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Screening for phenylketonuria and galactosemia among Egyptian newborns in Menoufiya governorate

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ABSTRACT

Aim of the Work: Was to study the prevalence of phenylketonuria and galactosemia in Menoufiya Governorate newborns. Among 3000 newborns, their mean ages were 9.3±2.43 days; mean weight was 3.1 ±0.82 Kg. Among them 1800(60%) males and 1200 (40%) females who attended the central hospital and medical units for BCG vaccination in the duration from March 2005 to May 2008.

Results: The results showed that the mean of phenylalanine level was 3.19 ±1.82 mg/dl and the mean total galactose level was 3.34 ± 2.23mg/dl, among the 3000 neonates, 2183 (72.8. %) had phenylalanine levels ranging from 2-5 mg /dl, 705 (23.5%) had levels ranging from 5-7 mg/dl, 111(3.7%) had levels ranging from 7-10 mg/dl and one newborn (0.033%) had phenylalanine level of 22 mg/dl. The results for galactosemia screening assay showed that 2528 neonates (84.3%) had galactose levels ranging from 2-6 mg/dl, 450 (15%) had levels ranging from 6-8 mg/dl, 21(0.7%) had levels ranging from 8-12 mg/dl and one newborn (0.033%) had galactose level of 19 mg/dl. The child was reassayed and was found to be true hypergalactosemia 120mg/dl.

Conclusion: We concluded that the prevalence of each of phenylketonuria and galactosemia in Menoufiya Governorate in the 3000 newborn tested was 1/3000 (0.03%). So, we estimate that about 333 neonates are affected every year with PKU and 333 with galactosemia as one million babies are born yearly, which could be prevented. The prevention of such treatable disorders depends on planning an efficient screening programme especially within three weeks after birth. So we recommend multicenter studies to encourage national neonatal screening programmes specialy for these treatable diseases.

Key Words:

Phenylketonuria, galactosemia, hepatomegaly, neonatal screening.

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INTRODUCTION

Neonatal screening programmes for PKU and galactosemia were introduced in many European and North American countries about 30 years ago. To prevent disability and handicapped through simple tests and subsequent dietary treatment[1]. If undiagnosed and untreated, many of the affected children will be hospitalized for extensive periods and require intensive care. There will be a

major impact on the families concerned and on health care provision.[2]

Phenylketonuria (PKU) is an autosomal-recessive disorder most commonly caused by a mutation in the gene coding for phenylalanine hydroxylase, an enzyme responsible for the conversion of phenylalanine to tyrosine. Sustained phenylalanine concentrations higher than 20 mg per dl usually correlate with classic symptoms of PKU.[3]

Unless the condition is detected and treatment is initiated soon after birth, phenylalanine builds up in the blood and body tissues and is toxic to the brain, usually resulting in severe mental retardation left to clinical ascertainment, diagnosis is often late and may be difficult to achieve. Frequently severe physical, developmental and neurological damage has occurred or begun by the time of diagnosis. It is characterized by hyperphenylalaninemia and manifest primarily as neurological damage.[4]

Infants with PKU appear normal at birth and have fair skin and hairs than other members of their family. Untreated PKU patients usually first become noticeable between 6 to 12 months of age, by which time up to 50 I Q points will have been lost[5]. This mental retardation may be associated with microcephaly, seizures, eczema, delayed speech, behavier abnormalities, gait disturbance and even autism.[6]

Only rarely, symptoms lead to diagnosis before this stage. Effective therapy to lower raised blood phenylalanine levels by dietary restriction of phenylalanine prevents progressive, irreversible brain damage but does not reverse pre-existing damage. The early treatment is commenced for the better ultimate outcome. Most early treated children with PKU fall within the normal range of ability and attend ordinary schools.[7]

Reports of neurological deterioration in young adults off dietary supervision have led to the recommendation of treatment supervision for life.[8]

Galactose is the major sugar found in milk. It is normally converted to glucose, another simple sugar, but in galactosemia, the enzymes needed for this conversions (galactose-1-phosphate uridyltransferase GALT, galactokinase GALK and galactose epimerase GALE)) are missing. Galactose accumulates in and damages the body's cells and organs. Clinical manifestations of galactosemia include lethargy, hypotonia, jaundice, hypoglycemia, elevated liver enzymes and coagulopathy, severe mental retardation, growth defeciency, overwhelming infection and death.[9]

