

Introduction:

For more than forty years, the Guthrie bacterial inhibition test has formed the basis for successful national neonatal screening for PKU (1). Similar methods were adopted for disease such as MSUD, hypermethioninemia Homocystinuria, and Galactosaemia (2,3). The Discovery of new metabolic disease likes organic acedemias and fathy acid oxidation (FAO) defects that are like- threatening to the neonate necessitates the development of newer techniques for their detection (4,5). The development and the relatively recent introduction in the overall approach to neonates screening for may intermediary defect (6).

Ms/Ms is a device that separates and quantifies ions based on their mass/charge $M/2$ ratios. The devices consist of two chamber and a third collision chamber. The mixture of the analysis of the sample undergoes a soft ionisation procedure (Fast atom bombardment or Electro spray) to create quasimolecular ions, which are injected, in the first chamber to separate parent ions. The are then passed (in order of their M/Z ratio) to the reaction chambers where they are fragmented. The M/Z ratios of the nagements are then analysed in the second chamber. The whole procedure including data acquisition by a computer talles about two minates (7). Filter- paper blood specimens are used and as little as one nanomole of analyate per 1ml of whole blood may be sufficient.

This technique has been applied to the metabolic profiling of acyl- carmtines from blood spots. The profiles obtained are highly diagnostic for organic academies, FAO defects and a number of amino acid defects like PKU, MSUD and urea cycle defects. Thus identification of > 20 inborn errors of metabolism is feasible in a single assay (7).

In January 1999, the regional centre for hereditary metabolic diseases in Padova Hospital in Italy has started a selective neonatal screening using this technique (8).

Objectives:

Through a link between our department of pediatrics and / health in the University of Khartoum and the department of pediatrics in Padova University in Italy, we had an access to Use the ESI- MS/MS to do a short term neonatal screening for some aminoacidurias (PKU, MSUD, NKH, ASS,ASL). Organic acidurias (propionic acidemia, MMA, IVA, Glutaric acidemia I Hydromethyl glutaric acidemia, Isolated 3. Methylcrotonylglycinemia) and FAO defects (SCAD, MCAD, LCAD, VLCAD, Glutaric acidemia type II carnitine palmitoyl transferase (I and II) deficiency.

Material and Methods:

Patients: All newborns admitted to the nursery in the maternity or police Hospital during the period starting at the first of September and ending by 30th of October 2002 were included in the screening after the Approval of their parents.

Samples:

Blood samples were collected at the age of 6 hours up to 5 days by a head prick as spots onto Schleicher and Schuell 903 filter paper (as shown in figure 1). After recording full name, date of birth, date of sample collection, birth weight and the sex of the baby. Samples were left to dry for few hours before being kept frozen until delivery to the laboratory by the author on the fifth of November 2002.

Sample preparation:

Two 3/16-inch circles from each blood spot were punched out using a standard hole punch. Those punched out spots were then put into SMALL centrifuge tubes and shaken with methanol, already mixed with stable isotope labeled internal standards, for 30 minutes before centrifugation at 14000 rpm for 10 minutes. The supernatants then obtained to glass vials and evaporated to dryness using a gentle nitrogen stream. To the residues butanolic HCL was added. The new mixtures were again dried in the same way. Each residue was finally reconstituted in a mixture of acetonitrile and water, now being ready for Ms/Ms analysis (7).

The Machine:

The analysis was done using an Ms/Ms (Quattro II, micromass, Manchester, UK) equipped with a phoenix 40 HPLC (Thermoquest, Milan, Italy)- Samples were injected automatically through an (As 800 Thermoquest, Milan, Italy).

Scan range and speed of the first chamber were M/Z 210-550 per 3 seconds for acylcarnitines and 120-300 per 1.5 seconds for amino acids (7).

Computerization:

The machine was enrolled by a digital workstation using (Windows NT) and masslynx software (micromass Ltd, UK). Spectra were initially interpreted by Nedyx software (Micro mass ltd, UK). Various analytical ratios were checked against their predefined reference range).(7)

Results:

All 282-sample taker for neonatal screening were found negative for aminoacidemias and organic academies test. One sample showed an abnormal acylcarnific pattern (see figure 2) with raised C4 (Butyryl), C6 (hexanoyl) C8 (Octanoyl), C10 (Decently), C12 (Dodecanoyl), C14 (tetradecanoyl) and 14:1 (Myristoyl) and 16:1 (Palmitotoyl) (see also figures 3-10).

The pattern is very much characteristic of Multiple acyl CoA delydrogenas defect (MADD) of the severe variety (See figure No.11).

- As 285 samples were analysed for same aminoacedemias, organic academies and FAO defects, Sudanese reference values with means and cut- offs of normality (being demonstrated by lines in figures 3-10) are now available.

