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Evaluation of Milk as a Source of Human DNA in Lactating Women Using Polymerase Chain Reaction

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Abstract: Somatic cells in milk from lactating women were used as a source of human DNA and as a substrate for Polymerase Chain Reaction (PCR). The DNA yield varies with the different stages of lactation as compared with that of peripheral blood leukocytes. Using primers targeting the human mitochondrial cytochrome-b gene, the PCR produced a 157 Base Pair (BP) PCR product from milk samples as well as from peripheral blood. However, amplification products were not detected when the PCR was applied to DNA from animal species including, sheep and cattle. The scientific data presented in this study indicated that DNA extracted from human milk could serve as substrates for direct sequencing of genes or a fragment of the human genome. Since collection of milk is a non invasive procedure, it can often substitute for blood as a source of DNA. The technical convenience of milk as a source of DNA can be expected to increase the field of application of marker-based methods for genetic analysis of the human genome.

Key words: Milk, somatic cells, human, nucleic acids, DNA, PCR

INTRODUCTION

It is well documented that peripheral blood leukocytes are the usual source of DNA for genotyping (Lwein and Stewart-Haynes, 1992). However, obvious technical difficulties exist in collection of blood samples from large numbers of individuals. These difficulties may limit the application of marker-based methods for genetic analysis in humans and for genetic improvement of economic traits in dairy cattle. Human milk contains a large but highly variable number of somatic cells, depending on the stage of lactation and health. These cells are predominantly leukocytes but also include a small proportion of epithelial cells (Brooker, 1980). The use of these cells would facilitate obtaining DNA samples from lactating women. Collection of milk is a non invasive procedure and does not require trained personnel or special help. In addition, milk samples can be collected and conveniently send to a central laboratory where they are tested for protein, fat, lactose and somatic cell contents. Moreover, milk could serve as a valuable source of biological specimen for diagnosis of microbial diseases in infected individuals (Ziccheddu *et al*., 2002). The literature on milk as a source of human DNA, in lactating women, is scanty and no information is available as to whether or not DNA extracted from milk is wholly or partially of somatic origin. The objective of the present study was to evaluate milk as a source of human DNA in lactating women and as a substrate for PCR amplification, using human mitochondrial cytochrome-b gene as a target DNA for PCR amplification.

MATERIALS AND METHODS

This study was conducted at the Molecular Biology Laboratory, Faculty of Medical Laboratory Sciences of the National Ribat University, Khartoum, Sudan, during the period from October 2008 to May 2009. Twenty lactating women were considered in this study and samples were collected with informed consent. Ethical clearance was obtained from the scientific committee of the University.

DNA extraction from milk samples: Extraction of DNA from human milk and peripheral blood was made possible using a commercially available QIAamp tissue kit

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(QIAGEN Inc. Chatsworth, CA) according to the manufacturer's Instructions. Briefly, milk samples were centrifuged and 200 μL of the supernatant, 20 μL of proteinase K stock solution and 200 μL of lysing buffer were pipetted into 1.5 mL eppendorf tube and the mixture was incubated at 37°C for one hour and then at 70°C for 30 min. Total 200 μL of absolute ethanol was then added to the sample and mixed by vortexing. The mixture was then transferred to the QIAamp spin column and placed in a clean 2 mL collection tube and centrifuged at 8000 RPM for 1 min at room temperature. The QIAspin column was washed twice using 500 μL of washing buffers and spinning for 1 min. The QIAamp spin column was placed in a clean 1.5 mL eppendorf tube and the DNA was eluted with 200 μL of double distilled water preheated at 70°C at room temperature. A maximum DNA yield was obtained by spinning at 12,000 RPM for 1 min at room temperature. The DNA concentration was determined by spectrophotometer at 260 nm wave length. Five microliter of the suspended nucleic acid was used in the PCR amplification.

