**The pH of the human nail plate**

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**ABSTRACT**

In this Chapter, measurements of the nailplate pH are reported. Measurements were conducted *in vivo* in 37 volunteers with healthy finger and toe nails, using a skin pH meter. The pH of unwashed and washed fingernails and the big toenails was measured and the influence of washing, anatomical site (fingers/toes), side (left/right), finger digit (digits 1-5) and gender were determined. The pH of the nail plate surface was around 5. There was no significant difference between the sides i.e. right or left hand/foot, among the ten fingernails, and between the two great toenails. However, toenails had a significantly higher pH than fingernails. Washing the nails caused an immediate, but transient increase in pH, which was not sustained with time, and pH returned to pre-washing levels within 20 minutes. In males, washing did not significantly influence finger or toe nailplate pH. In females however, washed fingernails had a significantly higher pH than unwashed ones, while there was no difference in the pH of the toenails. The pH of the nail plate interior, measured after tape-stripping, was found to be slightly lower than that at its surface.

## INTRODUCTION

In contrast to the large body of literature on skin pH and its alterations in certain skin disorders [1-4], and on nail properties [5, 6], there is very little literature on the pH of the nailplate. An understanding of the nailplate pH is however extremely important. It is possible that nailplate pH has a role in the health and antimicrobial defence of the nail unit, in the same way that the acidic nature of the skin is vital to the latter's barrier properties and integrity. In this context, the pH of the nailplate was measured in our laboratory, in volunteers with healthy nails, to determine the baseline (i.e. in health) values [7]. The influences of gender, anatomical site (finger/toe), side (left/right), digit (1-5 of fingernails), washing, on the nail plate pH, was explored. In addition, pH of the nailplate interior was measured.

The nailplate's surface pH was measured potentiometrically using an apparatus (skin pH meter PH 905®, Courage and Khazaka GmbH, Germany) that is commonly used to measure skin pH. A hydrated planar glass electrode connected to a pH meter was placed on the nailplate. It is expected that water-soluble components of the nailplate would be extracted out of the nailplate and into the liquid interface between the nail and the pH measuring probe. The pH of this solution would then be read by the glass electrode and would give an indication of the pH of the nailplate surface. The pH value thus obtained due to the extraction of only the water-soluble components out of the nailplate should be regarded as an apparent pH, and interpreted with caution, as has been suggested for skin pH [8].

Measuring the pH of the nailplate surface using an apparatus originally designed for the skin presents a number of challenges. Unlike the skin, the nailplate is a hard surface, such that it is difficult to create a perfectly good interface between the nail plate and the flat glass probe. Certain nail plates, such as the little fingernails and all the toenails (except for digit1) are too curved or have too small an area, while others are too ridged, to allow measurement. Nevertheless, it was possible to measure the nail plate pH in many nailplates in 37 individuals (16 females and 21 males, aged 22-69 years old) recruited for the study.

**MEASUREMENTS**

Measurements were performed *in vivo* following ethics approval, in an air-conditioned room where the temperature ranged from 20.1 $^{o}$C to 25.2 $^{o}$C (mean 22.8 ± 1.1 $^{o}$C), and the relative humidity ranged from 22% to 35% (mean 28.0 ± 3.4%). Volunteers rested in the room for 20 minutes prior to measurement to acclimatise. The pH probe was rinsed with distilled water and applied at right angle to the nail plate with gentle pressure for about one minute before the pH was read. Multiple readings were taken to ensure the pH had stabilised and the stable pH value was then recorded. To determine the influence of washing, volunteers washed their hands with tap water and Cussons Carex® (sensitive) handwash (whose pH was 4.02), and blotted them dry using paper towels. The pH of the washed nailplates was measured immediately afterwards, and then at time intervals, to investigate for changes in pH with time. It was found that following washing, the nail plate pH stabilised within 20 minutes. Subsequently, a larger study was conducted to measure the pH of washed finger and toe nail plates, where the volunteers washed their hands and feet as described above, and rested in the room for 20 minutes prior to measurement.