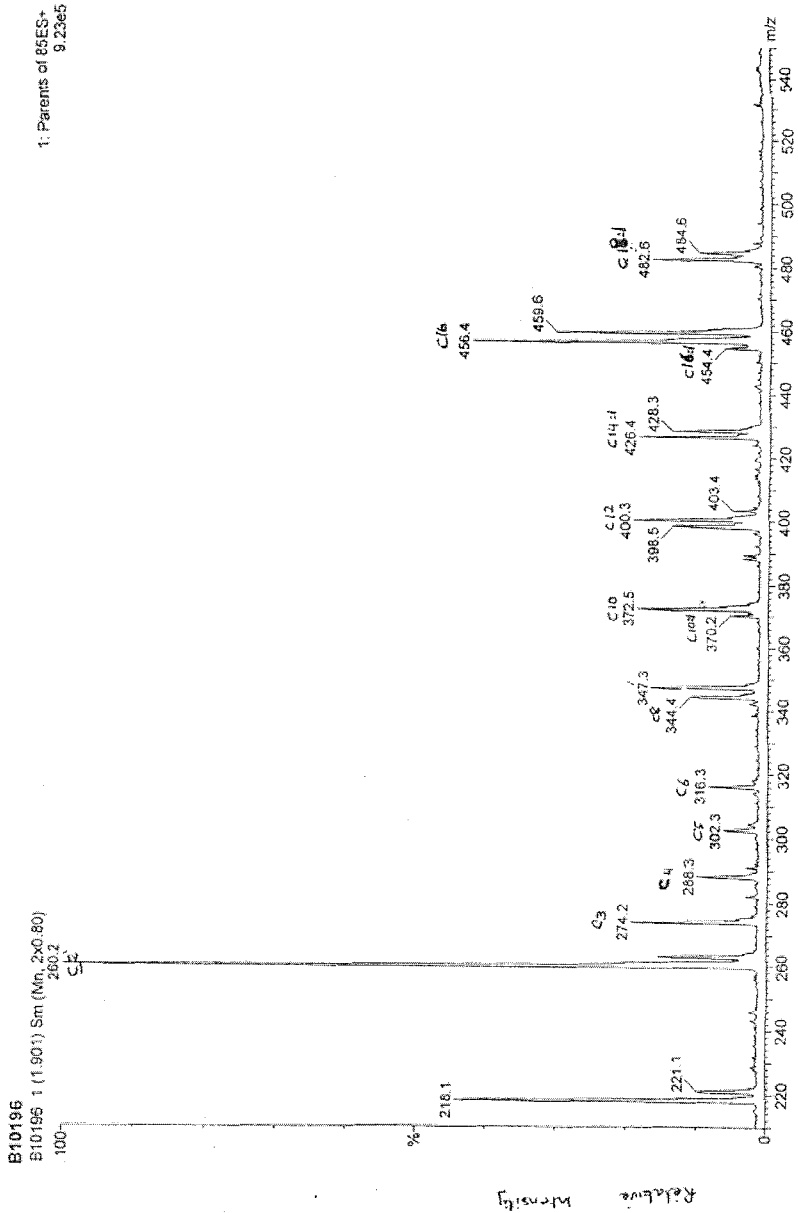


Figure 2: Blood Arginine profile of a normal with a MADD defect.