Selection of the primers for PCR amplification: For PCR amplification, a pair of primers was selected from the published sequences of the human mtcyt-b gene and used in the PCR assay. Primers (HSL1 and HSR2) were selected for the synthesis of the primary PCR product. A fragment of human cyt b gene was amplified from extracted DNA using a pair of primers. Primer HSL1 (5)-TAGCAATAATCCCCATCCTCCATATAT of the positive strand and primer HSR2 of the complementary strand (5)-ACTTGTCCAATGATGGTAAAAGG will be expected to produce a 157 bp PCR product from human DNA (Anderson *et al*., 1981).

The primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of MilliporeBurlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA) as per manufacturer's instructions.

Polymerase chain reaction: A stock buffered solution containing, 150 μL 10x PCR buffer, 100 μL of $MgCL_2$, 50 μL of 10 mM DNTPs, was prepared in 1.5 mL eppendorf tube and the volume was brought to 1500 μ. The primers were used at a concentration of 20 pg μL^{-1}. Two microliter of the primers, 5.0 μL of the target DNA and 42 μL of the stock solution were added onto 0.5 mL PCR tubes and mixed by vortexing. One microliter of Taq DNA polymerase (Perkin Elmer) at a concentration of 5.0 U μL^{-1} were used. All PCR amplification reactions were carried out in a final volume of 50 μL. The thermal cycling profiles were as follows: A 2 min initial incubation at 95°C, followed by 40 cycles of 94°C for 1 min, 55°C for 30 sec and 72°C for 45 sec and a final incubation at 72°C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ). Following amplification, 15 μL from each PCR containing ampilicons were loaded onto gels of 1.0% SeaKem agarose (FMC Bioproduct, Rockland ME) and electrophoresed. The gels were stained with ethidium bromide and the human-specific PCR ampilicons were easily identified following visualization under UV light.

RESULTS AND DISCUSSION

Human DNA was successfully extracted from milk samples of lactating women used in this study. The DNA yield varies with the different stages of lactation as compared with the yield from peripheral blood leukocytes. The described PCR-based assay afforded sensitive and specific identification of human mtcyt-b DNA. Using the pair of primers (HSL1 and HR2), the sensitivity of PCR-based assay detected the 157 bp PCR product from not <1.0 pg of human mtcyt-b DNA (Fig. 1).

The specificity studies indicated that the described PCR assay failed to amplify the specific PCR product from DNA extracted from animal species including, sheep, goat, cattle, deer, donkey, pig and camel (Fig. 2). Using primers HSL1 and HSR2, amplification of the human-specific 157 bp PCR product was produced from different milk samples collected from lactating women in Khartoum, Sudan (Fig. 2). There is no debate that breast feeding of infants is necessary for normal somatic and psychological growth and development of the infant. There are many studies, which proved that the human breast milk is superior to other milks as it contains all essential nutritional and non-nutritional elements that infants need with suitable concentrations. Many researchers believe that the human milk contains other factors that affect the cell growth and division and thus alters the gene expression (DNA). Tapper *et al.* (1979) have discovered in human breast milk a mitogen that stimulates DNA synthesis and induces division in cells grown in culture. This mitogenic activity is concentration dependent and also depends on time since lactation. No similar mitogenic activity is found in commercially available formulas or cow's milk. The human milk mitogen might be involved in the growth and development of cells in the neonate (Tapper *et al*., 1979). Milk is a body fluid that is involved in nutrition, growth and development. It contains certain factors that help the human infants compensate for the immaturity of some of their tissues. For example, human milk has high levels of secretory IgA (Lonnerdal, 1985). The IgA and other antibodies help to confer passive immunity to the infant. It has been demonstrated that

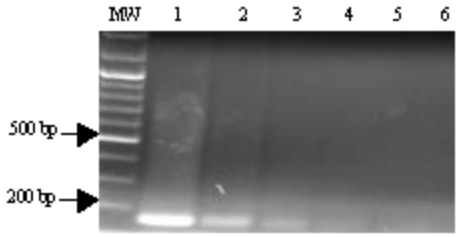

Fig. 1: Sensitivity of the PCR assay for the detection of the human-specific 157 bp PCR product, using primers HSL1 and HSR2. Visualization of the 157 bp PCR ampilicons on ethidium bromide-stained agarose gel from 100 fg of bovine DNA. Lane MW: Molecular Weight marker (100 bp ladder); lanes 1-6: human DNA at concentrations of 1 ng, 100 pg, 10 pg, 1 pg, 100 fg and 10 fg, respectively

