771 |

Like other studies our study shows a good correlation between glucometer and laboratory values when all blood glucose values were considered irrespective of hypoglycemia.

We can also infer that for every increase of one unit of glucometer value there is an increase of 0.68 units in the lab value. Thus lab value can be calculated by the formula:

Lab value = 0.68 x Glucometer + 6.64.

**Example:** if the glucometer value is 80 mg% then by this formula lab value will be

0.68 x 80 + 6.64 = 61mg%

Accuracy of this formula is only 62.5%.

The results show that the correlation between the lab and glucometer is good when the gold standard value is >45mg% [0.756] and when the value is <45mg%, there is just a moderate correlation [0.417]. This further stresses the inefficacy of glucometers in detecting hypoglycemia in newborns.

**CAPILLARY BLOD GLUCOSE ESTIMATION:** For the last 50 cases, blood glucose estimation was done by laboratory method [venous sample] and by glucometer [both venous and capillary blood]. This comparison was done because in many centers, capillary blood glucose estimation by glucometer is being done to screen neonatal hypoglycemia.

Results show that blood glucose estimation using capillary blood has a poor pick-up rate of detecting neonatal hypoglycemia. More than 50% of the proven cases of hypoglycemia [proved by lab method] were missed when capillary blood glucose estimation with glucometer was done. Though the pick-up rate of hypoglycemia by venous blood glucometer [28%] was slightly better compared to the capillary blood glucometer value [22%], the overall pick-up rate of hypoglycemia by glucometer is very low compared to the laboratory method.

**CONCLUSION:** There is a variable detection rate of hypoglycemia by glucometer in the studies mentioned and also in our study. These 'point of care' devices had a very low sensitivity and negative predictive value and for detecting hypoglycemia in newborn but had good specificity and positive predictive value. The correlation between the glucometer and laboratory values is good when the gold standard i.e. lab value is >45mg% and when the value is <45mg%, there is just a moderate correlation. Thus excluding hypoglycemia, these glucometers had a very large correlation with the laboratory measured values. These glucometers as a sole measuring device to screen neonatal hypoglycemia is not satisfactory and confirmation with the laboratory measurements of plasma glucose is still of up most importance.

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