Dynamics of Changes in Quality of Life and Local Levels of TNF-α and IL-1β in Patients with Acute Viral Rhinitis

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Acute viral rhinitis is one of the most common pathologies among the population of the globe. Although, at first glance, this pathology seems simple and well-studied, but due to the high incidence, the load it creates on the health care system encourages in-depth study.

Aims: To examine the dynamics of changes in quality of life and local levels of TNF-α and IL-1β in patients with acute viral rhinitis in different treatment regimens.

Methodology: The prospective study included 84 patients with a confirmed diagnosis of acute viral rhinitis. In accordance with the purpose and objectives of the study, all patients on days 1, 5 and 10, in addition to routine studies, were determined by local levels of TNF-α and IL-1β, as well as to determine the quality of life.

Results: In patients of the study group, only partial recovery of local levels of TNF-α and IL-1β and indicators of the physical component of quality of life was observed during 10 days of treatment. The emotional and functional components of quality of life in patients of this study were more correlated than with the physical component and local levels of proinflammatory cytokines, which are objective indicators of the activity of the inflammatory process. Complementing the traditional treatment regimen with 0.25% oxolin in the form of an ointment has reduced the duration of the

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disease, reduced the incidence of bacterial complications and improved the quality of life of patients with acute viral rhinitis in the short term.

Conclusions: The dynamics of local levels of TNF-α and IL-1β reflects the nature of the inflammatory process in the nasal mucosa and clearly correlates with the physical component of quality of life.

Keywords: Acute viral rhinitis; TNF-α; IL-1β; quality of life.

1. INTRODUCTION

Acute viral rhinitis is one of the most common pathologies among the population of the globe. Human rhinoviruses cause about half of the cases of upper respiratory tract infections or "colds" [1,2].

After entering the respiratory tract, the viruses infect, first, the epithelium of the nasal mucosa, and then other components of the upper and lower respiratory tract. Although oral inoculation may be an alternative source of virus transmission, the risk is relatively low [3].

It is believed that the common cold lasts 5-10 days, after which the symptoms either persist or worsen with the development of post-viral acute rhinosinusitis [4]. Joining the bacterial microflora causes the development of purulent rhinosinusitis, bronchitis, pneumonia [4,5,6,7].

According to European Position Paper on Rhinosinusitis and Nasal Polyps treatment of acute viral rhinitis includes paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs); second-generation antihistamines with short-term benefit in reducing symptoms the first 2 days; nasal decongestants with small effect in nasal congestion in adults; combination of analgesics and nasal decongestants; ipratropium bromide for reducing rhinorrhea; probiotics; zinc when administered the first 24 h after the onset of symptoms; nasal saline irrigations; vitamin C, in selected patients with suspected deficit or with high levels of physical activity; and some herbal medicines (BNO 1016, cineole, and Andrographis paniculata SHA-10) [4].

Although, at first glance, this pathology seems simple and well-studied, but due to its high incidence, the burden on the health care system encourages an in-depth study of it and the search for new and more effective approaches to its treatment [8,9].

The aim of the study was to study the dynamics of changes in quality of life and local levels of TNF-α and IL-1β in patients with acute viral rhinitis in different treatment regimens.

2. MATERIALS AND METHODS

Prospective study was approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Vinnytsia, Ukraine (Protocol № 10, 2.12.2021). The Bioethics Committee considered that research was performed in accordance with the World Medical Association Declaration of Helsinki on the ethical principles for medical research involving human subjects, the Council of Europe Convention on the Human Rights and Biomedicine, relevant laws, orders of the Ministry of Health of Ukraine. Each subject of the study were provided with all details about medical procedures and given the opportunity to discuss any questions with healthcare professionals, and then signed a detailed form of informed consent to conduct the research.

The study included 84 patients aged 18 to 60 years (39.4 ± 12.1 years) with a confirmed diagnosis of acute viral rhinitis (J00 according to ICD-10). There were 45 women (53.6%) and 39 men (46.4%).

The criteria for inclusion in the study were:

1. Confirmed diagnosis of acute viral rhinitis.
2. The time to seek medical attention is no more than 24 hours from the onset of symptoms.
3. Age from 18 to 60 years.
4. Absence at the time of inclusion in the study of emergencies and/or exacerbation of chronic diseases.
5. Absence in the anamnesis of allergic reactions to the offered medicines.
6. Absence of mechanical damage to the mucous membrane and skin of the nasal wings with/or without signs of bacterial infection.
7. No need for constant medication.
8. No pregnancy or breastfeeding.
The exclusion criteria were:

1. Refusal to further participate in the study.
3. Development of a severe allergic reaction to the proposed drugs.
4. Occurrence of emergencies and/or exacerbation of chronic diseases after inclusion in the study.

According to European Position Paper on Rhinosinusitis and Nasal Polyps the diagnosis of acute viral rhinitis was established in the presence of two or more nasal symptoms (nasal congestion obstruction, rhinorrhea, facial pressure, or loss of smell). If necessary, the diagnosis was confirmed endoscopically (mucosal edema or rhinorrhea) [4]. When ocular symptoms occurred, to exclude conjunctivitis, an ophthalmologist was consulted.

