

Brief Communication

3-Methylcrotonyl-CoA carboxylase deficiency. A long-term outcome

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We are describing the long-term follow up of a 6 and half-year old patient from Saudi Arabia with Biotin-Resistant 3-Methylcrotonyl-coenzyme-A carboxylase (MCC) deficiency. She presented with acidosis, lower respiratory tract infection, vomiting, diarrhea and failure to thrive at 3 months of age. The diagnosis initially reached by tandem metabolic stroke (MS) and then confirmed by enzyme analysis. She was placed on appropriate therapy. During the next 6 years, she had no recurrence of the disease except she had a thrombocytopenic episode at 18 months. This case illustrates that isolated MCC deficiency of early onset shows a severe clinical course. However, the clinical symptoms and signs can be reversed with suitable therapy.

The patient at present is a 6 and half-year-old girl who came to the service at the age of 2.5 months from a peripheral hospital. She was the product of full term spontaneous vaginal delivery and was discharged home at 2 days. She came back to the hospital with a chest infection at 8 days of age. The mother attributed tachypnea at 35 days of age, which was attributed to bronchiolitis. She was found to have gastro-esophageal reflux, diarrhea and acidosis. At that time, a blood sample sent for tandem MS revealed the MCC deficiency. Parents are first-degree cousins. The mother had 2 miscarriages and 3 normal children (one boy, and 2 girls). Physical examination at the time of first encounter at 2 months of age was unremarkable. There were no organomegaly and no neurological findings. She had a nappy rash with satellite lesions. She was placed on maple syrup urine disease formula with additional isoleucine and valine. She was prescribed Polycose, Polycitra 1 ml/kg/ tid, Carnitine 200 mg/kg/day and Glycine 500 mg/kg/day. Nystatin ointment was given for the nappy rash. When seen again at 3 months, the mother gave a history of loose motions. She was otherwise, unremarkable. She was normal at 4, 6, 8, 12 and 14 months of age. At 17 months: following a severe respiratory tract infection, she developed a repeat episode of mild acidosis and was found to have thrombocytopenia, 13,000/ml. A bone marrow examination showed increased megakaryopoiesis, but normal erythropoiesis and normal granulocyte

series. Cardiolipin antibodies, immunoglobulin A and immunoglobulin G were normal. However, immunoglobulin M was elevated (58 [normal <12.5]). One month later, the thrombocytopenia resolved spontaneously; her platelet count was 123,000 and blood smear showed large platelets. At the time, she was found to have mild iron deficiency anemia. No evidence for alpha-thalassemia was detected. All of her hematological parameters normalized at 2 years of age. She has been followed as outpatient frequently and remained normal thereafter until the present at 6 and half years of age. The EEG, visual and brain stem evoked potentials were normal at 3 months of age. The brain 18F-fluorodeoxyglucose positron emission tomography at 5 months revealed minor changes as somewhat increased uptake and somewhat decreased uptake in different cortical areas. The MRI of the brain at 4 months of age, showed cavum septum pellucidum, and slightly delayed myelination. At the time the magnetic resonance spectroscopy of the basal ganglia showed slightly decreased N-acetylaspartate, normal choline and creatine peaks with no lactic acid accumulation. Her EEG was normal at 4 months. Blood and urine studies showed a normal amino acid profile at 4 months with a blood lactic acid of 4.6-mM (normal <2 mM). Biotinidase was measured twice and was found to be normal, 4 and 8 units (normal between 4 and 8 units). Blood tandem MS on at least 4 occasions revealed an elevated hydroxy-C5 carnitine and absent propionyl-carnitine. At the time of initial diagnosis, the free carnitine was very low. Subsequently, it was elevated since she was on oral carnitine treatment. The qualitative urine GC/MS, showed highly elevated 3-hydroxy-isovaleric acid and highly elevated 3-methylcrotonyl-glycine. There were no intermediates of propionic acid metabolism. When the patient was challenged with oral leucine (500 mg/kg/dose), the excretion of the aforementioned compounds increased significantly, while oral L-Isoleucine had no effect. The blood tandem MS studies of sibs and parents were found to be normal. The results of the enzyme analyses in her fibroblasts revealed an isolated MCC deficiency (**Table 1**). Isolated biotin-resistant deficiency of MCC is an autosomal recessive disorder of leucine catabolism associated with elevated excretion of 3-hydroxyisovaleric acid (3 HIVA) and 3-methylcrotonylglycine (3 MCG) in the urine. Biotin supplementation does not influence the urinary metabolite pattern or the clinical progress. The introduction of tandem MS has increased significantly its diagnosis. The clinical findings in approximately 20 patients have been reported. The clinical presentation has a wide range of severity

Table 1 - Results of the enzyme analyses in the patient's fibroblasts.

