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Review Article

SCOPE OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN INBORN ERRORS OF METABOLISM SCREENING: A REVIEW

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ABSTRACT

Inborn errors of metabolism (IEM) arise from inherited errors in enzyme in any of the metabolic pathways. Symptoms are developed due to the accumulation of biosynthetic precursors or product that baby cannot excrete. IEM screening is a field that is undergoing a revolutionary change in its all aspects. Technological advances are transforming the way in which we can diagnose the whole population of IEM. In last few decades, evolution of gas chromatography-mass spectrometry (GC-MS) has improved our diagnostic capabilities in many fields. It is now possible to screen rapidly, simultaneously and inexpensively for a number of very rare disorders using GC-MS. Recent developments in the field of analytical and separation science and MS has brought about great improvement in GC-MS efficiency and sensitivity. The use of this technique in newborn screening made it possible in the present scenario, to screen for a wide range of previously unscreened IEM, including aminoacidemias, fatty acid oxidation disorders, and organic acidemias. Early diagnosis at appropriate time may help to decrease mortality and morbidity rates in children with IEM. In fact, the technique has tremendously improved the efficacy of neonatal screening programs. Uses of GC-MS demonstrate the importance of early identification and treatment of infants with disorders that would if go unrecognized, lead to irreversible clinical damage. The growing realization of the significant role of GC-MS in IEM screening resulted in extensive research in this area. Several excellent publications over the recent years, describe the crucial role played by the technique in fast high-throughput screening. The contributions by Matsumoto and Khurana together with the most recent work are the excellent areas for anyone intend to enter this field.

Keywords: Inborn errors of metabolism, Gas chromatography-mass spectrometry, Inborn errors of metabolism screening through gas chromatography-mass spectrometry, Neonatal screening.

INTRODUCTION

Inborn errors of metabolism (IEM) are a group of disorders arising from inherited errors of biological pathways, which can be of intoxication type (accumulation of intermediates of metabolism) or energy deficiency type pathways [1]. Certain pathological alterations in normal catabolic path of amino acids, carbohydrates, lipids or biogenic amine often result in abnormal excretion pattern of organic metabolites that are normally absent or present in very small concentration [2]. These IEM currently in human beings exceed 500. Of them 100 alone are the errors of aminoacids metabolism. Among these phenylketonuria (PKU) and maple syrup urine diseases (MSUD) are severely inherited [3].

The clinical conditions are generally manifested by various life threatening episodes including metabolic acidosis, hyperammonemia, recurrent vomiting, failure to thrive, neurological retardation, etc. In many of these disorders timely intervention and appropriate management can prevent the pathological events happening in the body. However, the therapeutic efficacy will be reduced if timely treatment and management are not preceded. For example PKU and MSUD not being detected and managed in early infant life, could result in severe mental retardation which is irreparable [4]. Therefore IEM screening in early infant life is very important in order to manage the disorders and save life. Various neonatal screening programs for early diagnosis of IEM are running in various developed countries and at very low pace in developing countries for prevention and significant reduction of clinical symptoms [5].

Garrod for the first time in early 1900 introduced the term "IEM" to describe the hereditary deficiency or alterations in the enzyme reaction [6]. Alkaptonuria disorder was firstly coined as "inborn error" [6]. In those times, there was no proper understanding of metabolic pathways. Only on the basis of observations of gross abnormalities such as darkened urine in alkaptonuric, disorders were originally recognized in individuals who were severely ill or showing dysmorphic features or mental deviations. These abnormalities were understood to be inherited on the basis of their recurrence noticed among the family members. With gradual evolvement in the field of metabolism, our knowledge about inherited metabolic disorders and identification of concerned enzymes had broadened the spectrum of IEM [7]. Finally, it was concluded that various body fluids can be exploited for analyzing and identifying the unwanted accumulated substances which are products of pathways in response to enzyme block [8]. These IEM can have deleterious effects on the life of human beings. So there is a requirement of comprehensive diagnostic neonatal screening in order to avoid any physical or neurological damage to the body. Early detection always aims to treat clinically important disorders in order to minimize morbidity and mortality in early childhood.

CASCADE OF EVENTS IN IEM SCREENING

It was the Guthrie and Susi in 1963 [9], for the first time introduced the method applicable to whole population screening for PKU. In 1962, the state of Massachusette instituted the first newborn screening program for PKU [1]. Till that time, it was very much clear fact that early detection of PKU and early introduction of diet low in phenylalanine resulted in a significant decrease in morbidity. For last two decades, mass spectrometry (MS) has been proved to be an important tool for diagnosing metabolic errors. Apart from gas chromatography-MS (GC-MS), electrospray MS, electrospray ionization (ESI)-MS-MS, liquid chromatography (LC)-MS were also been frequently used without derivatization of metabolites and their chromatographic separation [10]. In fact before the advent of MS, most analytical biochemistry procedure relied upon lengthy cumbersome procedures. These included an initial chromatographic separation followed by

identification of separated compound through suitable means of colorimetry, fluorimetry, or radioisotopic measurement [10].