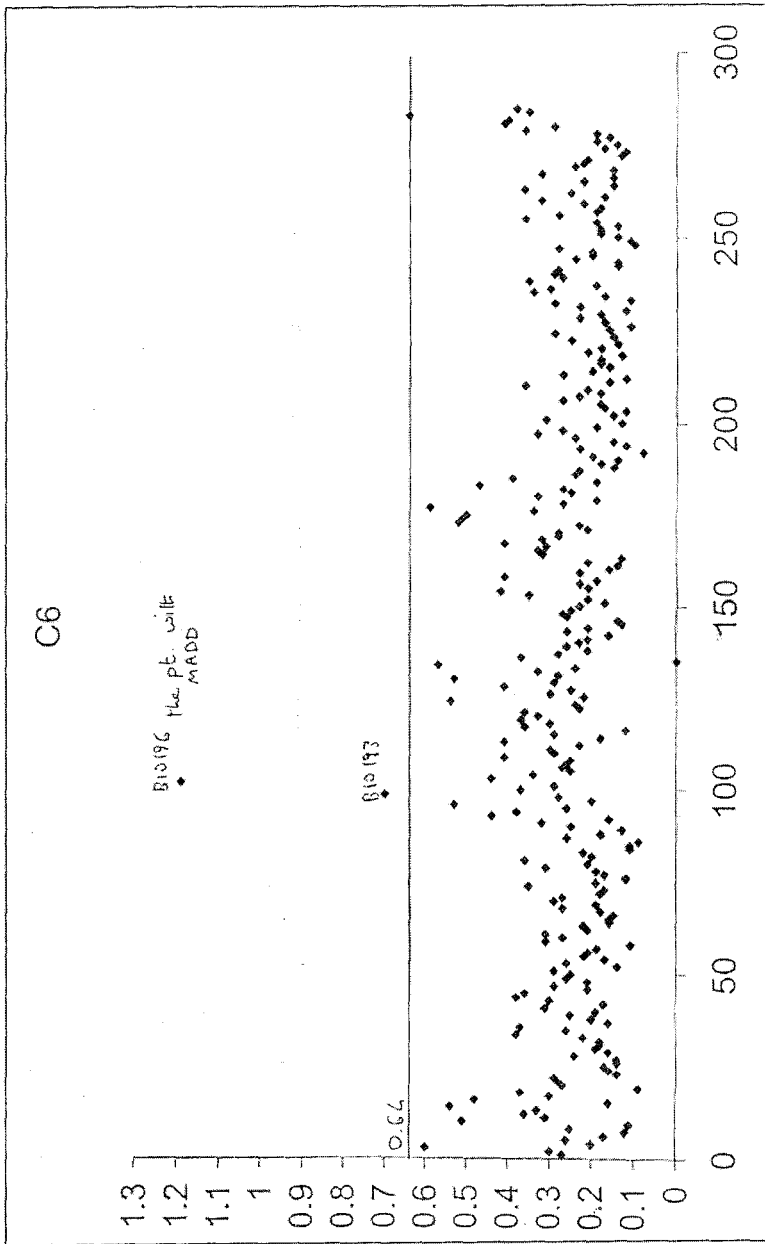


Figure 3: Levels of C6 in Samples of F. the neonatal screening

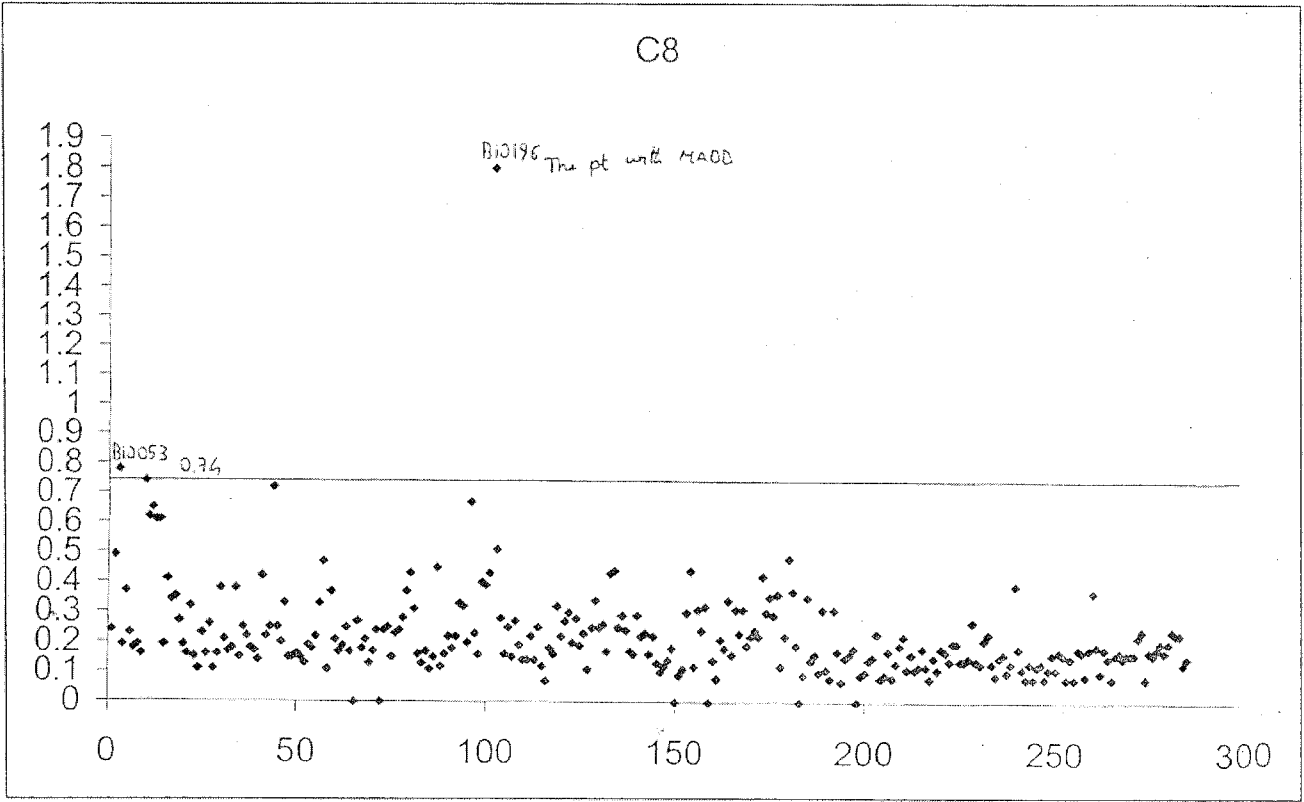


Figure 4: levels of C8 in the samples of the screening

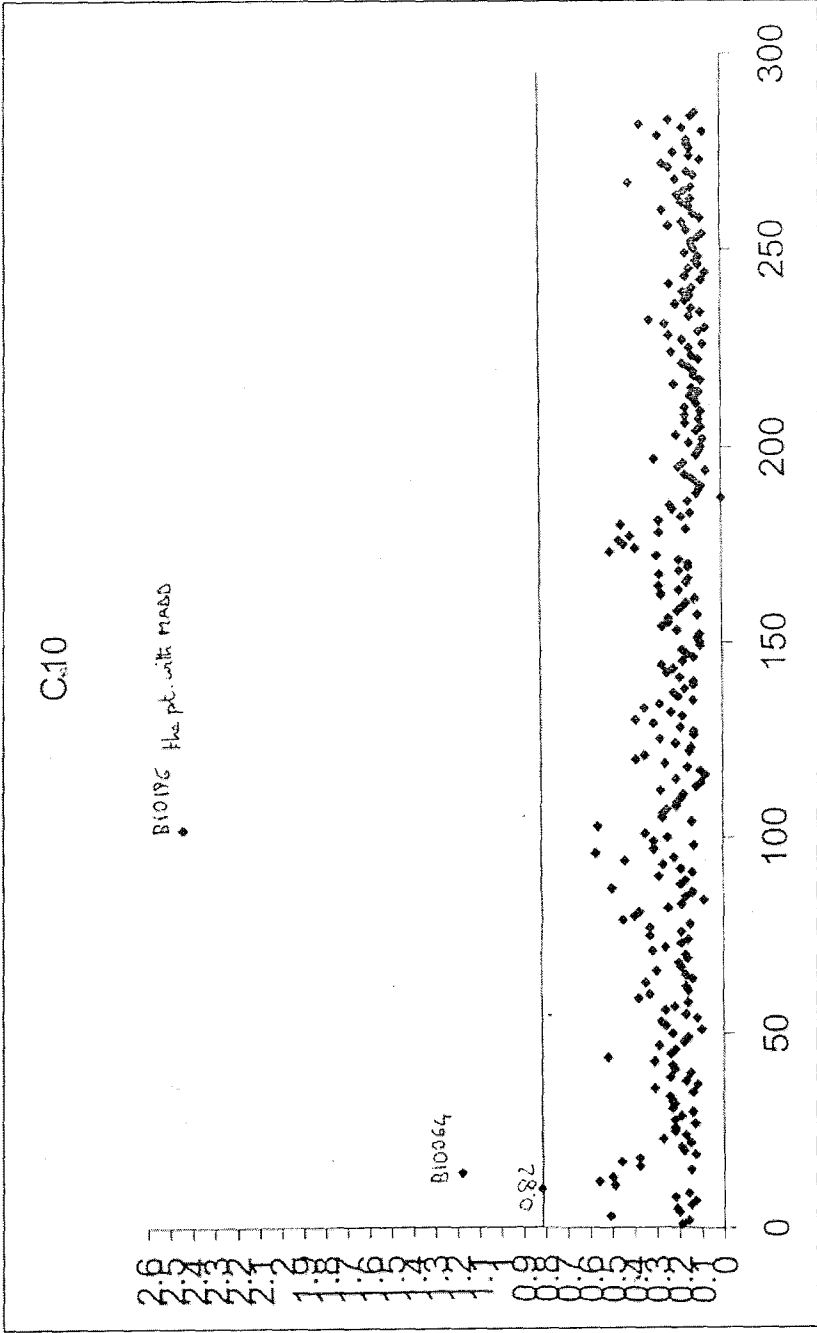


Figure 5: Level of C10 in the samples of the screening

NEONATAL SCREENING FOR INBORN ERRORS OF METABOLISM, A NEW EXPERIENCE WITH TANDEM MASS SPECTROMETRY.

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Summary

Through a link between the department of pediatrics in the University of Khartoum Sudan and University of padova in Italy, a neonate screening was carried out using electrospray tandem mass spectrometry (ESI-MS/MS). 2&5 neonates admitted the nursery in the maternity and police Hospitals during the period from the beginning of September to the end of October 2002, were screened for some aminoacidemias, organic acedemias and fatty acid oxidation defects. One neonate (0.35%) was found to be positive, and had a severe form of multiple acyl- Co A dehydrogenase (MADD) defect.

Abbreviations:

PhenyL ketonuria (PKU), maple syrup urine disease (MSUD), non-ketotic hyperglycemia (NKH), citrullinemia (ASS), Arginisussinic hyase (ASL) Methyl L maloric Acedemia (MMA), isovaleric academia (IVA), Glutaric acid type 1 (GAI), Medium chain AcylCoA dehydrogenate (MCAD) long-chain acyl CoA dehydrogenate deficiency (LCAHD), Multiple AcylCoA dehydrogenate deficiency (MADD). Mass spectrometry (FAB-Ms/MS) fast atom bombardment tandem mass spectrometry (FAB-MS/Ms) Electrospray ionization mass spectrometry (ESI- Ms/Ms).