Fibroblast	Propionyl-Coa Carboxylase ^a	3-methylcrotonyl Coa Carboxylase ^b	Pyruvate Carboxylase ^c
Patient	250	0	317
Control	128 - 537	71 - 250	93 - 365
Control ^d	310	113	473

^aLow biotin, 6 Nmole/l in culture medium; Pmol/min/mg. ^bLow (6 Mmol/l) and high (1000 Nmole/ml) in culture medium, yielded the same result both in patient's and in control fibroblast run simultaneously; Pmol/min/mg. ^cLow biotin, 6 Nmole/l in culture medium; Pmol/min/mg. ^dControl fibroblast activity run simultaneously with the patient's fibroblasts

and onset. The onset can be as early as the first week of life or as late as more than 4 years of age. Asymptomatic adult individuals have been reported from Amish population.¹ Symptoms and signs include upper and lower respiratory tract infection, muscular hypotonia, failure to thrive, seizures, developmental delay or regression. Bannwart et al² reported that severe case might expire. The MS in basal ganglia has been reported.³ The MCC is a heteromeric enzyme with alpha (biotin-containing) and beta subunits.⁴ Their c-DNA's have been identified and mutations in either chain were established. There is no clear correlation between the mutations established and the phenotype.

Our patient with isolated 3-MCC deficiency was unusual in its clinical presentation. She manifested thrombocytopenia, incidental at the peak of her second metabolic crisis. She responded well to therapy with a decrease of her urinary metabolites. Long-term follow up revealed a healthy child with appropriate age neuro-developmental milestones. These observations emphasized the variable nature of presentation in isolated MCC deficiency.

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Profile of baseline CD4 T-lymphocyte and viral load levels in HIV infected treatment naive patients in Lagos, Nigeria

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Sub-Saharan Africa bears the greatest brunt of the HIV pandemic with over 75% of global reported cases.¹ In Nigeria, the disease has been diagnosed in all the local government areas of the country. As at the end of 2001, it was estimated that 3.8 million people were living with the virus in the country.² The development of potent antiretroviral (ARV) drugs cast a ray of hope in the clinical management of HIV infections. Indeed, the use of a combination of ARV drugs has been documented to have a profound beneficial impact on morbidity and mortality of HIV-infected individuals. The reported benefits of ARV drugs have encouraged their use in the clinical management of HIV in most of the developed and some developing countries. Essentially, the proper use of a suitable combination of ARV drugs has been documented to achieve a sustainable suppression of viral replication in the infected patients. The drug combination also achieved significant improvement in the immune status of the patient through the reversal of the immunodeficiency that is characteristic of HIV infection. However, achieving these desired results of ARV treatment has been reported to be influenced by a myriad of factors. Among these factors are the threshold baseline levels of the CD4 cell counts and viral load prior to initiation of treatment. It has been reported that higher CD4 counts and lower viral load

prior to initiating therapy was associated with an increased ability to obtain and sustain virological and immunological response to treatment.³ In addition, viral load at baseline was reported to be predictive of the rate at which HIV-1 RNA levels decline during ARV therapy. In 2001, the Federal Government of Nigeria initiated a national ARV treatment program. Under this program 10,000 adults living with the virus were to be treated in 25 centers across the country. Prior to treatment, the baseline CD4 values of all the patients were determined. However, as a result of lack of adequate facilities, the baseline viral load levels of these patients were not all determined. This study was therefore carried out to determine the profile of the baseline levels of CD4 cells and viral load of infected patients prior to initiation of treatment. This information will be useful in the subsequent evaluation of treatment outcome amongst the patients. The study was carried out between January 2002 and December 2003.

The study was carried out at the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos which is one of the 25 treatment centers under the National ARV program. HIV positive patients diagnosed in several health facilities in Lagos and its environs, are referred to the Institute for ARV treatment. Within the period of the study, an estimated 1200 patients were referred to the Institute for treatment. Out of these, 448 adult patients of both sexes who met the CD4 requirements for treatment (<350 cells/ μ l) and had given informed consent to participate in the study were enrolled. These 448 patients constituted the study population, and prior to initiation of treatment, a questionnaire was completed for each of them to obtain their relevant biodata. Approximately 5 ml of blood sample was also obtained in EDTA bottles from each of the patients. While 125 μ l of the whole blood was assayed for the CD4 cells, the remaining blood was separated and the plasma was stored at -70°C until assayed for viral load. The CD4 T-lymphocytes were estimated by the Dyna bead Technique (Dyna AS, Oslo, Norway). This is a manual technique that involves the use of a light microscope and Dynal mechanical rotator. It is an immunogenic cell isolation method which uses Dyna beads magnetic particles coated with antibodies to CD4 antigen, to capture and isolate CD4 T-lymphocytes from whole blood. The isolated cells were used, and the nuclei were stained with Turks solution. The stained nuclei were counted in a Neubaur counting chamber (Weber Scientific International) using a light microscope. The results were expressed as CD4 cells/ μ l of whole blood. The viral load on the plasma samples was estimated using the Roche Amplicor HIV-1 monitor tests, Version 1.5

