Background: Neonatal screening is a preventive medical measure to screen infants shortly after birth for treatable metabolic disorders and endocrinopathies. The present study aimed to evaluate and compare the accuracy of laboratory samples in the screening programs for metabolic diseases in Mashhad city, Iran with international guidelines.

Methods: This observational study was conducted on all the patients referring to the health centers of Mashhad, Iran during two weeks. In total, 220 infants were enrolled in the study and divided into three groups of health center 1, 2, and 3. A checklist was prepared based on the international guidelines to evaluate the neonatal screening procedures.

Results: In total, 220 neonates aged 3-14 days (90 girls and 130 boys) were enrolled in this study. Mean weight and height of the neonates was 3215.90±485.12 grams and 49.85±2.04 centimeters, respectively. In all the cases, mode of nutrition was breastfeeding, and sampling was performed within 36 hours after birth. Neonates had no history of corticosteroid administration, catecholamine administration, parenteral nutrition, and blood transfusion. Heels of the neonates had no contact with the filter paper, and the samples were dried away from direct sunlight and heat. Two of the health centers used disinfectants and dried the heels of infants afterwards prior to drawing the samples.

Conclusion: According to the results, the studied health centers followed the protocol properly. However, two of the health centers used disinfectants in such way that we were not sure whether the heels of the neonates were dried completely.

Keywords: Health centers of Mashhad, Metabolic diseases, Neonatal screening

Introduction

Neonatal screening is a preventive medical measure to screen infants shortly after birth for treatable endocrinopathies and metabolic disorders. Inborn errors of metabolism are associated with carbohydrates, amino acids, organic acids (organic acidurias), purine or pyrimidine, urea cycle disorder, fatty acid oxidation, and mitochondrial and steroid disorders. In many countries, neonatal screening is a routine care procedure for congenital hypothyroidism and phenylketonuria.

Incidence rate of phenylketonuria (PKU) is one case per 13,500-19,000 births (2001) (1). In a study conducted over a 20-year period (1981-2002) based on a French screening program, incidence of permanent congenital hypothyroidism (CH) was reported to be 1:4000. In Fars province (Iran), incidence rate of PKU has been calculated to be 1:4698 (2). According to statistics, the national incidence rate of CH is 2.2 cases per 1,000 screened neonates in Iran.

The main goal of neonatal screening is the universal early diagnosis of treatable, but not clinically evident, endocrine and metabolic diseases in infants, followed by the timely start of an effective treatment. Initially, the criteria of neonatal screening programs were based on the guidelines established by JMG Wilson and F. Jungner in 1968 (3). With the instigation of the neonatal screening international guidelines in 2002, organizational measures were incorporated into the decision-making of the Joint Federal Committee (4, 5). Regional screening centers include primary treatment centers, public health departments, and screening laboratories, which carry out the related

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tests with the aim of performing neonatal screening of assured quality as an integrated overall care service.

Neonatal screening tests are most commonly performed by using the whole blood samples collected on specially designed filter paper. The filter paper is often attached to a form containing the required data on the infant and parents (date and time of birth, date and time of sample collection, weight and gestational age of the infants). In addition, this form contains the information on the history of blood transfusion and additional nutrition modes of the newborn (e.g., total parenteral nutrition). Most neonatal screening cards also include the contact information of the physician in the cases where follow-up screening or treatment might be needed.

For neonatal screening, samples are collected at 72 hours to seven days after birth, with the requirement that the infant must be fed at least once. Samples can be collected in the hospital or by midwives. If a sample is obtained from an infant aged less than 24 hours, the laboratory often requests a repeated specimen after 24 hours. Samples are sent daily to the laboratory responsible for the screening tests.

In neonatal screening, the infant must be evaluated by a qualified physician, who attempts to confirm the diagnosis by repeating the tests using a different method, in another laboratory, or by performing other confirmatory tests. Confirmatory tests in this regard vary depending on the positive results on the initial screening, and abnormal findings must be reported no later than 72 hours after obtaining the specimen.

In the past few decades, it has shown that about two-thirds of all the errors in neonatal screening programs occur due to the substandard performance of the healthcare organizations. The present study aimed to evaluate the accuracy of the laboratory samples used in the neonatal screening programs for endocrine and metabolic diseases in Mashhad city, Iran.

Methods

This observational study was conducted in three health centers in Mashhad, Iran during two weeks. Most of the neonatal screening tests were performed by measuring the metabolites and enzyme activity in the whole blood samples collected on specialized filter papers. Criteria of valid and invalid specimens are presented in Box 1.

