Trends of congenital hypothyroidism and inborn errors of metabolism in Pakistan

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Abstract

Background: Metabolic disorders are heterogeneous group of genetic disorders that are responsible for significant neonatal and infant morbidity and mortality worldwide. In developing countries like Pakistan where infant mortality is high current population based studies are unable to gauge contribution of metabolic disorders in causing mortality and morbidity. It is essential to address this gap by a review of available scattered Pakistani data related to metabolic disorders specifically congenital hypothyroidism and inborn error of metabolism to calculate probable burden of these disorders.

Main body: Unfortunately currently in Pakistan newborn screening which identifies these illnesses at birth as a preventive strategy are not available. For current review data was collected through a systematic search of published articles (including data related to screening in certain subgroups of patients admitted to pediatric/neonatal intensive care units, patients with developmental delay/mental retardation).

Conclusion: The primary aim of this review was to get an estimate of the disease burden in the Pakistani population as true prevalence of Congenital Hypothyroidism and Inborn Errors of Metabolism in Pakistan is not available. This systematic review will help us to identify the rough idea about the scale of problem in Pakistan.

Keywords: Metabolic disorders, Inborn errors of metabolism, Challenges, Pakistan

Background

Metabolic disorders like congenital hypothyroidism (CH) and inborn errors of metabolism (IEM) are considerable cause of disease and death among children in both the advance and developing nations of the world with the prevalence of CH is about 1 per 3000–4000 and IEM is 1 in 800–2500 births in West [1, 2]. Metabolic disorders are genetically inherited biochemical disorders of specific enzymes or proteins causing a block in a normal metabolic process of protein, carbohydrate or fat metabolism. Classification is challenging as it can occur in every biochemical pathway. Based on pathophysiology, there are three main sub groups of IEM; conditions that cause intoxication, conditions of energy metabolism and conditions of complex molecules [3]. Severity of the symptoms experienced by affected individuals of IEM varies relying on the genetic and mutation history [4]. Due to absence of neonatal screening facilities in Pakistan children born with IEMs are usually diagnosed on the basis of clinical symptoms including lethargy, poor feeding, apnea or tachypnea, and recurrent vomiting. That makes it difficult to manage childhood morbidity and mortality due to these disorders in an effective manner. Multiple studies in selected scattered groups of Pakistani patients have confirmed the high prevalence of IEMs is due to the high rate of inter-marriages and large family sizes [5]. To classify CH we can use etiological basis of disease according to which there are two main categories of these disorders (a) disorders of thyroid gland development (Group of dysembriogenesis or thyroid dysgenesis) or (b) defects in any of the steps of thyroid hormone synthesis (group of dyshormonogenesis) [6, 7].
Status of IEM diagnosis and management in Pakistan
Though some information regarding mental retardation and new born screening (NBS) for CH in Pakistani population is available since 1987, but a true prevalence of IEM is yet to be determined [5, 8]. Relative or true incidence of IEM in the population or even among the critically ill newborns through systematic reviews is lacking as NBS is not available in Pakistan. Mostly IEM is diagnosed either through biochemical tests on specific population mostly pediatric population. Mostly studies have bias of screening for IEM in high risk patients either in intensive care of pediatrics or medicine [9].

To determine true prevalence of IEM in Pakistan one important and ideal strategy needs to be applied, i.e., Prospective data collection from a universal and expanded screening of a low risk population. Mass spectrometry is considered to be ideal for screening metabolic IEM but very few centers in Pakistan are offering these services either in collaboration with tertiary care hospitals of Pakistan or abroad for few large cities [5, 10, 11].

Material and methods
The purpose of this study was to learn about the various forms of metabolic disorders across Pakistani population. For current review data was collected through a systematic search of published articles on metabolic disorders including congenital hypothyroidism and inborn error of metabolism among Pakistani population. Data related to screening in certain subgroups of patients admitted to pediatric/neonatal intensive care units, patients with developmental delay/mental retardation was included. Studies from Pakistan in which metabolic disorders were evaluated for the markers, technologies and modalities to reach the diagnosis were specifically included. Search was done to find any possible study with published data considering the overall prevalence of metabolic disorders in general population of Pakistan. For the current project unpublished data or data from institutions was not collected at national or individual hospital levels performing CH and IEM related biochemical or genetic tests locally or abroad. Studies, in which probability of IEM was not confirmed by biochemical or molecular testing, were excluded. Data from all studies included in this review was summarized to obtain an overall picture of the selected disease burden from studies based on selected subgroups (Tables 1, 2) and from studies mentioning about various forms of IEM (Table 2).

