Blood and virus detection on barber clippers

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Background. Bleeding from the popular clean-shave ’chikop’ haircut was recently reported as prevalent in South Africa (SA), a country with 6.9 million HIV-infected people.

Objectives. To investigate the prevalence of barber hair clipper contamination with blood and HIV and hepatitis B viruses.

Methods. Fifty barbers from three townships in Cape Town, SA, were invited to participate. One clipper from each barber was collected immediately after it had been used for a clean-shave haircut. Each clipper was rinsed with phosphate-buffered saline and then submerged in viral medium. The polymerase chain reaction (PCR) was used to identify the blood-specific RNA marker haemoglobin beta (HBB), hepatitis B virus (HBV) and HIV.

Results. The clean-shave haircut was the most common haircut requested by clients (78%). Of the clippers collected, 42% were positive for HBV, confirming detection of blood, none were positive for HIV, and 4 (8%) were positive for HBV. Two clippers (clippers 16 and 20) were positive on qualitative HBV PCR. HBV DNA from clipper 16 clustered with genotype A sequences from SA, India, Brazil and Martinique, while clipper 20 clustered with SA genotype D sequences. The clipper 20 sequence was identical to a subtype D sequence (GenBank accession AY233291) from Gauteng, SA.

Conclusion. This study confirms that there is significant contamination of barber hair clippers with blood and blood-borne viruses. Hepatitis B was detected with enough DNA copies to pose a risk of transmitting infection. Although HIV was not detected in this small study, the risk of transmission should be quantified. Further studies to investigate barber clipper sterilisation practices and whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus infections are warranted. Public education on individual clipper ownership (as is the case with a toothbrush) should be advocated for clean-shave and blade-fade haircuts.


Human scalp hair varies significantly in curvature, and individuals of African ancestry have hair with the tightest curl.1-3 Population data suggest that hair follicles with this texture are prone to specific forms of alopecia (hair loss) that predominantly affect women (traction alopecia) and central centrifugal cicatricial alopecia (and men (folliculitis keloidalis nuchae)).

Hairstyle trends for men have changed over the years, from the ‘big afros’ of the 1960s and 1970s to the ‘blade-fade’ haircut or German cut (originally worn by working class men in the 1920s and popular among Hitler’s Wehrmacht soldiers in Germany, hence the name) that became popular in the 1990s and the current clean-shave or ‘chikop’ that became fashionable at the turn of the last century and is worn by 70% of black men in Cape Town townships.4 The chikop is worn by the majority of black men in South Africa (SA) and the African diaspora. Besides being a trend, this haircut is part of certain cultural rituals in various African and Indian (toshube) tribes. The haircut is achieved either by using a razor blade or by pressing the metal shears of an electric clipper directly onto the scalp without using the manufacturer-supplied plastic stages (combs). This gives a clean-shave haircut similar to that achieved using a razor blade.

Shaving pimples, more commonly seen in the beard area, also occur on the scalp as transient papules and pustules. However, when these pimples evolve into permanent keloids on the back (nuchal) scalp they are characteristic of folliculitis keloidalis nuchae (FKN), also commonly and incorrectly called acne keloidalis nuchae, which has a prevalence of 10.3% in males and 0.1% females aged >18 years.5,6 The prevalence of FKN was found to be highest in individuals whose hair had been cut with razors (10.7%), followed by clippers (5.9%), while no case was seen in those who used depilatory cream.7

A history of haircut-associated bleeding as a result of clean-shave haircuts was an unexpected finding in 32% of male participants from a population study8 in which HIV status was unknown. This was later confirmed in a later study where a history of bleeding was reported in 24.8% of HIV-positive men.9 Invisible bleeding was recently detected from scalp swabs after professional clean-shave haircuts (with no visible injury on the scalp when examined by a dermatologist) in 37% of participants using genetic testing for blood-specific RNA markers (albumin and haemoglobin beta (HBB)).10 The potential transmission of blood-borne viruses such as hepatitis B (HBV) and HIV is most concerning. A study in Ethiopia reported that sharing shaving equipment in barber shops is common practice,11 and an accidental scratch by sharp equipment in barber shops may create an opportunity for HIV and other blood-borne pathogens to enter the body.12 Currently there is no study in the literature that has looked at informal barber practices in SA townships. The prevalence of contamination of haircut clippers or razors with blood and blood-borne viruses in our setting is unknown. Methods of sterilisation...
or disinfection of barber instruments in our townships have been speculated on but not verified.