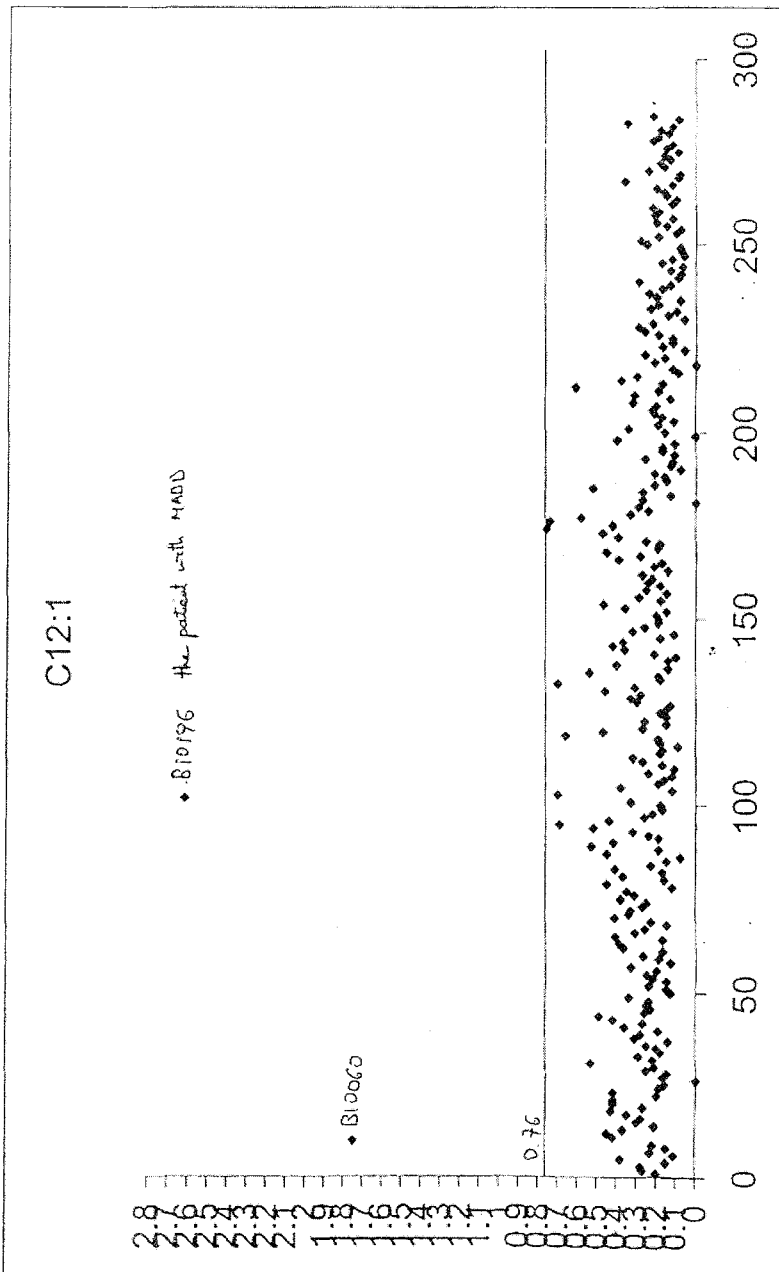


Figure 6 :- Levels of C12:1 in the samples of the screening.