It is important to distinguish the galactosemia disease genotype (G/G) from asymptomatic variant genotypes (e.g., G/D, G/N, D/D), which can be picked up as "positive" in newborn screening. Galactosemia is treated by excluding milk and milk products from the diet. Children treated with this special diet usually show satisfactory general health and growth. But most patients develop abnormalities despite dietary treatment.[3]

Infants with classical galactosemia, who have near total absence of GALT activity, exposure to dietary galactose results in acute deterioration of multiple organ systems, including liver dysfunction, coagulopathy, poor feeding and weight loss, renal tubular dysfunction, cerebral edema, vitreous hemorrhage and Escherichia coli sepsis and even death.

This "neonatal toxicity syndrome" can be reversed by withdrawal of dietary galactose. So most national screening programs have included galactosemia in their newborn screening programs, anticipating that early detection and intervention would prevent long-term complications such as mental retardation, premature ovarian failure which is nearly universal in females with galactosemia, speech delay and cerebellar symptoms.[10]

Cataract has been observed within few days after birth. Some patients have more chronic course with presentation few months after birth with delayed development, hepatomegaly and cataracts.

Early introduction of a galactose free diet will cause the resolution of few manifestations of the disease, including cataract and prevent their recurrence. Unfortunately this has not proven to be true, probably because of the significant endogenous turnover of galactose which is independent of manipulation of dietary intake.[11]

There are different views in the literatures of the value of neonatal screening for galactosemia for a number of reasons: there is little evidence that the early institution of treatment allowed by neonatal screening improve long term outcome[12-14]. The long term outcome remains poor inspite of best available current treatment and there are yet uncosted difficulties and uncertainties posed by the allelic variants producing GALT deficiency.[15]

Hypergalactosaemia identified by neonatal mass screening has several aetiologies, including portosystemic shunt[16-20]. Recently it has been reported that citrin deficiency also causes hypergalactosaemia in infancy[21-23]. Thus hypergalactosaemia should not be thought of solely in terms of possible enzyme deficiency, but also as a reflection of portosystemic shunt or liver dysfunction, e.g. citrin deficiency and Fanconi-Bickel syndrome. However, the incidence of these different causes of hypergalactosaemia has not been determined[11]. The benefits of screening are currently related to the possible prevention of acute neonatal illness in some cases, the facilitation of collection of epidemiological and outcome data and improved patient support.

PATIENTS AND METHODS

The study included 3000 newborns, their mean ages was 9.3 ± 2.43 days, mean weight was 3.1 ± 0.82 Kg, among them 1800 (60%) males and 1200 (40%) females who attended the central hospital and medical units for BCG vaccination in Menoufiya Governorate from March 2006 to May 2008.

Every newborn was subjected to: Through history using special card as shown below (Fig. 1):-

* Birth date.
* Date of sampling.
* Governorate.
* Code number for every sample either the first sample or repeated for follow up.
* Full name.
* Sex.
* Single or twin.
* Full mother name.
* Address and telephone.
* Site of birth (hospital, private clinic or home birth).

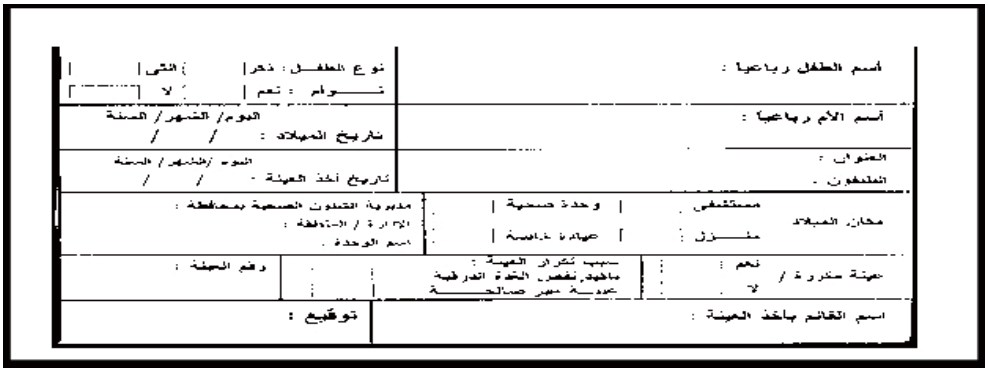

Fig. 1: Card used record to cases in the study.

