Many studies have shown that viruses and viral proteins may affect mitochondrial function, induce mitochondrial abnormalities or affect apoptosis, sometimes by effects mediated at the mitochondria. Mitochondria play a crucial role in apoptotic process, which can be viewed as part of the host cell response to infection. Since cell suicide would reduce the yield of any infecting virus it would be widely applicable as a defence mechanism. Apoptosis also plays an essential role in pathogenesis of many diseases caused by viruses. Several viruses are also known to affect apoptotic pathways which permit the maintenance of latent viral infections or enhance the efficiency of viral replication. The basic hypothesis for this study was that viruses may play an important key role in aetiology of diseases in which energy generation is impaired. One example of such a situation might be provided by chronic fatigue syndrome (CFS). CFS remains a poorly understood and controversial disease, because no causal agents have been identified. Diagnostic laboratory tests have poor sensitivity and specificity. The aetiology and pathogenesis of CFS still remains unknown thus CFS could be a state of viral infections, most probably persistent infections. Measles virus (MV) is an extremely
contagious agent inducing acute illness\textsuperscript{16}, belongs to the genus \textit{morbillivirus} within the family \textit{Paramyxoviridae} with a non segmented single-stranded RNA molecule of negative sense\textsuperscript{17}. Several studies have revealed that MV can induce apoptosis in infected cells\textsuperscript{18-23}. This might well proceed by mitochondrial interaction and therefore the purpose of this study was to determine any possible change/s in cellular oxygen consumption post MV infection in Hela cells to determine any mitochondrial effect of this virus.

\textbf{Material & Methods}

Measles virus (Edmonton strain, kindly provided by Prof. V. ter Meulen, Wu\'rzburg, Germany) was grown in HeLa (human cervical epithelial carcinoma) cells. VERO (African green monkey kidney) cell line was used for viral titration\textsuperscript{24}. All cell lines were obtained from European Collection of Cell Cultures (ECACC, UK). Cell lines were grown in growth medium [Foetal bovine serum heat inactivated (FBS), penicillin/streptomycin (P/S), non essential amino acid (NEA) and minimal essential medium (MEM), (all obtained from Gibco, USA)] and were incubated at 37°C in a humidified incubator in an atmosphere of 5 per cent CO\textsubscript{2} until confluent. For harvesting of the cells, monolayers were detached by rinsing in PBS (Invitrogen) and then in 5 ml trypsin-versin solution (Invitrogen, UK). Most of this solution was then poured off and the cells allowed standing in the residual liquid until detached. The cells were added into 10 per cent FBS medium and collected by centrifugation for 5 min at 1500 × g before re-suspension in fresh medium. Cells were transferred to fresh flasks achieving a subculture ratio of 1/10 and were grown as above. Confluent monolayers of cell in 75 cm\textsuperscript{2} tissue culture flasks were rinsed with PBS and infected with 1 ml virus inoculum (10 pfu/cell). The infected flasks were kept at room temperature for 1 h. Cells were incubated in their respective media supplemented with 2 per cent FBS at 37°C in incubator until cytopathic effect (CPE) was observed (3 days for measles). Cell associated virus was released by one cycle of freeze-thaw: flasks were placed at -70°C for 1 h to freeze and allowed to thaw on the bench at room temperature. Cell debris was thoroughly re-suspended using a pipette and clarified at 377 × g for 5 min. Supernatant for inoculation was collected and snap frozen for storage at -70°C (2.5 cm\textsuperscript{2}). Dishes were seeded with cells, incubated overnight at 37°C, and checked for confluence. The plaque assay method\textsuperscript{25} was used for titration of the virus. For oxygen measurement procedure, the cells were infected with MV (10 pfu/cell) and allowed to adsorb for 1 h at room temperature with gentle rocking. Inocula were replaced with MEM supplemented by 2 per cent FBS and incubated for the desired time periods (6, 12, 24, 48 h). At each time point infected and control cell sheets were gently detached from the flasks using a scraper, re-suspended in 0.4 ml PBS and transferred into the oxygen electrode (OE) chamber. The rate of oxygen consumption or total cell respiration (TCR) was measured by a Clark-type OE according to manufacturer's Instruction (Oxyg32, Hansatech Instruments Ltd, UK).

\textbf{Results}

Cultures were assessed visually for the appearance of CPE. Typical MV-induced CPE was seen as cell fusion yielding syncytia (Fig. 1). The CPE changes commenced after 60 h post-infection and considerable

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/figure1.png}
\caption{Mock-infected (A) and measles virus (MV) infected HeLa cells (B). Confluent monolayer of HeLa cells was infected with MV at a multiplicity of 10 pfu/cell. Considerable CPE changes appeared after 3 days post-infection. Large syncytia are shown (Arrow) X40.}
\end{figure}
CPE appeared after 3 days post-infection. The respiratory rate was measured after 6, 12, 24 and 48 h post MV infection, when no CPE was evident in the cultures (Fig. 2). Basal respiration in mock-infected and infected HeLa cells was not significantly different.

Discussion

Studies done on viral effects on cell respiration in vitro showed that poliovirus and human herpes virus type one (HHV-1) infections of the cells caused a rapid decrease in total cellular respiration, which was related to mitochondrial dysfunction. Probably such effects will be of limited significance within the context of an active, productive and lytic infection, where the host cell has no long-term future. However, infected cells clearly survive in the context of persistent infections; this must indicate that apoptosis is blocked in such cases and in these instances; long-term reduction in a cell’s ability to generate energy might adversely affect cell function. Thus, the hypothesis for present study was that similar effects might be induced by diverse viruses and may underlie conditions where no single virus aetiology has been proven. This study was done to determine whether viruses can indeed affect mitochondrial energy generation. It has been shown that MV induces mitochondrial structural abnormalities in dendrites during persistent infection of the brain termed subacute sclerosing panencephalitis (SSPE) and also has been associated with apoptosis. Results of the present study showed that MV infection did not decrease total cellular respiration in HeLa cells. Further studies need to be done to clarify this issue.

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Reprint requests: Dr Mohammad Derakhshan, Assistant Professor of Clinical Virology, Microbiology & Virology Research Centre Avicenna Research Institute & Department of Clinical Bacteriology & Virology, Ghaem University Hospital Faculty of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran e-mail: derakhshanm@mums.ac.ir, m.derakhshanmd@yahoo.com