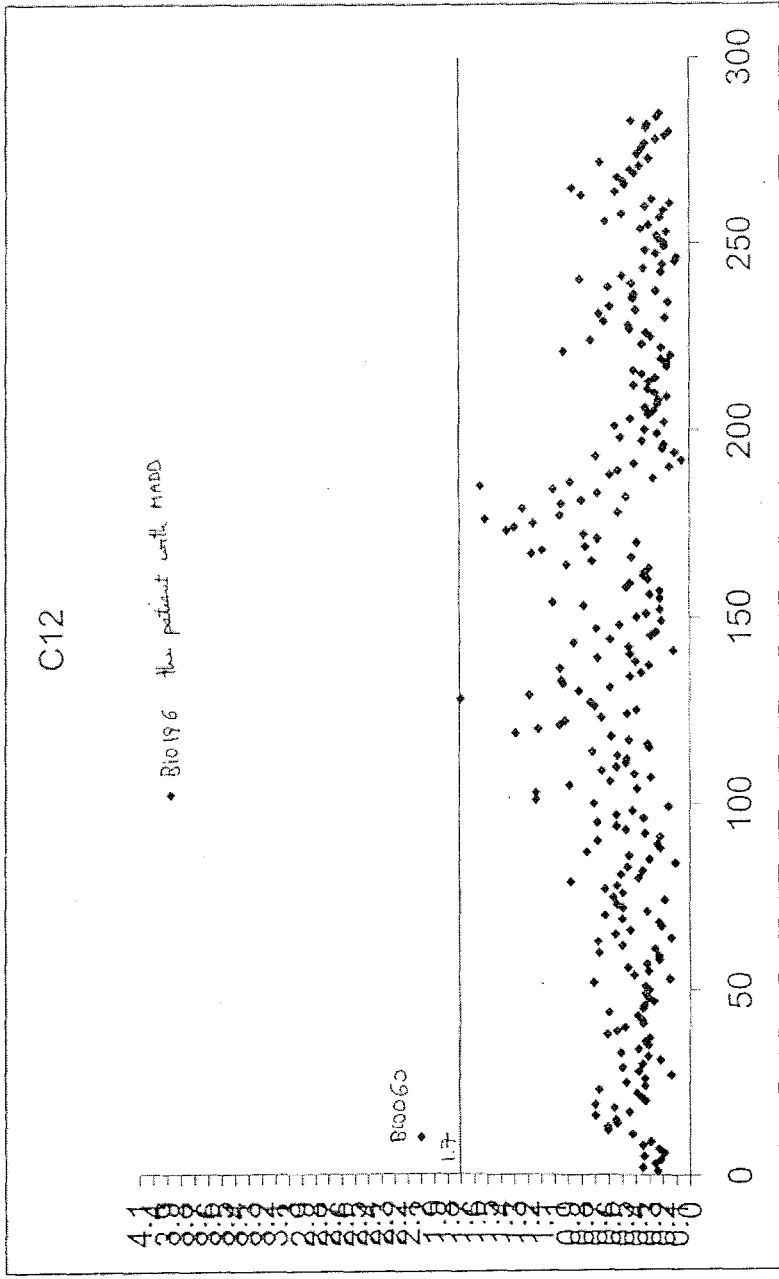


Figure (7) - Levels of C12 in the samples of the screening.