Box 1. What makes a good spot?

- Blood should soak all the way through the filter paper. Complete saturation is necessary for accurate testing.
- Collect blood from one side of the filter paper only.
- It is important NOT to superimpose or layer the blood drops on top of each other since it may yield false results.
- Collect blood for each circle on the filter paper/collection card. It is to properly collect four circles than inadequately fill all the five circles.
- Let each drop touch the paper within three millimetres from the previous drop.
- Avoid contamination

Invalid specimens:
- Insufficient quantity for testing:
  - Removing the filter paper before blood has completely filled the circle or soaked through;
  - Touching the filter paper before or after collecting the specimen or contamination of the filter by gloves, hand lotion, or antiseptics
- Specimen appears scratched or abraded:
  - Applying blood with a capillary tube or other devices
- Specimen has not dried before mailing:
  - Allow specimen to dry for 2-4 hours before sending to the laboratory
- Specimen appears supersaturated:
  - Applying excess blood to the filter paper, usually with a device;
  - Applying blood to both sides of the filter paper
- Specimen appears dilated, discoloured or contaminated:
  - Squeezing or milking of the puncture site
  - Contamination
  - Blood spots exposed to direct heat
- Specimen exhibits serum rings:
  - Puncture site was not cleaned with alcohol/antiseptic before puncture;
  - Contamination;
  - Squeezing or milking the puncture site;
  - Improper drying;
  - Applying blood with a capillary tube or other devices
- Specimen appears clotted or layered:
  - Touching the same circle to the blood drop several times (layering);
  - Applying blood to both sides of the filter paper
- No blood:
  - Failure to obtain the specimen
Abnormal findings must be reported no later than 72 hours after obtaining the specimen. In the past few decades, it has been reported that about two-thirds of all the errors in neonatal screening tests occur due to the substandard performance of the healthcare organization. Nonetheless, some of the individual sources of error are patient-related factors and external generators of laboratory artifact, such as the very early collection of the specimen, premature birth, medical treatments (e.g., corticosteroid administration, catecholamine administration, parenteral nutrition, iodine exposure, and blood transfusion), and potential generators of laboratory artifact (e.g., heating of the screening card and disinfectant contamination) (10).

Sample size of the present study consisted of 220 neonates aged 3-14 days, who were referred to the selected health centers. A checklist was prepared in accordance with the international guidelines of the neonatal screening protocol. Neonatal data in the checklist included the age, gestational age, gender, weight, height, parental family history, type of feeding, mode of delivery, neonatal age at the time of sampling, corticosteroid administration, catecholamine administration, parenteral nutrition, blood transfusion, interval between collecting and using the specimens, potential generators of artifact (heating of the screening card and disinfectant contamination), and interval between collecting and sending the samples to the laboratory.

Data analysis was performed in SPSS version 11.5.

**Results**

In total, 220 neonates aged 3-14 days (90 girls and 130 boys) were evaluated in this study. Mean weight and height of the infants was 3215.9±485.12 grams and 49.85±2.04 centimeters, respectively (Table 1). Data on the gestational age, parental family history, type of feeding, mode of delivery, and sampling time after birth are presented in Table 2.

All the studied infants were breastfed, and sampling time was 36 hours after birth. None of the neonates had a history of corticosteroid administration, catecholamine administration, parenteral nutrition, and blood transfusion. Heels of the infants had no contact with the filter paper, and the obtained samples were dried away from direct sunlight and heat. Two of health centers used disinfectants, and the samples were drawn when the heels of infants had dried completely. However, one of the health centers did not use disinfectants for sampling (Table 3).

**Discussion**

Neonatal screening is a public health program used to assess infants shortly after birth for various treatable conditions that may not be clinically evident. Some of the conditions included in neonatal screening programs are only detectable after causing irreversible damage to the health of newborns, and in some cases, sudden death might be the first manifestation of the undiagnosed disorder. In Iran, neonatal screening is a routine care procedure for congenital hypothyroidism, PKU, and various other disorders. In Iran, the screening program for CH began in 2005.

In many neonates, serum level of the thyroid-stimulating hormone (TSH) remains high. Moreover, as a consequence of transplacental dialysis, the disease-specific metabolite profile of a metabolic disease may not be evident within the early hours of birth. Therefore, screening must be repeated for all the neonates whose first screening specimen is obtained within less than 36 hours after birth.