**Objectives**

The primary objective was to test clippers that had just been used for a haircut for blood and viruses. Our intention was to detect the prevalence of clipper contamination with blood, HIV and HBV, to ascertain whether barbers who mainly cut clean-shave *chiskop* haircuts caused more or less haircut-associated bleeding than barbers who did longer haircuts, i.e. at a distance from the scalp, and to investigate the cleaning methods used by barbers.

**Methods**

This was a prospective cross-sectional study. Aerial maps were used to sample 50 barber shops from three townships, the populations of which reflect the racial (apartheid) segregation of the previous SA government. Langa and Gugulethu townships have a population of predominantly black African ancestry and Bonteheuwel a population of predominantly mixed-race ancestry. The maps of Gugulethu and Langa, the two larger townships, were divided into four, then five barbers were selected from each section. The smaller Bonteheuwel township was divided into two, and five barbers were selected from each section. Basic demographic data were collected. Consenting barbers then performed one haircut, immediately after which they gave their clipper to the investigator; in exchange they received a new clipper. Methods used by the barbers to clean the clippers after use on each client were documented.

Each clipper was placed into a ziplock bag at room temperature, after which the bag was sealed and taken to a laboratory for virological sampling of the clipper. At the laboratory the clipper was submerged in a petri dish with 1.5 mL of viral medium, plugged into an electrical port and switched on to allow maximum wash through the teeth. The viral medium was then aspirated using a pipette and the residue was placed into a tube and stored at –80°C until viral analysis.

Total nucleic acid was extracted from 800 µL of wash using the EasyMag system (bioMérieux, Netherlands). For HIV testing, qualitative nested reverse-transcription polymerase chain reaction (RT-PCR) was performed to detect HIV RNA present, as follows. A 160 bp region of gag (nucleotide position 1494 - 1653 on the reference HIV HXB2 genome) was amplified by nested PCR using SuperTherm *Taq* DNA polymerase (Separation Scientific, CA) in a 50 µL final reaction volume. First-round primers were as follows: HIV1 (forward) TGGGACACAGAAGCTAC; HIV2 (reverse) GAACCTGAGCCACACG; the final primer concentration was 0.4 µM. Nested PCR primers were as follows: HIV3 (forward) AAATCCGATTGACACCTCA; HIV4 (reverse) TCTCAGTGGGAGTTG; the final primer concentration 1 µM. Cycling conditions were identical for both rounds, and were as follows: 94°C for 2 minutes, followed by 40 cycles of 94°C for 30 seconds and 72°C for 45 seconds, with a final extension step of 72°C for 7 minutes. PCR products were analysed using UV light after 2% agarose gel electrophoresis. Both qualitative HIV and HBV PCRs were performed on the Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems, USA).

HBV PCR products obtained were sent to Inqaba Biotechnical Industries (SA) for bidirectional Sanger sequencing. Chromatograms were edited in FinchTV (Geospiza, USA). Sequences were aligned using ClustalX[14] and manipulated in BioEdit.[15] The maximum-likelihood tree was drawn from an alignment of 188 nucleotide positions in MEGA6.[16] using the Tamura-Nei evolutionary model.[17] Support bootstrap was calculated with 1 000 replicates.

For comparison, HBV sequences representing most genotypes were selected from GenBank, including sequences from SA. The following sequences, with GenBank accession numbers listed by genotype, were chosen to represent the different genotypes: genotype A: AF010309, AF010329, AY233291, AY233295, AY576434, EU594430, FJ904447, GQ184322, GQ205389, HE974375, HQ646554, HQ646556, JF784220, KC752150, KF476003, KF476015, KF476018 and X51970; genotype B: AB033554, AF100309 and D00329_JP; genotype C: AB644284, AB644286 and GQ184326; genotype D: AF280817, AY1723291, AY1723295, AY576433, EU594430, FJ904447, GQ184322, GQ205389, HE974377, JX898722, KC012652, KF170740 and KF476030; genotype E: AP007262, DQ060824 and HE974380; genotype F: AB116654 and AF223965; genotype G: AP007264 and GU565217; genotype H: AB298362 and AY090454; genotype I: FJ023674 and FJ882615. Orangutan HBV, AF193864, was used to root the tree.