Clinical examination including: Weight, length, head circumference, presence of jaundice, suckling, Moro reflex and systematic examination of chest, heart and abdomen.

From each newborn a heel prick was taken after sterilization and a sample of blood of 1 cm in diameter is absorbed on filter paper (Schleucher and Schlaull 903 = S & S 903) which is specific filter papers for neonatal screening, left to dry and then two punches were taken (Fig. 2) using specific puncher with certain diameter for phenylalanine (6 mm) and total galactose assay (4 mm) using quantitative colorimetric enzyme assay test.

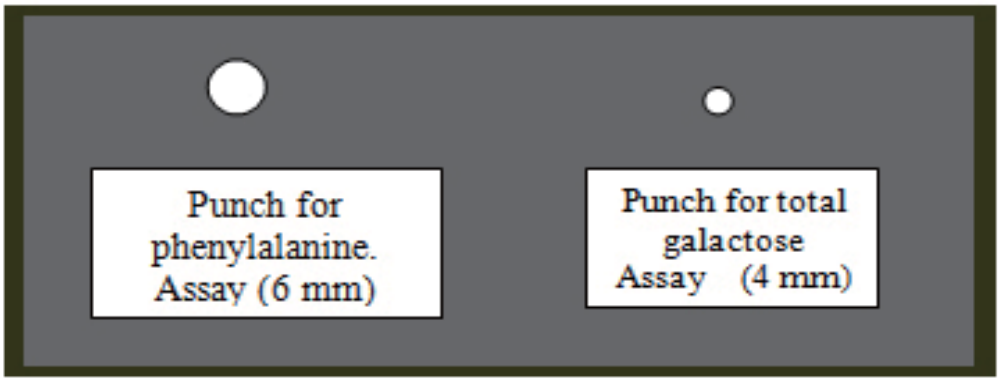

Fig. 2: Size of punches for PKU and for galactosemia.

A- Determination of the amount of phenylalanine in dried blood spot using Quantitative Colorimetric Enzyme Assay test:

1. 6 mm diameter blood spot is punched into the ELISA plates well from each sample. 200 ul 3% trichloroacetic acid. Standard and controls should be punched in duplicate.
2. Incubate for 30 – 60 minutes at 18 – 26ºC.
3. Pipette 15 µl of 0.5 M NaOH solution into the wells of a clean flat bottom microtitration plate
4. Add 80 µl of each blood spot eluate or standards, controls and specimens to the wells cotaining 15 µl 0.5 M NaOH.

5. Add 100 µl working enzyme reagent to each well, mix and incubate for 30 minutes at 18 – 25ºC.

6. Add 100 µl colour reagent to each well, mix and read the absorbance at 570 nmand 690 nm 2-5 minutes after colour reagent addition.

7. Calculate the concentration of controls and unknowns from the absorbance of the standard curve.[24]

B- Determination of total galactose (Galactose and galactose -1- phosphate) in dried blood spot using Quantitative Colorimetric Enzyme-linked immunoassay test:

1. Punch 4 mm spots into microtitration plate wells. Pipette 10 µl precipitation reagents into each spot and allow to stand at room temperature for 10 minutes.

2. Pipette 100 µl of PBS or saline into each well and incubate at room temperature for 30 minutes.

3. Pipette 40 µl standard/control into designate wells of a clean flat bottom microtitration plate.

4. Transfer 40 µl of the blood spot eluate from the elution plate to the equivalent position of clean flat bottom microtitration plate and add 100 µl working enzyme reagent.