kit. This method involves 4 major steps; namely: a) reagent preparation, which involves preparing the master mix, b) specimen and control preparation, the HIV-RNA is extracted at this stage, c) reverse transcription and amplification, this involves the transcription of the RNA into cDNA and amplification occurs in the region of the HIV-1 genome between the primers only, d) detection reaction, this allows for the quantification of the RNA in the specimen. The EPI-INFO version 6.04 was used to analyze the data.

Data on the age and sex distribution of the study population showed that 232 (52%) of the patients were males while 216 (48%) were females. A total of 346 (78%) of the population were within the age bracket of between 25 and 44 years. Sixty (13%) of the patients were within the age group of 45-54 years, 28 (6%) within the age group of 15-24 years, while 14 (3%) were 55 years and above. The age distribution was similar in both sexes. Data on viral load estimation indicated a median viral load level of 172,641 HIV-1 RNA copies/ml amongst the patients. Further analysis showed that 90 (20%) of the patients had HIV-1 viral load levels of below 10,000 copies/ml, 95 (21%) had values between 10,000-100,000 copies, while 263 (59%) had values of above 100,000 copies. The blood samples obtained from the patients were also assayed for their CD4 T- lymphocyte levels. A review of the overall data reflected that 54 (12%) of the patients had CD4 cell counts of below 200 cells/ μ l, 299 (68%) had CD4 counts of between 200-299 cells/ μ l while 95 (20%) had counts of between 300-350 cells/ μ l. **Figure 1** shows the pattern between viral load categories and corresponding CD4 counts. The higher the viral load the lower the CD4 cell count.

Relatively complete viral suppression is required to prevent the emergence of viral resistance. Therefore, in all treatment naïve patients the principal goal of treatment should be to achieve virologic suppression to undetectable levels. Some patients, especially those with high initial viral load levels such as observed in the majority of the studied patients, where 59% of them had values of above 100,000 RNA copies/ml, may not be able to achieve this level of success. The ability to achieve undetectable levels of virus is mainly dependent on the baseline viral load, hence, the higher the baseline viral load, the more difficult it is to achieve undetectable levels of virus.⁴ Where such cases occur, to achieve optimal suppression may require more expensive drugs, more complex regimen and increased chance of non-adherence.⁴ In a general sense, the intensity of the regimen is proportional to the stage of infection and the baseline viral load level. For example, for patients with relatively low viral load values (for example, $<70,000$ copies/ml), triple

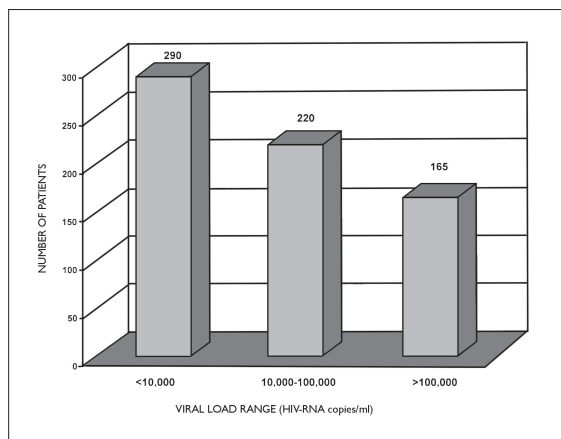


Figure 1 - Viral load categories and corresponding CD4 cell levels.

nucleoside reverse transcriptase inhibitors (NRTI) may be used. For patients with more advanced disease and higher viral load values such as observed in this study, a 4-drug “boosted” protease inhibitor (PI) - containing regimen may be optimal.⁵ However, interest in PI-sparing regimens as initial therapy has increased, along with the desire to reserve that class of drug for subsequent therapy. In Nigeria, this class of drug is not available for first line therapy. Hence, approaches that will enhance early diagnosis of new cases and possible treatment of such cases when they have met recruitment criteria should be encouraged. Clinical benefit can however be realized with less than maximal suppression of viral load.⁴ A reduction in HIV RNA levels of $0.5 \log_{10}$ copies/ml below baseline is associated with relative maintenance of the CD4⁺ T lymphocyte count over time, provided that this degree of viral suppression is sustained.⁴ Treatment started at moderate CD4⁺ T-lymphocyte counts (for example, 350 cells/ μ l) can be accompanied by preservation of immune competence, whereas treatment that is initiated late in the course of disease (for example, at CD4⁺ T-lymphocyte counts of less than 100 cells/ μ l) is associated with a poorer outcome.³ In this study, since most of the patients had counts of between 200-350 cells/ μ l, it is expected that their immune competence will be preserved. It is interesting to note that studies have shown little disease progression among patients who initiated therapy with moderate CD4⁺ T-lymphocyte counts (for example, 350 cells/ μ l), even if their viral load values at the time of treatment initiation are high (for example, >100,000 RNA copies/ml).³ However, it must be quickly added that longer-term follow-up of the subjects in those studies was lacking. It is therefore pertinent to advocate for enhanced health education in the signs of