Data related to studies
Based on review of these studies data we were be able to include total of 19 IEM specific studies 8 with CH and 11 mentioning about various forms of IEM in a single article. Out of 19 studies related to IEM five were regarding congenital adrenal hyperplasia (CAH) mainly focusing about the diagnosis, research and management based findings. Eight studies were about lysosomal storage disorders, three were about carbohydrate storage disorders including Galactosemia, Glycogen storage disorders, Fructose 1, 6, Bisphosphate. Two studies specifically mentioning about organic acidemia and one about disorders of aminoaciduria. Total numbers of patients mentioned in various studies were found to be around 313. Out of which 78 patients with carbohydrate storage disorder, 66 with lysosomal storage disorder, 47 with organic acidemia and aminoaciduria and 122 with congenital adrenal hyperplasia. In almost all studies biochemical analysis was discussed to reach the diagnosis but significant delay in diagnosis was due to lack of specific diagnostic facilities. Eleven studies specifically mentioned about the use of genetic test for research purpose. Few basic genetic based diagnostic tests done in Pakistan like PCR, RFLP. But sequencing based data mentioned in studies usually obtained from the international labs working in collaboration with academic institutions. None of the study mentioned regarding the availability or use of advance forms of management options like gene editing etc. (Table 1).

8 studies specifically discussed about congenital hypothyroidism. Seven were on biochemical profile of patients and in one survey tool was designed and implemented to collect data from 400 multiparous women. Number of Patients with congenital hypothyroidism tested through biochemical analysis was 212. None of these studies mentioned about genetic tests use and availability for patients with CH (Table 2).

In 11 studies disorders were investigated with the aim to find about various varieties of IEM. Total number of patients were about 536 including 188 patients from various types of carbohydrate storage disorders like Galactosemia, Glycogen storage disorders, 188 with Lysosomal storage disorders including Mucopolysaccharidosis. 220 with organic acidemia, 64 with Aminoacid disorder, alkapturia, tyrosinemia. 4 were with congenital adrenal hyperplasia, 7 with FA oxidation and Carnitine defect, 7 with Ketogenesis and ketolytic defect and 19 with various forms of IEM. Biochemical Analysis was done in all while genetic analysis was not available in any study (Table 2).