5. Incubate for 30 minutes at room temperature.

6. Add 100 µl colour reagent to each well and read the absorbance at 490/690 (dual wave length) 5 minutes after the addition of the colour reagent. The galactose concentration of the patient blood spot is calculated by reference to the absorbance values of the standards. A standard curve is constructed by blotting the absorbance at 490/690 nm of the standards of vertical (Y) axis against galactose concentration of the standard curve on the horizontal (X) axis.[24]

RESULTS

The study included 3000 newborns. Their mean ages was 9.3 ± 2.43 days and mean weight was 3.1 ± 0.82 Kg. They were 1800 (60%) males and 1200 (40%) females from Menoufiya Governorate.

Table 1: Phenylalanine and galactose levels among screened newborns.

Phenylalanine level mg/dl	Number	%	Total galactose mg /dl	Number	%
2 - 5	2183	72.8	2 - 6	2528	84.26
5 - 7	705	23.5	6 - 8	450	15
7 - 10	111	3.7	8 - 12	21	0.7
22	1	0.033	19	1	0.033
Total	3000	100	Total	3000	100

Table 2: Frequencies of Type of Labor, Gender and Consanguinity among the Screened Newborns.

Parameters	Number	%
Type of labor		
Cesarean	1080	36
Vaginal	1920	64
Sex		
males	1800	60
Females	1200	40
History of consanguinity		
Positive	1710	57
Negative	1290	43
Total	3000	100

DISCUSSION

Although newborn screening programmes started about 30 years ago in many countries, they are still lacking in a larger number of developing countries. The aim of such screening programmes is the prevention of disability and handicapping through simple tests and subsequent dietary treatment. As we are dealing with autosomal recessive disorders, the incidence is expected to be higher with increased consanguineous marriages in our population.

In our study, which included 3000 newborns, the mean age of sample collection was 9.3±2.43 days and the mean weight was 3.1±0.82 Kg. This is a relatively high age for sample collection since; the screening programs collect the samples between 3rd - 5th day after delivery. Our study included 1800 (60%) males and 1200 (40%) females. Male predominance has been encountered in other studies in our population. Positive consanguinity was found in 57% of the samples.

The consanguinity rate might be higher than Prof. Temtamy's study because in this study Menoufiya Governorate is the only source of the cases. This is a rural area with many villages following old customs this is the cause of the high consanguinity rate.

64% were delivered by vaginal sections and 36% by cesarean delivery. The phenylalanine level ranged from 1.2 – 22 mg/dl with a mean of 3.19 ± 1.82 mg/dl and the galactose level ranged from 2 – 19 mg/dl with a mean of 3.34 ± 2.23 mg/dl.

This study showed that among the 3000 neonates, 2183 (72.8. %) had phenylalanine levels ranging from 2-5 mg /dl, 705 (23.5%) had levels ranging from 5-7 mg /dl, 111(3.7%) had levels ranging from 7-10 mg/dl and one newborn (0.033%) had phenylalanine level of 22 mg /dl which. The child appeared clinically normal at the time of diagnosis. On repeating the test, it was found to be a true case Phenylketonuria (PKU). Those with a phenylalanine levels ranging from 7-10 mg /dl (3.7%) were reassayed again after one week and found to be transient hyperphenylalaninemia.

Hyperphenylalaninemia is a common finding in many previous studies. This is a transient condition, due to low birth weight or immature liver.

By repeating the test after one week they should be all reverted to normal. Persistence of the high value or even rise in the phenylalanine level means a case of PKU which needs immediate management.

Galactosemia screening showed that 2528 neonates (84.3%) with galactose levels ranging from 2-6 mg/dl, 450 (15%) had levels ranging from 6-8 mg/dl, 21(0.7%) had levels ranging from 8-12 mg/dl and one newborn (0.033%) had galactose level of 19 mg /dl. The child was reassayed and found to be to true galactosemia with jumping in the total level to 120mg/dl. That newborn presented with mild jaundice which progressed to a picture similar to neonatal sepsis in the form of poor suckling, vomiting, hepatomegaly and failure to gain weight. All signs and symptoms improved on using lactose free milk.

This child was rescued by the study, because by the time of the appearance of the signs and symptoms the diagnosis was confirmed and dietary treatment with lactose free formula was immediately started. Otherwise, this newborn would have been treated as neonatal sepsis and might have died without a final diagnosis is reached.