For the blood-specific markers, hair clippers were rinsed with phosphate-buffered saline (PBS) and RNA was extracted from the PBS using the QiAmp RNeasy kit (Qiagen, SA). Briefly, total RNA was extracted according to the manufacturer's instructions. The eluted RNA was used for cDNA synthesis with a reverse-transcription enzyme (Fermentas, SA) and random hexamer primers. Conventional PCR was performed using primer sets for the blood-specific marker, with HBV RNA primer sequences as follows: (forward) CAC CTG AGA ACA CAC AGG GA AAG GTG A-3’ and (reverse) 5’-TCTCTAAAGGGTTCCTTTGG-3’ each at 0.6 µM, and 10 µL eluted RNA was used for cDNA synthesis with a reverse-transcription enzyme (Fermentas, SA) and random hexamer primers. Conventional PCR was performed using primer sets for the blood-specific marker, with HBV RNA primer sequences as follows: (forward) CAC CTG AGA ACA CAC AGG GA AAG GTG A-3’ and (reverse) 5’-TCTCTAAAGGGTTCCTTTGG-3’ each at 1 µM, with 2 µL prenested PCR product. PCR cycling conditions were the same as described above. PCR products were analysed under ultraviolet (UV) light after 2% agarose gel electrophoresis.[60]

For HBV testing, 600 µL of wash was tested with the quantitative Roche COBAS AmpliPrep/COBAS TaqMan HBV Test, version 2 (Roche Diagnostics, Germany). Samples with detectable HBV DNA were then tested on a qualitative nested PCR[11] to obtain products for sequencing, as follows. A 189 bp region of the HBV pre-S1 gene was amplified by nested PCR using SuperTherm *Taq* DNA polymerase (Separation Scientific) in a 50 µL final reaction volume. First-round primers were as follows: HBV1 (forward) TGGGACACAGAAGCTAC; HBV2 (reverse) GAACCTGAGCCACACG; the final primer concentration was 0.4 µM. Nested PCR primers were as follows: HBV3 (forward) AAATCCGATTGACACCTCA; HBV4 (reverse) TCTCAGTGGGAGTTG; final primer concentration 1 µM. Cycling conditions were identical for both rounds, and were as follows: 94°C for 2 minutes, followed by 35 cycles of 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 45 seconds, with a final extension step of 72°C for 7 minutes. PCR products were analysed under UV light after 2% agarose gel electrophoresis.

Ethics clearance

Ethics approval for this study was obtained from the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town (ref. no. 208/2013).
Results

All invited barbers agreed to participate, and a total of 50 clippers were collected. Twenty-one (42%) of all clippers were positive for the blood RNA marker HBV (5/20, 7/10 and 9/20 from townships 1, 2 and 3, respectively). Clippers from township 2 had a significantly higher prevalence of blood contamination at 72%, compared with 33% of those from townships 1 and 3 (p=0.023). Table 1 shows the characteristics of the study participants according to hairstyle.

No clippers were positive for HIV. Four clippers (8%) were positive for HBV. Two clippers (clippers 16 and 20) were positive on qualitative HBV PCR. HBV DNA from clipper 16 clustered with genotype A sequences from SA, India, Brazil, and Martinique, while clipper 20 clustered with SA genotype D sequences. The clipper 20 sequence was identical to a subtype D sequence (GenBank accession AY233291) from Gauteng, SA[15,16] (Fig. 1).

In the communities with a predominantly black African population, townships 1 and 3, the chiskop haircut was the style most commonly requested by clients. In township 2, the population of which is predominantly of mixed-race ancestry, no client requested a chiskop; instead 6 had a brush cut, 3 a blade-fade and 1 a German cut (the scalp margins are shaved with a blade in the blade-fade and with a clipper in the German cut; however, the result is the same). All the barbers cleaned the clippers after each client, but the cleaning agents varied. Most barbers (82%) used disinfection with methylated spirits after using a brush to remove hair, and 8% used an open flame. All the barbers who used an open flame to clean their clippers (8% of the total sample) were from township 2 and usually cut the blade-fade style (p=0.013).