Discussion
According to this data we can say that access to Screening is primarily available at a cost from private laboratories within Pakistan and abroad [5, 10, 11]. Through tandem-MS technology we can simultaneously test for more than 40 disorders. Conventional methods help us to diagnose common disorders like CH, congenital adrenal
| Diagnosis                                              | Population                  | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis          | Biochemical methods for diagnosis | References |
|--------------------------------------------------------|-----------------------------|-------------|---------------------------|---------------|----------------------------------------|-----------------------------------|------------|
| Congenital adrenal hyperplasia                         | Karachi                     | 26 patients | Not mentioned             | Not mentioned | Genetic ARMS-PCR (amplified refractory mutation system) | Not mentioned                     | [18]       |
| Congenital adrenal hyperplasia                         | Karachi                     | 63          | 1 day to 12 year          | 33 cases (52.3%) | Not mentioned                          | Enzyme assays mentioned           | [19]       |
| Congenital adrenal hyperplasia                         | Karachi                     | Case series 3 cases | 47, 20, 24 year          | positive      | Genetic analysis through PCR           | Progesterone, testosterone levels done | [20]       |
| Congenital adrenal hyperplasia                         | AFIP Rawalpindi             | Case report  | 5 years                   | positive      | Not mentioned                          | Progesterone, testosterone levels done | [21]       |
| Congenital adrenal hyperplasia                         | AKU Karachi                 | 29 patients | Not mentioned             | positive 65%  | Mutation analysis done                  | Progesterone, testosterone levels done | [22]       |
| Lysosomal storage disorder: Gaucher's Disease          | Aga Khan University, Karachi, Pakistan, with different forms | 2 patients | Not mentioned             | Not mentioned | Not Done                               | BM, Hematological parameters, acid phosphatase level, visceral volumetric CT and MRI, xray, DEXA | [23]       |
| Lysosomal storage disorder: Gaucher's Disease          | AKU Karachi                 | Case report | Not mentioned             | Not mentioned | Not Done                               | Acid phosphatase level done        | [24]       |
| Lysosomal storage disease                              | Peshawar                    | 22 patients | Not mentioned             | Not mentioned | Not mentioned                          | A total of 413 bone marrows were aspirated in 2 months | [25]       |
| Gaucher's Disease                                      | National Institute of Blood Disease and Bone marrow Transplantation | 5 patients out of total 19 patients (10 parents 4 control) | Not mentioned | Not mentioned | Identification of GBA Gene Mutations | B-glucosidase enzyme Levels rather than On bone marrow Morphology | [26]       |
| Diagnosis               | Population                  | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis | Biochemical methods for diagnosis | References |
|-------------------------|-----------------------------|-------------|---------------------------|---------------|-------------------------------|----------------------------------|------------|
| Lysosomal storage disease: Gaucher's Disease Type I | Civil Hospital, Karachi Case report | 18 months | Not Done                  | Not Done      | Long lediostasis of acid β-glucocerebrosidase activity, plasma chitotriosidase, and the presence of Gaucher cells in bone marrow biopsy. The disease was treated with intravenous replacement of the enzyme Imiglucase (Cerezyme) and was followed. | Low leukocyte glucocerebrosidase activity, raised plasma chitotriosidase and the presence of Gaucher cells on bone marrow biopsy. The disease was treated with intravenous replacement of the enzyme Imiglucase (Cerezyme) and was followed. | [27] |
| Niemann-Pick disease | Children's Hospital Lahore Total seven sporadic patients | Not mentioned | Not mentioned | Unrelated patients from consanguineous families | We have mapped five different mutations in SMPD1 gene of enrolled patients with a novel homozygous missense variant (c.1718G > C) (p.Trp573Ser) in one patient, a missense mutation (c.1267C > T) (p.His423Tyr) has been identified in three unrelated patients, a nonsense mutation (c.1327C > T) (p.Arg443Ter) and one missense mutation (c.1493G > A) (p.Arg498His) mapped in one patient each. A compound heterozygous mutation has been mapped in one patient (c.740G > A; c.1493G > A). A novel variant has been predicted through in-silico analysis and has not been reported in general overall population in the globe | [28] |
| Diagnosis | Population   | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis | Biochemical methods for diagnosis | References |
|-----------|--------------|-------------|----------------------------|---------------|-------------------------------|----------------------------------|------------|
| MPS       | KPK, Punjab, Baluchistan, FATA | 8 families | Not mentioned | Not mentioned | DNA extraction Sanger sequencing Insilico (QAU) Linkage analysis followed by sequence analysis of the gene detected four novel (p.Phe216Ser, p.Met38Arg, p.Ala291Ser, p.Glu121 Argfs*37) and two reported (p.Pro420Arg, p.Arg386Cys) mutations in the eight families. In silico structural and functional analysis predicted that these mutations disrupt the function of GALNS protein through fluctuating its three-dimensional structure, stability, and binding affinity and produce severe phenotypes | Not mentioned | [29] |
| MPS       | Pakistan     | Thirteen MPS1-affected children from 12 unrelated cohorts were enrolled | Not mentioned | Not mentioned | Results Six IDUA gene mutations were mapped co-segregating with the recessive pattern of inheritance including a novel variant. A novel missense variant c.908 T>C (p.L303P) was mapped in two affected siblings in a cohort in the homozygous form. The variant c.1469 T>C (p.L490P) was mapped in five unrelated patients and c.784delc (p.H262Tfs*55) was mapped in three unrelated patients, while mutations c.1598C>G (p.S533R), c.314G>A (p.R105Q) and c.1277ins9 [p. (A394-L395-L396)] were mapped in a single patient each | Not mentioned | Mapping of IDUA gene variants in Pakistani patients with mucopolysaccharidosis type 1 |
| Diagnosis                        | Population                                                                 | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis | Biochemical methods for diagnosis | References                                                                 |
|---------------------------------|-----------------------------------------------------------------------------|-------------|---------------------------|---------------|-------------------------------|----------------------------------|---------------------------------------------------------------------------|
| Type 1 Galactosemia (Classical and Duarte) | Department of Pediatric Gastroenterology and Hepatology, Children's Hospital and Institute of Child Health, Lahore | 8 Families  | 1.6–15 months             | 6 Families    | Detection of common mutations in the GALT gene through ARMS | Done locally                    | Not mentioned [13]                                                          |
| Galactosemia                    | Department of Pediatric Gastroenterology and Hepatology at The Children's Hospital and Institute of Child Health, Lahore | 22 patients | Mean age 112 days with a range from 8—510 days | Not mentioned | Not Done                      | Benedict's test (urine), Dipstick (Glysinuria) Enzyme analysis GAL-1 PUT  | [30]                                                                        |
| GSD Type 1a                     | Department of Pediatric, division of Gastroenterology & Hepatology of the Children's hospital, Lahore | 40 pts with GSD out of 360 with liver disorder | 25.6 months | Not mentioned | Not Done                      | Clinical and Biochemical test based diagnosis                           | [31]                                                                        |
| Diagnosis                        | Population                               | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis | Biochemical methods for diagnosis | References                                      |
|---------------------------------|------------------------------------------|-------------|---------------------------|---------------|-------------------------------|-----------------------------------|-----------------------------------------------|
| Methylmalonic aciduria:         | January 2013 to April 2016 at the Aga Khan University Hospital, Karachi | 1,778 patients 50(2.81%) were detected with methylmalonic acidurias. | Not mentioned | Not mentioned | Not Done                      | Methyl malonic aciduria is a biochemical finding present in patients with MMA, Cb1-RD, SUCL deficiency and serum B12 deficiency thus all patients with mmauria should be further investigated with PAA, thcy, B12 and FA levels for the correct diagnosis. A correct diagnosis allows clinicians to prescribe appropriate treatment, leading to better outcome |
| Tyrosinemia Type 1 and Fructose-1, 6 Bisphosphatase Deficiency | Pakistani cohorts Children hospital Lahore | 4 cohorts Hepatorenal tyrosinemia type 1 (HT1) and 8 cohorts fructose 1,6-bisphosphatase deficiency (FBPD) | Not mentioned | Not mentioned | Mapping of two recessive mutations in FAH gene for HT1; c.1062 +5G > A(VS12 + 5G > A) in three families and c.974C>T(p.E281K) in one. We identified three mutations in FBP1 gene; c.841G>A(p.E281K) in five FBPD families, c.472C>T(p.R158W) in two families and c.778G>A(p.G260R) in one | Not mentioned | Genetic Analysis of Tyrosinemia Type 1 and Fructose-1, 6 Bisphosphatase Deficiency Affected Pakistani Cohorts Muhammad Yasir Zahoor, Huma Ashraf Cheema, Sadaqat Ijaz, Zafar Fayyaz. Published in Fetal and pediatric pathology 2019 Medicine |
| Alkaptonuria                    | Mayo hospital Lahore                     | 2 Cases     | Non-consanguinity         | Not Done      | Urine analysis HGA            | Biochemical assays                | Alkaptonuria – case report and Review of literature Muhammad Nafees1, Muhammad Muazzam. Pak J Med Sci 2007 Vol. 23 No. 4 www.pjms.com.pk |