As for the 21 cases, which had total galactose levels ranging from 8-12 mg/dl, they were all retested after one week from the first test and reverted to normal levels. Galactose is a very sensitive liver marker. Any liver insult will increase the total galactose level. In a previous study on Galactosemia done on high risk Egyptian children with hepatomegaly found that liver insult raises the total galactose level, being a very sensitive marker to liver damage.

The actual prevalence of PKU and galactosemia in this study were 1/3000. This is higher than that reported by Temtamy[25], who found in a pilot study on 15,000 newborns in 3 governorates in Egypt that the incidence of PKU was 1/ 7500 and of galactosemia 1/2350[25]. Other studies were done in different populations to determine the prevalences of PKU and galactosemia as shown in (Table 3).

The latest consensus in Egypt showed that at least one million babies are born every year. Our study postulates that per year 333 newborns will have PKU and 333 newborn have galactosemia. Both are treatable disorders and if diagnosed early handicapping caused by these two disorders could be prevented. The prevention of such treatable disorders depends on planning an efficient screening program; aware of the problems which might face our population. One of these problems is that the patients after delivary due to many social and cultural habits and lack of awareness will not come again to the health service unit just for newborn screening, especially when the newborn looks healthy. There is variation between countries as to the exact time of screening but there is general agreement to be during the first three weeks of life as we did in our work and it is advisable to repeat the screening tests if done during the first 3 days of life. This high incidence in Menoufiya Governorate needs urgent planning of a neonatal screening program to save the expected affected numbers of diseased children and to find out the real prevalence of such disorders in this governorate with dense population number in the Delta region.

Table 3: Prevalence of PKU and Galactosemia by Different Authors.

Author	Year	Number studied	Prevalence	Population
Durad [26]	1992	12000 PKU 15000 galactosemia	1/12000 1/15000	UK
Scriver [1]	1995	25000 20000 12000	1/25000 1/20000 1/12000	Turkey California Massachusetts
Temtamy [25]	1998	7500 PKU 2350 galactosemia	1/7500 1/2350	Egypt
Fateen [27]	2001	437	5/437	Egypt
Zylkovicz [28]	2001	160,000	7/160,000	England
Kimura [29]	2001	309914	4/309914	Japan
Torrs [30]	2002	10,000 PKU 30,000 galactosemia	1/10000 1/30000	Spain
Abu Shahla [31]	2004	100,000 PKU	28.3/100000	Gaza Strip

In the present study the only baby with true PKU (1/3000, 0.033%) had phenylalanine level of 22 mg/dl. Clinically, he was a male baby of low birth weight 2.05 kilogram. Otherwise, he appeared clinically normal. By follow up and repeating the test, he was cofirmed to be classical phenylketonuria and dietitically managed using phenylalanine free formula. His growth, physically and mentally went within the normal variation.

Those with a phenylalanine levels ranging from 8- 12 mg / dl (12 %) were tested after two weeks and found to be to transient hyperphenyalaninemia. May be due to prematurety and immaturity of liver and others due to low birth weight. But on follow up and repeating the test they returned to normal levels within one month and needed no treatment. All positive screening tests must be repeated by taking a new sample. If phenylalanine levels decreases, this is a case of transient hyperphenyalaninemia that does not need treatment. But if the case showed increase in the phenylalanine level, treatment must be instilled immediately.

There were highly significant negative associations between phenylalanine and age of newborns and weight. But positive associations with sex, being higher in males; type of labor either vaginal or cesarean being higher with vaginal delivery. This is attributed to higher number of vaginal deliveries than cesarean ones.

The results were in accordance with Abu Shahla 2004 who reported that approximately 60 % of PKU patients had consanguinous parents (first cousins), while 40 % showed no consanguinity.[31]

Outcome data from the continuous follow-up study showed that IQ of PKU patients was inversely correlated with

blood phenylalanine levels. Accordingly, new treatment guidelines were issued that involved more stringent restriction of phenylalanine levels with the recommendation to start dietary treatment within 20 days postpartum.[32]

