HBV DNA Can Be Detected from Nail Clippings of HBs Ag Positive Patients

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Hepatitis B virus (HBV) DNA is detectable not only in serum, but also in many other body fluids, e.g. urine, saliva, sputum, tear, seminal fluid, synovial fluid, and cerebrospinal fluid from patients with HBV infection [1-5]. To our knowledge, it has not yet been examined whether or not HBV DNA is detectable in nails, but it has epidemiological importance to know if it does. We tried to amplify the HBV DNA in fingernail samples from patients with HBV infection using the Polymerase Chain Reaction (PCR) technique.

Serum and fingernail samples were taken from 20 chronic hepatitis patients who were hepatitis B surface antigen (HBsAg) positive, and 10 healthy controls who were HBsAg negative, after informed consent was obtained. One hundred microliter of serum was mixed with 300 µl of Tris-HCl buffer (13.3 mM, pH 8.0) containing 6.7 mM EDTA, 0.67 % (w/v) sodium dodecyl sulfate and 133 µg/ml of proteinase K and was then incubated for 4 hrs at 55 ºC. After approximately 30 mg of nail clippings in a 1.5 ml microtest tube were rinsed once with 1.0 N NaOH and twice with distilled water, the nails were mixed with 300 µl of 4 M guanidine thiocyanate, 0.5% (w/v) sodium N-lauroyl salcosinate, 25 mM sodium citrate, and 0.1 mM 2-mercaptoethanol [7], and were incubated at room temperature over night. Nucleic acids from serum or nail were extracted with phenol/chloroform TE buffer (Tris-HCl buffer (10 mM, pH 8.0) containing 1 mM EDTA (pH 8.0)) and then precipitated with ethanol and sodium chloride. After centrifugation, the pellet from the nail sample was dissolved in 12 µl, while that from the serum was dissolved in 20 µl, of TE buffer diluted 1:10. Ten microliter of the genomic DNA from nail, or 0.5 µl of genomic DNA from serum, was subjected to 30 cycles amplification with a first set of primers, as described previously [8], and then a second round amplification of 0.5 µl of the first PCR product was performed with a second set of primers [8]. The PCRs were conducted in a final volume of 25 µl, containing 50 mM potassium chloride, 10 mM Tris-hydrochloric acid (pH 8.3), 200 µM dNTP mix, 0.5 µM each of the two oligonucleotide primers, 1.5 mM (1st PCR) or 2 mM (2nd PCR) magnesium chloride, and 0.625 U of Taq DNA polymerase (Nippon Gene). PCR variables were: 94 ºC for the first 4 min for denaturation, followed by 30 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min, and extension at 72 ºC for 1 min. After the final step of amplification, each amplified DNA fragment underwent electrophoresis in a 3% of agarose gel, and was visualized by ethidium bromide staining. The size expected for the second PCR of HBV DNA was 233 base pairs. HBV DNA was detected in 14 of 20 (70%) fingernail samples (Fig. 1) from the 20 HBsAg positive patients, whereas no band of HBV DNA was detected in 10 normal HBsAg negative subjects. In comparison, HBV DNA was detected in all 20 serum samples from the HBsAg positive patients (Fig. 2).

Although a nested PCR is a very sensitive technique, false positive results were unlikely for the present nested PCR because it was performed under the standard precaution procedure against false positive results and many runs of PCR on negative
controls always produced negative results.

The positive results obtained by the present nested PCR persisted when it was applied to the nails which were rinsed four times with 1N NaOH and dissolved in water. It may indicate that the positive results were not due to contamination by HBV DNA of the nail surface but to HBV DNAs in nail tissues.

We have therefore shown that nucleic acids were successfully extracted from nail clippings by the present guanidine method, that the method of DNA extraction we used was successful even with manicured or smudgy nails, and that nails may be a suitable material for genomic DNA analysis with PCR without contamination in a population study [6,9]. Nails may also be a possible material for detecting HBV DNA of patients with HBV infection.

REFERENCES