CH is not reliably detectable in the neonates born earlier than the 32nd week of gestation. A normal finding must be followed-up by a second screening at 32 weeks of the corrected gestational age. In the present study, the first screening

<table>
<thead>
<tr>
<th>Table 1. Basic Neonatal Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age (day)</td>
</tr>
<tr>
<td>Weight (gr)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>All values expressed as mean ± SD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Demographic Data of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Gestational Age</td>
</tr>
<tr>
<td>&lt;32 weeks</td>
</tr>
<tr>
<td>≥32 weeks</td>
</tr>
<tr>
<td>Parental Family History</td>
</tr>
<tr>
<td>Consanguineous Marriage</td>
</tr>
<tr>
<td>Non-consanguineous Marriage</td>
</tr>
<tr>
<td>Mode of Delivery</td>
</tr>
<tr>
<td>NVD*</td>
</tr>
<tr>
<td>C/S**</td>
</tr>
<tr>
<td>Sampling Time after Birth</td>
</tr>
<tr>
<td>First Time</td>
</tr>
<tr>
<td>Second Time</td>
</tr>
</tbody>
</table>

*NVD: natural vaginal delivery, **CS: cesarean section
Table 3. Potential Error-Causing Factors in Health Centers of Mashhad

<table>
<thead>
<tr>
<th>Potential Error-Causing Factors</th>
<th>Incorrect</th>
<th>Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling Time after Birth (hour)</td>
<td>&lt;24</td>
<td>24-72/&gt;72</td>
</tr>
<tr>
<td>Gestational Age (week)</td>
<td>&lt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Corticosteroid Administration</td>
<td>Interval between &lt;2 Weeks</td>
<td>&gt;2 Weeks</td>
</tr>
<tr>
<td>Catecholamine Administration</td>
<td>Obtaining and &lt;2 Weeks</td>
<td>&gt;2 Weeks</td>
</tr>
<tr>
<td>Parenteral Nutrition</td>
<td>Using Samples &lt;4 Hours</td>
<td>&gt;4 Hours</td>
</tr>
<tr>
<td>Iodine Exposure</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Blood Transfusion/Administration of Fresh Frozen Plasma (FFP)</td>
<td>Before Transfusion (No Repeated Screening)</td>
<td>Repeated Screening 2 and 6-8 Weeks after Transfusion</td>
</tr>
<tr>
<td>Heating of Screening Card</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Disinfectant Contamination</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Contamination with Mother’s Milk</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>*EDTA Contamination</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*EDTA: Ethylenediaminetetraacetic Acid

specimen was obtained after 72 hours of birth, and if a neonate was born earlier than the 32nd week of gestation, the screening would be repeated in accordance with the guidelines.

Corticosteroid administration induces false normal findings, particularly for 17-OH-progesterone. Retesting should be performed two weeks after the discontinuation of corticosteroids. Furthermore, catecholamine administration is likely to yield false normal findings for TSH, possibly indicating low biotinidase activity. In such cases, retesting should be performed two weeks after the discontinuation of catecholamine.

In parenteral nutrition, non-specific elevation of amino acid levels might mask the disease-specific laboratory profiles in newborns. Lack of catabolism reduces the sensitivity of the detection of fatty acid oxidation defects. As such, drawing blood samples four hours after the temporary cessation of parenteral nutrition is advisable. In the current research, the interval between sampling and use of corticosteroids/catecholamine was two weeks, while it was four hours between sampling and parenteral nutrition.

Blood transfusion and/or administration of fresh frozen plasma (FFP) may lead to false normal findings in all screening tests. Therefore, blood samples for neonatal screening should be obtained before blood transfusion, even if less than 36 hours have elapsed since birth. Moreover, screening should be repeated two weeks after the transfusion. If no sample has been obtained before blood transfusion, two screening tests should be performed at two and 6-8 weeks after the transfusion. In the present study, screening tests in the selected health centers were performed after two weeks in the case of blood transfusion or FFP administration.

Heating of the screening card disrupts enzymatic activity, thereby generating false pathological findings for galactosemia and biotinidase. In addition, contamination with disinfectants has been shown to disrupt enzymatic activity (10). It is notable that in the current research, one of the health centers did not use alcohol for disinfection, whereas it was used by the other health centers, which might have caused errors in the findings.

Conclusion

According to the results of the present study, the selected health centers in Mashhad city properly complied with the protocol of neonatal screening. However, two of the health centers used disinfectants in such way that we were not sure whether the heels of infants could be dried completely, which might have caused errors in the findings.

Acknowledgments

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Conflicts of interests

None declared.

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