Discussion

Four clippers were contaminated with HBV. The HBV sequences identified are in keeping with other sequences from SA, where the main hepatitis B genotypes are A and D.[15,16] The use of only 188 nucleotide positions for phylogenetic analysis limits the conclusions that can be reached, but is adequate to show that our sequences cluster with sequences obtained from our region by other researchers. We found concentrations of HBV DNA of between <20 and 45 IU/ml in clipper wash. Welzel et al[17] calculated a ratio of 1 IU:10 DNA copies in their laboratory. The 2009 chronic hepatitis B practice guidelines[18] indicate a ratio of 1 IU:5 DNA copies. This amounts to approximately 300 DNA copies washed from clipper 16. Levels of HBV DNA in blood vary considerably, depending on the stage of infection, whether infection is acute or chronic, e-antigen positivity or negativity, and other factors. Viral loads are reported to reach as high as 10 log10 IU/ml serum/plasma,[19,20] and in our diagnostic laboratory we have seen occasional samples with an estimated viral load of up to 15 log10 IU/ml plasma. Viral loads (log copies/ml) of 4.3, 5.9, 6.2 and 5.2 have been reported in urine, saliva, tears and sweat, respectively.[21] Hepatitis B transmission has been linked to body fluids other than blood,[22,23] as well as to the sharing of toothbrushes and razors.[24] The risk of transmission between healthcare workers and patients has been well documented.[24-26] There is therefore a risk of clipper contamination from minor bleeding on the scalp. The infectious dose of hepatitis B has been estimated at 10 - 100 viruses. A study in chimpanzees determined the ID50 of HBV (the minimum infectious dose of HBV, or the dose at which 50% of chimpanzees would be infected)[27] in five chimpanzees to be ~10 DNA copies for both genotypes A and C.[27] In a subsequent study, the same team found that a dose of 2.6 - 4.6 DNA copies from pre-acute-phase chimpanzee serum caused infection in 3/3 chimeric mice with transplanted human hepatocytes, while a dose of 200 - 350 DNA copies from late acute-phase serum caused infection in only 1/3 mice,[28] suggesting that virus during early acute infection is more able to infect human hepatocytes.

Table 1. Characteristics of study participants according to hairstyle

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, N (%)</th>
<th>Clean-shave haircut barbers, n (%)</th>
<th>Other haircut barbers, n (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Township 1 (Langa)</td>
<td>20 (40.0)</td>
<td>19 (48.7)</td>
<td>1 (9.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Township 2 (Bonteheuwel)</td>
<td>10 (20.0)</td>
<td>0</td>
<td>10 (90.9)</td>
<td></td>
</tr>
<tr>
<td>Township 3 (Gugulethu)</td>
<td>20 (40.0)</td>
<td>20 (51.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>39</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age group of clients serviced (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - 17</td>
<td>6 (12.0)</td>
<td>6 (15.4)</td>
<td>0</td>
<td>0.317</td>
</tr>
<tr>
<td>18 - 60</td>
<td>44 (88.0)</td>
<td>33 (84.6)</td>
<td>11 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>39</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Cleaning methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brush and spirit</td>
<td>41 (82.0)</td>
<td>35 (89.8)</td>
<td>6 (54.5)</td>
<td>0.013</td>
</tr>
<tr>
<td>Spirit only</td>
<td>5 (10.0)</td>
<td>3 (7.7)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Open flame</td>
<td>4 (8.0)</td>
<td>1 (2.5)</td>
<td>3 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>39</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4 (8.0)</td>
<td>3 (7.7)</td>
<td>1 (9.1)</td>
<td>0.643</td>
</tr>
<tr>
<td>Negative</td>
<td>46 (92.0)</td>
<td>36 (92.3)</td>
<td>10 (90.9)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>39</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Blood marker (HBB)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21 (42.0)</td>
<td>13 (33.3)</td>
<td>8 (72.7)</td>
<td>0.023</td>
</tr>
<tr>
<td>Negative</td>
<td>29 (58.0)</td>
<td>26 (66.7)</td>
<td>3 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>39</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

HBB = haemoglobin beta.

*p-value based on χ2 test of association for difference between clean-shave and other hairstyles.

6 brush-cut, 3 blade-fade, 1 German cut.
**Fig. 1.** A maximum-likelihood tree constructed in MEGA6 using an alignment of 188 nucleotide sequences. Selected bootstrap support above 60% is shown. Study samples are shown in red. The tree is rooted on an orangutan HBV sequence. Sequences for comparison have names starting with **A**, **B**, **C**, **D**, **E**, **F**, **G**, **H**, **I**, **J**, **K**, **L**, **M**, **N**, **O**, **P**, **Q**, **R**, **S**, **T**, **U**, **V**, **W**, **X**, **Y**, **Z**.