In the year 1966, Tanaka *et al*. at the Massachusetts General Hospital was the first to detect isovaleric academia by GC-MS showing the importance of this method [11]. In Japan, a program was initiated to diagnose 22 inherited metabolic diseases in year 1977 at Kurume University. Since 1977, a number of studies were carried out; gradually improving chemical diagnosis through GC-MS [1]. Shoemaker and Elliott at St. Louis University in US reported that organic acids and sugars can be separated clearly by GC-MS [12]. His procedure however was invasive and time taking which was further simplified by Kuhara *et al*. [13]. As a result, the simultaneous analysis of amino acids, nucleic acid bases, nucleoside, sugars and sugar alcohols in addition to organic acids became possible. A GC-MS was used in the year 2007 for quantification of Phe, Tyr, Leu and Val in blood spots [14]. The method was minimum invasive, sensitive, rapid and inexpensive and allowed simultaneous quantitation of several amino acids in small blood spot. The success of the blood spot newborn screening in the USA and other developed countries led to early screening efforts in parts of the Asia Pacific Region from the mid-1960s onward. Later not only blood, but other body fluids i.e. urine were also frequently used for screening purposes. From the perspective of metabolic biochemistry, body excretes all unnecessary and toxic compounds in the urine that exceed the desired level in blood by homeostasis. Therefore, substances toxic to the body such as those compounds sought in neonatal mass screening, also excreted in the urine in a large amount make facilitated detection through GC-MS. Moreover, collection of urine is more facile over invasive method for collecting blood via sole puncture.

Though the exact incidence and prevalence of most of the disorders is not known as large population-based studies were not done. Some information is available to have an idea about the disease burden in India also. A pilot newborn screening project was carried out on 125,000 newborns. Homocysteneimia, hyperglycinemia, MSUD, PKU, hypothyroidism and Glucose-6-phosphate dehydrogenase (G6PD) deficiency were found to be the common errors [15]. Another pilot expanded newborn screening was started in 2000 at Hyderabad to screen amino acid [16] disorders, congenital adrenal hyperplasia, G6PD deficiency, biotinidase deficiency, galactosemia and cystic fibrosis [17]. Recent data from Kerala has suggested congenital hypothyroidism to be about 2.1 per 1000 live inborn babies [18]. Considering the available Indian data, there is a need for universal newborn screening for all newborns in India.

GC-MS AND IEM SCREENING

MS technique as proved to be an indispensible method for diagnosing metabolic errors. By using E-electrospray MS, ESI-MS-MS, methods were developed without derivation and chromatographic separation. Multiple reaction monitoring was used instead of constant neutral loss scanning. The detection of aminoacids and quantitative determination is done via isotope labeled internal standard [19,20].

GC-MS in itself is an invaluable tool in the field of IEM. GC-MS is typically used to quantify and identify primarily nonpolar metabolites influencing the practice that are <1 KD in mass [21,22]. GC-MS combines the separating power of GC, with the detection power of MS. MS is a wide-ranging analytical technique very efficient technique. It involves the production and subsequent separation and identification of charged species according to their mass to charge (m/z) ratio. GC has developed into a sophisticated technique since the pioneering work of Martin and Synge in 1952. GC is capable of separating very complex mixtures of volatile analytes. In GC, the mobile phase is a gas and the stationary phase is either a solid, known as “gas-solid chromatography” or it may be an immobilized polymeric liquid, referred as “gas LC” [23-25].

Additions of efficient technical advances in the lab medicine are of much pivotal importance, and their clinical application is tremendously influencing the field of medicine. Introduction of GC-MS to the diagnosis of IEM has been one such sophistication to the field of pediatric science. Being the most commonly used method for small molecule analysis, it enables the identification of key small molecules such as aminoacids in biofluids, particularly in urine and blood [1]. GC-MS is simple, inexpensive, easily operated and high-speed technique. MS in itself is a well-established quantitative tool for small molecules. MS also facilitates the identification of previously uncharacterized metabolite. MS-MS [26] instrument is very costly to be afforded by small hospitals. Moreover, it is not widely used for aminoacidemias, including PKU and MSUD in developing countries. In contrast GC-MS is quite simple, inexpensive and user-friendly technique. A more convenient and advanced method was introduced by Matsumoto and Khurana [1] for IEM screening using GC-MS enabled accurate chemical diagnosis through urine samples. The urine sample preparation takes 1 hr for I sample and 3 hrs for batch of 30 samples, followed by GC-MS analysis that takes 15 minutes per sample.

The great advantage with the method was the simultaneous analysis of aminoacids, organic acids, sugars, sugar alcohols, sugar acids and nucleic acid bases. Therefore, a large number of disorders can be screened together in a short span of time. Previous method is not well suited for such a broad category of compounds. The method was developed using urine samples for GC-MS analysis. Sample preparation involves urease treatment, deproteinization with alcohol, evaporation to dryness of supernatant, and finally trimethylylation. For neonates urine samples were collected into the filter papers, dried with air and for analysis, these dried samples were eluted with water [1]. Initially, GC-MS analysis was hampered by inadequate chromatographic separation using packed column and slower scan rate. Current advances in separation methodology in association with MS have brought about great improvement in GC-MS efficiency.

CONCLUSION

The last few decades have evolved the implementation of advanced methodologies in IEM screening in various developed and developing countries like US, Europe, China and Japan, etc. IEM screening is basically the process of testing newborn or any age group subject for treatable genetic, endocrinologic, metabolic and hematological diseases before the development of symptoms. In the Newborn period IEM can easily be misdiagnosed as sepsis or birth asphyxia. Therefore, fast screening at the earliest is of much importance. Use of GC methods for detection and diagnosis of IEM over the past few years became a more common through the use of a variety of samples for examples blood, urine, cells, and tissues, etc. Continuous advances in high resolution GC with MS, development of newer combination of efficacious techniques like GC-tandem MS, multidimensional GC-MS has dramatically changed the field of medicine and pediatric allowing better monitoring of IEM.

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