| Diagnosis          | Population                          | Sample size | Age range of presentation | Consanguinity | Genetic Methods for diagnosis | Biochemical methods for Diagnosis | References |
|--------------------|-------------------------------------|-------------|---------------------------|---------------|--------------------------------|----------------------------------|------------|
| Congenital hypothyroidism | Karachi                            | 4 out of 5000 | Neonate birth to 1 month  | Not mentioned | Not Done                      | Thyroid function tests           | [33]       |
| Congenital hypothyroidism | Karachi                            | 116 hypothyroid 46 with vitamin B12 deficiency | 19 year to 91 year | Not mentioned | Not Done                      | Thyroid function tests           | [34]       |
| Congenital hypothyroidism | Karachi                            | 80 hypothyroid 80 normal mothers | 1 to 3 month postpartum | Not mentioned | Not mentioned                  | Thyroid function tests           | [35]       |
| Congenital hypothyroidism | Pediatric department PIMS Islamabad | 3 babies out of 1337 had CH | Neonates less than 8 days | Not mentioned | Not mentioned                  | Thyroid function tests           | [36]       |
| Congenital hypothyroidism | Gynec/Obs and Pediatric Shaikh Zayed Hospital and Jinnah Hospital, Lahore | 2 out of 1357 cases | neonates | 2 patients of CH | Not Mentioned | TSH levels                      | [37]       |
| Congenital hypothyroidism | Department of Pediatrics Mayo hospital lahore | 4 out of 550 screened | Neonates 4th–7th day of life | Not mentioned | Not mentioned                  | Thyroid function tests           | [38]       |
| Congenital hypothyroidism | Pathology Department of Allama Iqbal Medical College, Lahore in collaboration with Pediatrics and Gynecology & Obstetrics Department, Jinnah Hospital, Lahore | 3 hypothyroid out of 770 screened neonates | Not mentioned | Not mentioned | Not mentioned                  | Serum TSH by immunoassay         | [39]       |
| Congenital hypothyroidism | Karachi                            | 400 multiparous women | < 26 to > 35 year | Not mentioned | Not mentioned                  | TSH screening done survey based  | [40]       |
hyperplasia (CAH) and Galactosemia etc. DNA based testing is not freely available for screening and diagnosis. Few research institutes in Pakistan are working with international collaboration and they are offering genetic tests for few common disorders wherein causal gene is already known [12–17]. But for a proportion of IEM, the causal gene or the mutations in various genes remain to be identified in our ethnic population and thus newborn screening (NBS) program encompasses a strong research component as well [5].