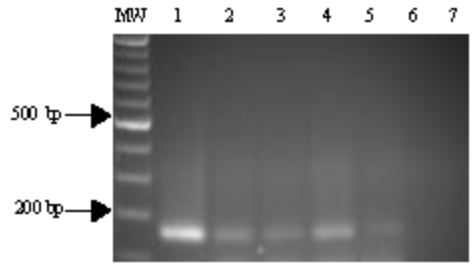

Fig. 2: Amplification of the PCR assay for the detection of the human-specific 157 bp PCR products in milk samples. Lane MW: Molecular Weight marker; Lane 1: 1.0 ng of human DNA (positive control); Lane 2-5: 4 different human DNAs extracted from milk samples; Lane 6: Sheep and Cattle DNA (negative controls)

human milk contains a polypeptide growth factor capable of inducing DNA synthesis and cell division in cultured cells. Human milk is capable of stimulating DNA synthesis and cell division in cultured mouse and human cells (Klagsburg, 1978). Thus, it is plausible that the human milk growth factor could be absorbed in active form from the gastrointestinal tract. Macromolecules like a-lactalbumin have previously been shown to be absorbed intact from the gastrointestinal tract of the newborn (Jakobsson *et al*., 1986). Therefore, the role of breast feeding and associated nucleic acid metabolism in infants gut needs to be investigated sufficiently. In the present investigation, DNA extraction was a simple procedure that takes only half an hour using QIAamp extraction kit. The electrophoresis, staining of the agarose gel with ethidium bromide and visualization of the specific PCR products usually takes 1 h. The specificity studies indicated that the primary 157 bp PCR ampilicon was not amplified even from 1.0 ng of DNA extracted from animal species including, sheep and cattle. Further specificity studies demonstrated that DNA from goat and camel, deer donkey and pig did not amplify the specific PCR product under the same stringency condition described in this study.

It is worth mentioning that the PCR, described in this study, could be used as a valuable tool in forensic medicine to authenticate the presence of human DNA in biological samples from victims or suspected individuals. In a previous report, it has been shown that heat treatment resulted in decreased sensitivity of the PCR assay (Meyer *et al*., 1995; Khairalla *et al*., 2006).

Nevertheless, in the present study, treatment of milk with high temperature did not affect the detection limit of the PCR assay. This could be attributed to the different methods of DNA extraction, which subsequently resulted in different DNA yield from heat treated samples. Thus, application of this PCR could be useful for detection of heat-treated milk. In previous studies, different genes were targeted for PCR amplifications including growth hormone gene; mtcyt-b gene and interspersed repetitive elements (Walker *et al*., 2004; Aradaib *et al*., 1998). In the present study, selection of the primers was based on the observation that the mtcyt-b gene has high copy numbers (hundred to thousand copy numbers per cell). In addition, the occurrence of conserved regions within the mtcyt-b gene provides high sensitivity for PCR amplification (Aradaib *et al*., 1998, 2001). Moreover, the described PCR assay does not require sophisticated laboratory equipments such like DNA sequencer and hybridization facilities. Further more, analysis of the PCR fragments with endonuclease enzymes to detect Restriction Fragment Length Polymorphism (RFLPs) was not employed in this study. The RFLPs is useful to differentiate humans and animals DNA. However, PCR-RFLPs is time consuming and expensive. The PCR, described in this study, could be used as a valuable tool in forensic medicine to authenticate the presence of ancient or modern human DNA. In the present study, we demonstrated that human milk can often serve as a convenient source of purified DNA. In addition, human milk can always serve as a substrate for amplification of specific DNA sequences using the PCR.

CONCLUSION

The scientific data presented in this study indicated that DNA extracted from milk could serve as substrates for direct sequencing of parts of the human genome. The technical convenience of milk as a source of DNA can be

expected to increase the field of application of marker-based methods for genetic analysis of the human genome. In addition, human milk could serve as a source of DNA in comparative genomics and investigative forensic medicine.

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