- **Genotype A**: AAY576434 ZA, AAY576430 ZA, AAY576430 ZA, AAY576430 ZA, AAY576430 ZA, AAY576430 ZA
- **Genotype B**: AB100029 CN, AB100029 CN
- **Genotype C**: AB100029 CN, AB100029 CN
- **Genotype D**: AB100029 CN, AB100029 CN
- **Genotype E**: AB100029 CN, AB100029 CN
- **Genotype F**: AB100029 CN, AB100029 CN
- **Genotype G**: AB100029 CN, AB100029 CN
- **Genotype H**: AB100029 CN, AB100029 CN
- **Genotype I**: AB100029 CN, AB100029 CN
- **Genotype J**: AB100029 CN, AB100029 CN
- **Genotype K**: AB100029 CN, AB100029 CN
- **Genotype L**: AB100029 CN, AB100029 CN
- **Genotype M**: AB100029 CN, AB100029 CN
- **Genotype N**: AB100029 CN, AB100029 CN
- **Genotype O**: AB100029 CN, AB100029 CN
- **Genotype P**: AB100029 CN, AB100029 CN
- **Genotype Q**: AB100029 CN, AB100029 CN
- **Genotype R**: AB100029 CN, AB100029 CN
- **Genotype S**: AB100029 CN, AB100029 CN
- **Genotype T**: AB100029 CN, AB100029 CN
- **Genotype U**: AB100029 CN, AB100029 CN
- **Genotype V**: AB100029 CN, AB100029 CN
- **Genotype W**: AB100029 CN, AB100029 CN
- **Genotype X**: AB100029 CN, AB100029 CN
- **Genotype Y**: AB100029 CN, AB100029 CN
- **Genotype Z**: AB100029 CN, AB100029 CN

- **Clipper 10**: D AY576434 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA
- **Clipper 20**: D AY576434 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA
- **Clipper 30**: D AY576434 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA
- **Clipper 40**: D AY576434 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA, D AY576430 ZA

**Antiviral sprays are used internationally in hair salons. Quaternary ammonium disinfectant cleaners have virucidal activity effective enough to kill herpes simplex virus (HSV) in the presence of blood.**

Many developing countries lack regulating bodies that monitor health practices at barber shops. In developed countries, activities of barbers are regulated through comprehensive training, licensing and monitoring programmes.
Conflicts of interest. None.

Acknowledgements. Simphiwe Khondo assisted with data collection in the communities.

Author contributions. Conception and design of the work: NPK, ZS; data collection: ZS, SK; laboratory data analysis: SK, KM, LMD, WZ, MA, MN; data capturing: SM; data analysis and interpretation: FG, NPK, ZS, MN; drafting the article: ZS, NPK, SK, MN; critical revision of the article: ZS, SK; laboratory data analysis: SK, KM, LMD, WZ, MA, MN; collection: ZS, SK; final approval of the version to be published: NPK.

References.


In the meantime, public education recommending individual clipper practices may require upscaling and access to sterilisation facilities. As HIV/HBV and HIV/HCV co-infections. Finally, barber hygiene as an important factor for HIV, HBV and hepatitis C virus (HCV) infections as well as HIV/HBV and HIV/HCV co-infections. Further scientific scrutiny is required to quantify infection risk and investigate whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus (HCV) infections as well as HIV/HBV and HIV/HCV co-infections. Finally, barber hygiene practices may require upscaling and access to sterilisation facilities. In the meantime, public education recommending individual clipper ownership for close-shave chopkop and blade-fade haircuts should be advocated.

Study limitations. There were several confounding factors that could have contributed to our results, such as the skill of the barbers in the different townships and the methods they used to cut the hairstyles. The small sample size of township 2 could also have skewed our results. The amount of time spent cleaning the clippers with the different methods was not measured. This is also a possible confounding factor and could have contributed to the results. A study with a bigger sample size could obtain results of better quality.

Conclusions. This study confirmed that there is significant contamination of barber hair clippers with blood and blood-borne viruses. Hepatitis B was detected with enough DNA copies to pose a risk of infection. Although HIV was not detected, the clinical significance of contamination of clippers with blood, especially with regard to the transmission of blood-borne infections, warrants further study. Further scientific scrutiny is required to quantify infection risk and investigate whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus (HCV) infections as well as HIV/HBV and HIV/HCV co-infections. Fin...