This result was in accordance with Fateen, 2001 who screened 437 newborn and found that phenylalanine was significantly higher in 5 newborns and galactose levels were high in 6 newborns and showed no significant change between cord blood sample and heel prick at third day of life but this high prevalence should be repeated at 2 weeks to exclude transient hyperphenylalaninemia in phenylalanine and galactose levels[33]. In this study, we used quantitative colorimetric enzyme-linked immunoassay method which also was used by Schulze et al.[34], who screened 400000 newborn for PKU and have clearly shown that enzyme assay is highly reliable, sensitive as compared to Guthri test[34] or Fluorescence intensity (FI) which is influenced by high temperature, humidity, duration between sampling and testing, and anemia. He stated that this method was useful, simple, and highly reliable for newborn mass screening of phenylketonuria.[35]

Prevalence of a disorder can only be estimated in large number of screened newborns. Still the high consanguinity rate is alarming and points to a high incidence of autosomal recessive disease in Menoufiya Governorate found in this study.

Our study neither prove prevalence nor frequency, it is only a small population study.

The whole Menoufiya Governorate should be screened before we can talk of prevalence or frequency. But the data obtained from this studied population sample is alarming and larger studies are needed to estimate the magnitude of the problem in each governorate separately.

Koch in 1999 reported the normal level of phenylalanine was 0.5 -1 mg/dl and some of the false negative PKU infants to the samples taken in first 24 hours of life. So PKU may be missed during early newborn screening. He stated that:-

- Transient hyperphenylalaninemia due to delayed maturation of enzymes required for amino acid metabolism was 4-10mg/dl

- Breast fed infants have a low phenylalanine level than formula fed infants as breast milk contain 12 -14 mg phenylalanine per ounce while formula fed 24 -28 mg phenylalanine per ounce.

- Positive results were not problems as confirmatory repetition of PKU test is done and false positive results easily discovered.[36]

In the present study, 84.26% (2528/3000) have galactose levels ranging from 2 - 6 mg/dl. Fifteen percent (450/3000) had total galactose level ranging from 6-8 mg /dl, 0.7% (21/3000) had total galactose level ranging from 8-12 mg /dl and found to have transient elevated galactose. One patient 0.033% (1/3000) had total galactose level of 19 mg/dl which when repeated for confirmations was found to be 120 mg /dl. The child presented by mild jaundice and then progressed to a picture like neonatal sepsis, week suckling, hepatosplenomegaly and failure to gain weight, which was improved on

galactose free diets. Cataract has developed inspite of the dietary treatment.

Dietary treatment: Babies with transient hypergalactosaemia were placed initially on a galactose-restricted formula. A galactose challenge test (blood examination after having about 100-150 ml normal milk) was performed and dietary restriction was lifted if their blood galactose level had normalized at three –four weeks of age.[9]

In our study the prevalence of true galactosemia was 1/3000 newborns which was higher than that remembered by Nishimra, 2004 who reported the incidence of galactosemia neonatal screening to be 0.16% (100/63542), from them 0.15% had transient galactosemia and 0.009% had persistennt galactosemia. Nishimra 2004 referred the etiology of transient galactosemia to delayed closure of the ductus venosus, heterozygous GALE (UDP-galactose 4-epimerase) deficiency, and heterozygous GALT (galactose-1-phosphate uridyltransferase) deficiency and due to undetermined causes. Also Nishimra 2004 explained the etiology of persistant galactosemia to hepatic haemangioendothelioma with portohepatic venous shunt, patent ductus venosus with hypoplasia of the portal vein, citrin deficiency, homozygous GALE deficiency and heterozygous GALE deficiency.[11]

False positive results in newborn screening for galactosemia are frequent and represent a substantial problem for screening programs. A common observation is the adverse affect that environmental factors and sample handling procedures (practiced at the site of specimen collection or during specimen transport) may have effect on the galactose level, resulting in low activity and in false positives.[37]

CONCLUSIONS

The estimated prevalence of each of phenylketonuria and of galactosemia in this study is 1/3000 newborns, while in a previous study on 15000 Egyptian newborns it was 1/7500 for PKU and 1/2350 for galactosemia.

Because early diagnosis and treatment can prevent the irreversible sequalae of the diseases and their complications, the prevention of such treatable disorders depend on planning an efficient screening programme especially within the first three weeks after birth.

So, we recommend multicenter studies in different governorates in Egypt to estimate the real prevalence data and the magnitude of the problem in our country.

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