HIV infection and expansion of voluntary counseling and testing centers. This will enhance the reduction of stigma and improve early diagnosis of new HIV cases in the country. Such individuals will benefit from the subsidized government treatment, particularly at a time where scaling up is being implemented.

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Cytotoxic potential of cervical mucus on *Trichomonas vaginalis* to prevent upper genital tract infections

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The flagellated parasitic protozoan *Trichomonas vaginalis* (*T. vaginalis*) is the causative agent of trichomonal vulvovaginitis, a sexually transmitted disease worldwide.¹ This pathogen colonizes the epithelial cells of the human urogenital tract. Mucus layer constitutes the first step defense against microorganisms in both physical and antimicrobiological aspects.² In order to colonize the underlying epithelium, it has to pass the mucus layer.

Trichomonad invasion of the mucus layer requires adhesins, mucinases, and motility.³ Although this protozoan has the potential to traverse the mucus barrier covering the epithelium, it usually does not pass the cervical mucus and invade the upper genital tract. The purpose of this study was to investigate if cervical mucus plays a cytotoxic role against *T. vaginalis* besides being a mechanical barrier.

Trichomonas vaginalis isolate used in this study was cultured from a symptomatic woman who was admitted to the outpatient clinic in Ankara, Turkey. Two vaginal swabs were taken from this patient with vaginal discharge. One of them was examined as a wet mount preparation, while the other was inoculated in cysteine, peptone, liver, maltose (CPLM) medium supplemented with 10% horse serum, penicillin (1000 U/ml), streptomycin (1 mg/ml), and fluconazole (1 mg/ml). After axenisation was achieved by consecutive passages in CPLM medium, trichomonas isolate was frozen and stored at -80°C until it was used. The *T. vaginalis* was thawed and 24 hours culture was used in the experiments. Thirty-five cervical mucus samples were obtained on the day of oocyte aspiration from women who underwent in vitro fertilization (IVF). No symptoms and signs of vaginal infection were present during IVF treatment. This group of patients were selected as the ovulation induction is an augmentation of the physiological hormonal state and the amount of the cervical mucus increases. Further more, cervical mucus samples from all patients were collected in the same period of their cycle in a standard manner by aspiration with an insulin injector from the cervical os. All samples were stored at -20°C until used.

The ability of cervical mucus to kill trichomonads was assayed by mixing medium containing *Trichomonas* (4×10^5) with cervical mucus at a 1:3 ratio on a slide.⁴ As a control preparation, *T. vaginalis* in CPLM medium mixed with phosphate buffered saline was prepared. After the preparations were covered by coverslips, they were incubated at 37°C for one hour. The remaining motile parasites were counted under microscope.

The results were expressed as the viability rates of the parasites using the following formula:

$$\left[\frac{\text{number of motile parasites in control} - \text{number in sample}}{\text{number of motile parasites in control}} \right] \times 100$$

Results were classified as 100% viability of organisms, 25-75% killing of the parasites or 100% killing of

trichomonads. Of 35 mucus samples collected from the IVF patients, 31 samples showed no cytotoxic effect against the trichomonas isolate that was used. Only 4 (11.4%) cervical mucus samples killed 25-75% of trichomonads.

Demes et al⁴ compared cytotoxic activity of cervical mucus with serum and menstrual blood and concluded that there was approximately 15% of mild to moderate trichomonocidal effect of cervical mucus samples, which was very less than the serum and menstrual blood and suggested that this situation partly depended on the lack of complement in cervical mucus. Cervical mucus plug of pregnant women were reported to exhibit antimicrobial activity against *Escherichia coli* and group B *Streptococcus* where as no detectable activity was shown against *Candida albicans*.² As to *Candida albicans*, in this study we found that cervical mucus samples obtained from women during IVF treatment lack significant activity against trichomonads, which is a result comparable with the results of Demes et al.⁴ As only 4 out of 35 mucus samples showed weak anti-trichomonal effect (25-75%), we thought that the importance of mucus as a mechanic barrier is superior to its role as a cytotoxic defender against *T. vaginalis* and there may be other uncharacterized factors that prevent this parasite's entry into the uterine cavity to cause an upper urinary tract infection.

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