To determine the pH of the nail plate interior, the thumb nailplates were used. Following acclimatization and an initial pH measurement, the nailplates were tape-stripped; an adhesive disc (D-Squame, Cuderm, Dallas, USA) was applied to the nailplate and firmly pressed by the operator for 30 seconds before being removed. This was repeated a number of times and the pH of the newly exposed nail plate surface was measured after every ten strips and at the end of the experiment, when the volunteer felt tingling and discomfort at the nail and stripping was stopped. The different volunteers experienced discomfort following different extents of tape-stripping. Thus, in the unwashed nails group, the volunteers received a total of either 15 (1 volunteer), 20 (1 volunteer), 30 (2 volunteers), 40 (1 volunteer) or 50 (1 volunteer) strippings. In the washed nails group, the volunteers received a total of either 15 (1 volunteer), 20 (5 volunteers), 30 (1 volunteer) and 40 (3 volunteers) tape strippings. The different tolerances of the volunteers to tape-stripping is likely due to inter-individual variability in the mechanical properties of nailplate, such that different amounts of nail cells/layers would be removed by each tape-strip. Such inter-individual variability reflects results by Tudela et al who reported variable amounts of nail protein removed in different individuals following 20 tape strips in each volunteer [9].

## RESULTS AND DISCUSSION

### pH of UNWASHED nail plate surface

The pH of the nail plate surface was found to be acidic (Table 1). The mean (±sd) pH of the surface of unwashed nail plates was 5.0 ± 0.5, with a minimum of 3.4 and a maximum of 6.3 measured in 157 fingernails and 33 big toenails in 15 males and 13 females. These pH values are expected to be due to the extraction of water-soluble components of the nail plate into the liquid interface between the nail plate and the pH measuring probe, as well as residual cosmetics such as hand creams, and the presence of sweat (which has a pH of 5-7 [10]) and sebum (a large proportion of which is made up of free fatty acids [11]) on the nailplate. Low levels of sebum have been measured on the nailplates [12]; sweat and sebum may be present on the nails following contact between skin and nail and/or their flow from surrounding skin onto the nail plate.

General linear model statistical analysis showed an influence of gender and anatomical site (finger/toe), with nailplate pH being higher in males and in toenails ($p < 0.01$), while side (right/left) had no influence ($p=0.2$). In addition, no significant differences were found among the pH of the ten fingernails ($p>0.01$, repeated measures ANOVA using pH values from 15 volunteers where it had been possible to measure all ten fingernails). A lack of difference in nailplate pH between right/left sides, and among the ten fingernails could be due to the similar environments experienced by the right and left hands or feet and by the digits.

It is not known why toenail pH was statistically higher than fingernail pH (Table 1). Toenail plates are thicker, grow at a slower rate compared to fingernails [13] and allow a smaller transonychial water loss than the fingernails [14]. It is interesting though, to note that the higher toenail pH (compared to fingernail pH) reflects the statistically higher foot skin pH compared to hand skin pH reported in a small cohort of Japanese men [15]. Although the reason for the higher toenail pH is unclear, it may have clinical significance. If the acidic nature of the nail plate does have a role in antimicrobial defence, the lower acidity of toenail plate (compared to the fingernails) might contribute to their greater susceptibility to onychomycosis (fungal infections of the nail). Indeed, a higher incidence of onychomycosis [16], greater recalcitrance to treatment and the need for longer treatment duration in toenails compared to fingernails is well known [17]. The higher toenail pH might also favour the production of fungal spores; the latter's production by *Trichophyton rubrum* – one of the most common causes of onychomycosis – was shown *in vitro* to increase when the medium pH was increased from pH 4.5 to pH 7.5, and to decrease thereafter [18]. A higher toenail pH could favour the presence of fungal spores, which provide a reservoir for fungal infection. The influence of gender on nailplate pH is discussed in the next section.