C14:1

◆ B10196 the patient with AIDS

◆ B10187

1.15

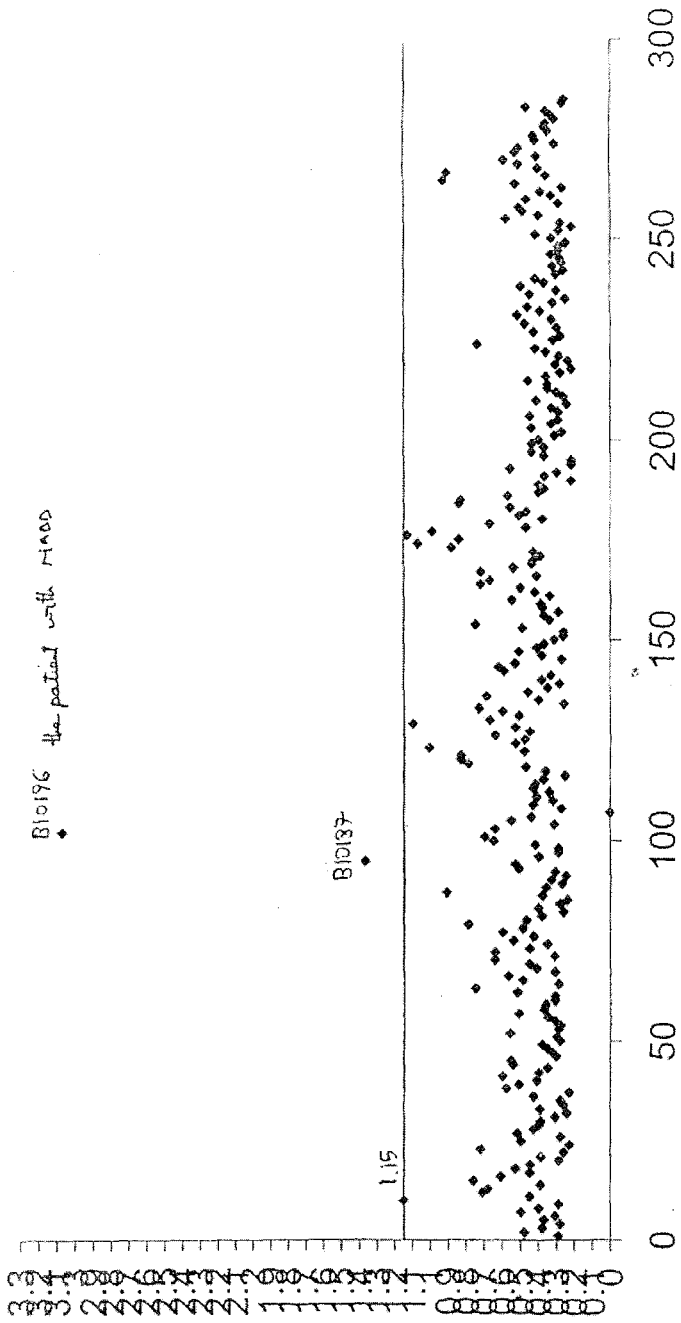


Figure no. (8) levels of Cavit in the samples of the screening

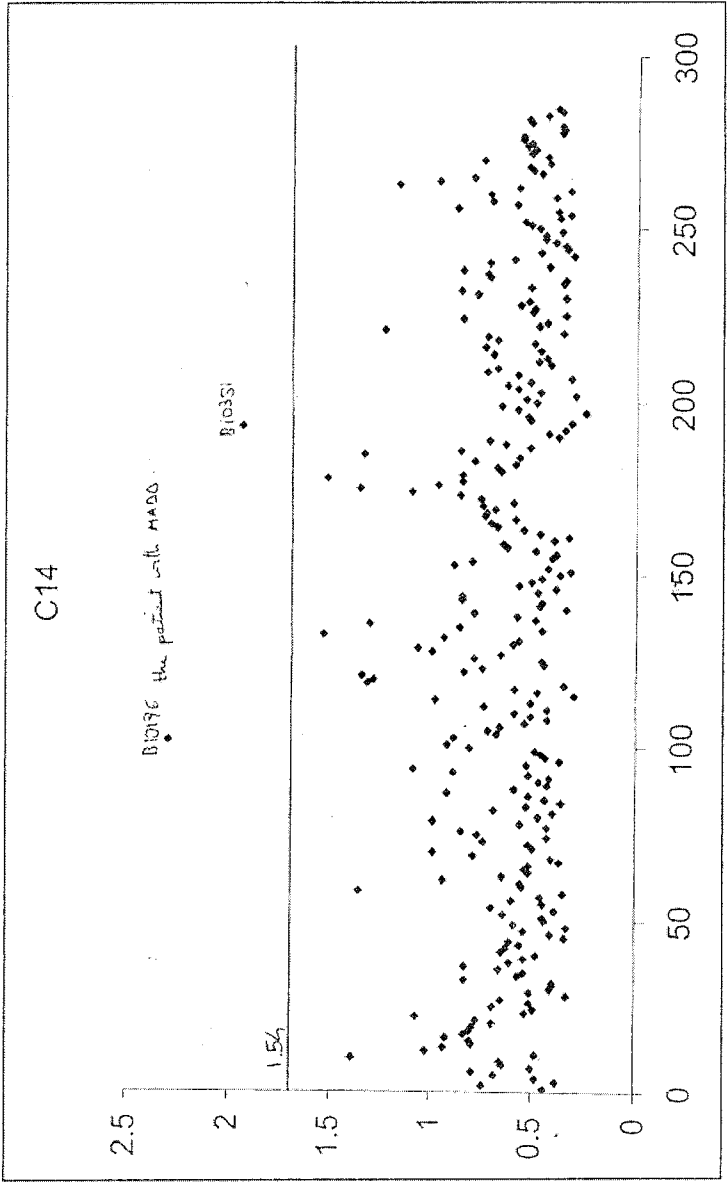


Figure no. (9) Levels of C14 in the samples of the screening

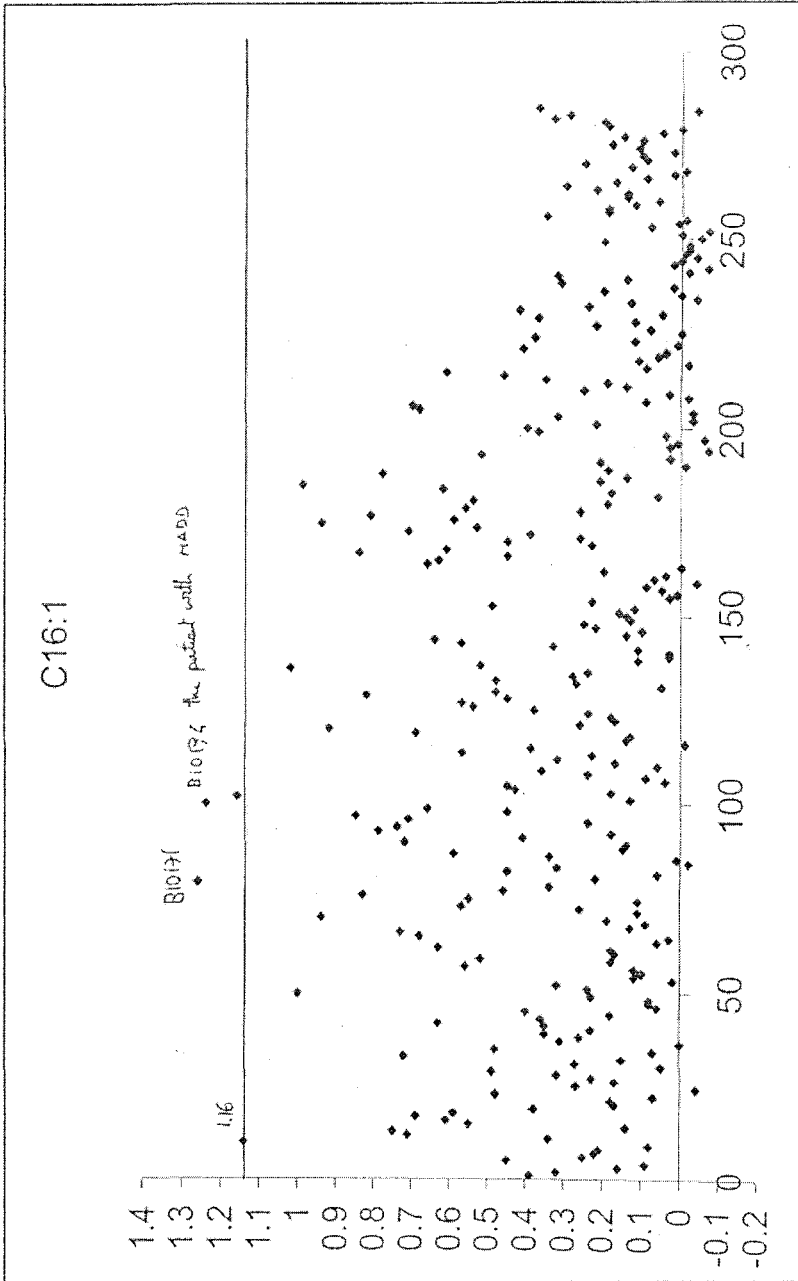
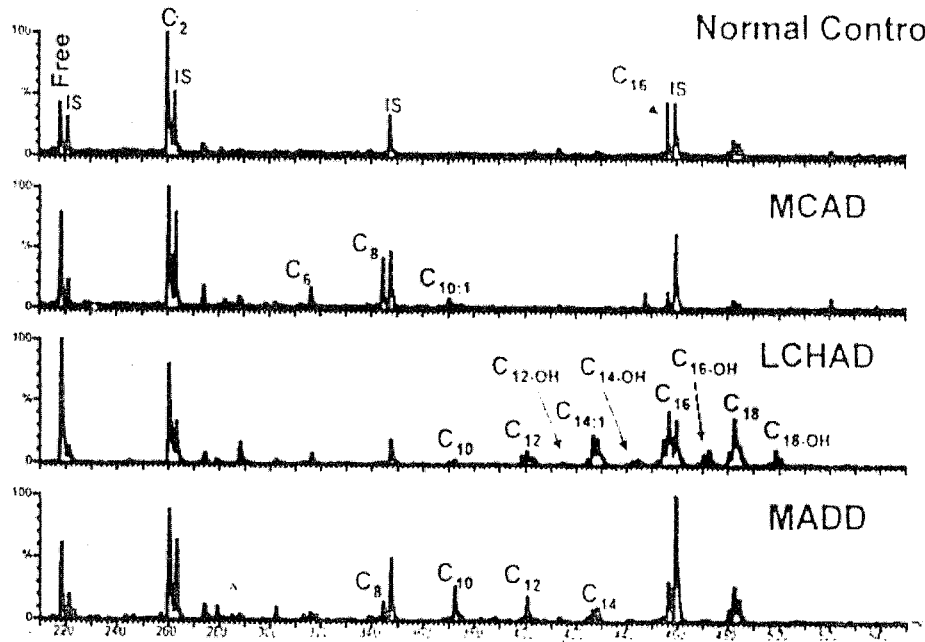


Figure no 10a levels of C16:1 in the samples of the screening

Fig. 11. Acylcarnitine profile of dried blood specimen from normal control patient, and from MCAD, LCHAD, MADD patients.



Discussion:

Although inborn errors of metabolism are rare disorder, the frequency of their-occurrence in this study was found to be 0.35%. This is comparable to the frequency reported from the center of hereditary metabolic diseases in padova, where the frequency reported was 0.2% (8) those frequencies are very much high when compared to the results obtained from certain states in America, where the frequency was found to be 0.025 for all metabolic disorders and 0.00015% for MADD.

Those differences are probably affected by the sample size our sample size was 285, the Italian sample was 500, but the American sample was 746.337, and for rare disorder a large sample size is very much recommended to avoid the possibility of the chance. Another exploration for our high frequency may be that, and because of a common occurrence of consanguineous marriage, these autosomal recessive disorders are commoner than in other parts of the world where consanguineous marriage is not common.

On studying the case of the neonate with MADD, we found that he was a boy and he was the product of a first degree cousin consanguineous marriage. This birth weight was 2500 gms. His blood sample was taken at day I, and he was quite well. On Inquiring the mother through the telephone after the diagnosis was made, we were told that he died at the 5 days of, fever, breast reluctance, convulsion and loss of consciousness with a diagnosis of neonatal septicemia not responding to Antibiotics. The F:H. revealed that the sister of the mother, who is also married to a first degree cousin, had all her three offspring dying during the early neonatal period of a similar presentation, and that she refused not to conceive again.

This history is very much suggestive of the presentation of the severe form of MADD.

Acknowledgements

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