In Pakistan earliest reports of IEM have been far and rare and mostly based on testing newborns with suspicion of IEM or referred to tertiary care centers for intensive care. Some studies included group of subjects who were tested for intellectual disability (Tables 1, 2) [11]. A number of studies from different centers and universities in Pakistan have considered the Pakistani population in the last 15 years for biochemical and genetic analysis and have diagnosed congenital hypothyroidism (CH), congenital adrena hyperplasia (CAH), carbohydrate storage disorders, lysosomal storage disorders and organic acidemias (Tables 1, 2). These disorders have been tested at a number of centers, and samples mostly collected from intensive care departments, pediatric units and from hematology units of tertiary care hospitals in Pakistan including Karachi, Lahore, Islamabad, Peshawar and Faisalabad. Mostly patients referred to these centers for diagnosis and better management [5, 12–15] (Tables 1, 2). It is apparent from the data compiled and presented that there is a need for establishing/identifying reliable labs for biochemical testing and genetic confirmation and taken together, warrant formulation of a standard protocol for testing and follow up of IEM positive cases in the country.

In summary, the present findings suggest that expanded NBS for all treatable and untreatable IEM is carried out at a very few centers in the country; NBS for common treatable conditions namely CH and few organic academia are being carried out in a limited number of hospitals at a few places such as Islamabad, Karachi and Faisalabad in pediatric cohorts with index of suspicion of IEM [5, 8, 10, 11]. Patients are tested on referral at several hospitals/private testing labs in the country; but epidemiological data for all the testable or untreatable IEM in Pakistan are not yet available.

Conclusion:
Keeping the genetic landscape of the Pakistani population (for example diversity) combined with socio-cultural practices (for example consanguinity), the need to carry out systematic prospective expanded screening for IEM in each of the Pakistani province to identify the prevalent disorders is imminent. Finally, ongoing and future prospective studies in Pakistan will be useful with moderate sample size in the country which will provide the much needed epidemiological data for this large group of genetic disorders. In future we must try to overcome challenges of IEM in our country including new born screening (NBS). Findings from these studies from different regions of Pakistan will help Pakistan’s health policy makers to mandate universal and expanded NBS in different provinces in the country.

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