**pH of WASHED nail plate surface**

In order to remove the possible influence of extraneous substances such as residual cosmetics on the nail plate pH, nails were washed. Immediately after handwashing and drying, the pH of the fingernail surface increased, from a mean ±sd of 5.1 ±0.4 to 5.3 ±0.5 (paired t test, $p<0.01$, n=140 fingernails in 14 volunteers). This could be due to the nails being exposed to high pH during washing. Although the liquid cleanser had a pH of 4.02, the pH of the washing solution (cleanser + tap water) would have been much higher, the pH of a 2%w/w aqueous solution of the cleanser in tap water being measured to be 7.90. The raised nailplate pH was however not sustained with time, as shown in Figure 1. Within 20 minutes of washing, the pH had decreased to pre-washing levels. The transient increase in the pH of the nail plate surface and subsequent return to pre-washing levels reflects the profile of skin pH upon washing [19, 20]

Subsequently, a larger study was conducted to measure the pH of washed, dried and rested (for at least 20 minutes) finger- and toe- nail plates. The mean (±sd) pH of the surface of washed nail plates was found to be 5.1 ± 0.6, with a minimum of 3.9 and a maximum of 6.9 measured in 204 fingernails and 32 big toenails in 13 males and 9 females. The pH of toenail plates was higher than those of fingernail plates (Table 1, general linear model, $p = 0.01$) while right/left side and gender had no influence ($p>0.2$). As for the unwashed nails, the pH of the ten fingernails did not differ significantly (repeated measures ANOVA, $p=0.2$, pH of all ten fingernails measured in 15 volunteers).

When the pH of washed and unwashed nails were compared (Table 1), it was found that washing did not significantly influence finger and toe nailplate pH in males (independent t tests ($p\geq0.5$, Table 1). In females however, washed fingernails had a significantly higher pH than unwashed ones ($p<0.01$), while there was no difference in the pH of their toenails ($p\geq0.5$). This suggests that washing removed something from the fingernails, such as handcreams. The fact that gender influenced the pH of unwashed nails, but not that of washed ones indicates that the lower pH in females' unwashed nails was due to an exogenous factor, such as residual hand creams. Use of cosmetics such as moisturizers has been suggested as a potential reason for reported differences in skin pH between males and females and for the conflicting reports on the influence of gender on skin pH [21], although a recent systematic study found consistently higher skin (forehead, cheek, neck, forearm and hand) pH in females compared to males [22]. Further studies where volunteers refrain from using topical products on the hands and feet and use the same cleansers for washing, for a run-in phase prior to measurement of nail plate pH could shed more light on the influence of gender on nailplate pH.

**pH of the nail plate interior**

Tape stripping removes nail plate cells, and the newly exposed nail plate surface appears somewhat 'flaky' (Figure 2) with volunteers complaining of a tingling sensation at the nail once a certain number of strips had been removed. Different volunteers had different numbers of tape stripping performed before they complained of discomfort, and before the experiment was stopped, hence, pre-stripping and post stripping pH for each volunteer were compared to investigate for differences, if any, between the surface and interior nail plate pH. For unwashed nails, a statistically significant difference (paired t test, $p<0.05$; $n=12$ thumbnails in 6 volunteers) was found, with the pH of the nail plate interior (4.1 ±0.7) being lower than that of its surface (4.7 ±0.7). For washed nails (20 thumbnails in ten volunteers), the pH of the nail plate interior was also slightly lower (4.8 ±0.6) than that at its surface which were 5.1 ±0.5 (before washing) and 5.2 ± 0.7 (after washing and resting).

The lower pH of the nail plate interior could be due to enhanced extraction of acidic, water-soluble nail components during the pH measurement, once the topmost nail layers had been removed by tape stripping.  The topmost layer of the nail plate is known to be the least permeable part of the nail plate and its removal results in increased permeation of chemicals into the nail plate [23].  Similarly, removal of the topmost nail layer is expected to increase the movement of molecules out of the bulk of the nail plate and into the liquid interface between the nail plate and the pH measuring probe.  A decrease in nail plate pH upon tape stripping reflects the **initial** reduction in skin pH upon tape stripping that has been observed prior to an increase in skin pH upon further tape stripping  [24-27].

**CONCLUSIONS**

The pH of the nail plate surface was measured to be around 5, with toenails having significantly higher pH than fingernails.  There was no difference however, among the ten fingernails or between the two great toenails.  Gender influenced the pH of unwashed fingernails - with women having lower pH compared to men - but had no influence on the pH of washed ones, suggesting the influence of an exogenous factor on women's lower fingernail pH.  Washing the nails with a liquid cleanser and tapwater transiently increased pH, which subsequently returned to pre-washing values within 20 minutes.  Tape stripping the nail plates in order to measure the pH of the nail plate interior revealed a lower pH inside the nail plate compared to its surface.

This study has shown that it is possible to measure the surface pH of nail plates using the Courage & Khazakha skin pH meter, although a long stabilisation time is needed, and the small area and high curvature of certain nail plates preclude their measurement.

## FUTURE WORK

The study has established pH values of healthy finger and toe nail plates. Much more needs to be done, to understand the origin and significance of the nailplate acidity, and any changes in the diseased state. The influence of factors such as aging, environment, systemic disease e.g. diabetes, on the nailplate pH could indicate how these factors predispose one to develop nail diseases such as onychomycosis. Changes, if any, in diseased nails should enable a greater understanding of these diseases, and investigations into new prevention and treatment modalities. For example, changes in nailplate pH in onychomycosis, if any, could shed light on factors which influence fungal colonisation and infections. Fungi are known to detect and respond to their environmental pH, secreting the appropriate proteolytic enzymes, which allows fungal invasion and subsequent infection [28]. The dermatophyte, *T. rubrum* has also been shown to alter the pH of the growth medium to pH values ranging from 8.3 to 8.9, irrespective of the initial medium pH [29]. Whether such a change in nailplate pH occurs *in vivo* in onychomycotic nails, remains to be seen. The difficulties of using the glass electrode of the pH meter was mentioned above. An electrode that is more flexible and smaller in area is needed to enable more accurate measurements on nailplates.

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Table 1 : Measured pH of unwashed and washed finger- and toe- nailplate surface in males and females. Reproduced with permission from [7].

| | pH of nail plate surface<br>**mean ± SD**<br>(minimum-maximum; N=number of nails in n=number of volunteers) | | | |
|---|---|---|---|---|
| Gender | Unwashed nails | | Washed and rested nails | |
| | Fingernails | toenails | Fingernails | toenails |
| Males | **5.1 ± 0.5**<br>(3.4-6.1; N=87 in n=10) | **5.3 ± 0.4**<br>(4.5-6.3; N=20 in n=10) | **5.1 ± 0.5**<br>(3.9-6.4; N=125 in n=13) | **5.3 ±0.7**<br>(4.1-6.9; N=20 in n=11) |
| Females | **4.8 ± 0.4**<br>(3.8-5.7; N= 70 in n=7) | **5.4 ± 0.5**<br>(4.4-5.8; N=13 in n=7) | **5.1 ± 0.6**<br>(3.9-6.5; N=79 in n=9) | **5.6 ± 1.0**<br>(4.7-6.9; N=13 in n=7) |

**Figure legends**

Figure 1 Change in nail plate surface pH upon washing and subsequently with time. The latter is given as an approximation, rather than a definite value as the time varied slightly for each volunteer and for each nail, depending on the durations of pH measurements. Reproduced with permission from [7].

Figure 2: The 'flaky' appearance of the nail plate surface after tape stripping. This volunteer received a total of 15 tape strips, at which point he felt discomfort and tingling. Reproduced with permission from [7].