All patients were equally randomized into two study groups.

Experimental group 1 included patients who were prescribed treatment in accordance with European Position Paper on Rhinosinusitis and Nasal Polyps. This treatment included nonsteroidal anti-inflammatory drugs (selective COX-2 inhibitors), nasal decongestants, 2nd generation antihistamines, vitamin C. Treatment (excluding antihistamines administered in the first 2 days) was prescribed until complete recovery, but for a period not exceeding 10 days. The age of patients in this group was 38.5 ± 12.9 years. There were 23 women (54.8%) in the group, 19 men (45.2%).

Experimental group 2 included patients whose treatment was supplemented by topical application of 0.25% oxolin in the form of nasal ointment three times a day. The age of patients in this group was 40.2 ± 11.3 years. There were 22 women in the group (52.4%) and 20 men (47.6%).

In accordance with the purpose and objectives of the study, all patients on days 1, 5 and 10, in addition to routine studies, were determined by local levels of TNF-α and IL-1β, as well as to determine the quality of life.

TNF-α and IL-1β levels were determined in nasal mucosal washes by enzyme-linked immunosorbent assay using the Human TNF-α ELISA kit and the Human IL-1β ELISA Kit (CUSABIO, China).

Quality of life was studied using the Rhinosinusitis disability index (RSDI) with the definition of key integrative indicators (questionnaire subscales), such as: Emotional, Functional and Physical.

To determine the reference values of the studied indicators, we studied a control group similar to the research groups in the number of respondents (n=42). This group included relatively healthy people who were once tested for TNF-α and IL-1β levels in nasal washes and assessed the quality of life according to a selected questionnaire. The age of patients in the control group was 36.8 ± 11.8 years. There were 21 women and men in this group.

The obtained data were processed using the statistical software package SPSS 20.0 for Windows. The significance of differences in disease duration and TNF-α and IL-1β was determined by the Student test. The significance of differences between groups in the number of complications was determined by the exact bilateral Fisher test. Wilcoxon T-test was used to compare the quality of life between groups. Mann-Whitney U-test was used to assess the relationship between the indicators was assessed using the Spearman correlation coefficient.

3. RESULTS

In statistical comparison, the control group and both research groups were gender-age-homogeneous.

All patients in both study groups tolerated the prescribed treatment well.

The use of oxolin was accompanied by isolated adverse reactions, which included irritation of the mucous membrane in 1 (2.4%) patient and exacerbation of rhinorrhea in 1 (2.4%) patient. Adverse reactions did not affect the general condition of the patients, were mild, and therefore all patients were recommended to continue to participate in the study, with which patients agreed. It should be noted that in patients with adverse reactions, all subjects were in the range of 25 to 75 percentiles at each time point.

The duration of the disease differed significantly between groups (P<0.001) and was 5.9 ± 0.8 days for experimental group 1, and 7.2 ± 0.9 days for experimental group 2.
The joining of bacterial microflora with the development of purulent complications occurred in 5 patients (11.9%) in study group 1 and 1 patient (2.4%) in study group 2. It should be noted that the statistical significance of differences between groups was not proven ($P=0.98$).

Local levels of TNF-α and IL-1β in patients in our study are shown in Table 1.

The dynamics of changes in local levels of TNF-α and IL-1β in the studied contingent generally had similar dynamics.

At the beginning of the study, TNF-α levels in experimental groups 1 and 2 significantly exceeded the control group in 17.1 and 16.3 times, respectively, and did not differ significantly ($P=0.35$). There was a progressive decrease in the levels of this inflammatory mediator throughout the study. Thus, on day 5, TNF-α levels were 1.6 times lower than baseline in experimental group 1 and 1.8 times lower in experimental group 2 and differed significantly ($P=0.003$). On the 10th day of the study, the levels of the studied indicator were lower than the initial values by 6.4 and 7.0 times, and on the 5th day by 4.0 and 3.8 times for the experimental groups 1 and 2, respectively. In addition, on the 10th day of the study, TNF-α levels in both study groups differed significantly ($P=0.004$) and exceeded the control values by 2.7 times in experimental group 1 and 2.3 times in experimental group 2.

At the beginning of the study, IL-1β levels in experimental groups 1 and 2 were significantly higher than those of the control group by 4.10 and 4.13 times, respectively, and did not differ significantly ($P=0.91$). There was a progressive decrease in the levels of this inflammatory mediator throughout the study. Thus, on day 5, IL-1β levels were 1.4 times lower than baseline in experimental group 1 and 1.8 times lower in experimental group 2 and differed significantly ($P<0.001$). On the 10th day of the study, the levels of the studied indicator were lower than the initial values by 2.2 and 2.8 times, and on the 5th day by 1.63 and 1.57 times for the experimental groups 1 and 2, respectively. In addition, on day 10 of the study, IL-1β levels in both study groups differed significantly ($P<0.001$) and exceeded the control values by 1.8 times in experimental group 1 and 1.5 times in experimental group 2.

Table 1. Local levels of TNF-α and IL-1β in the studied contingent

<table>
<thead>
<tr>
<th>The term of the study</th>
<th>TNF-α, pg/mL</th>
<th>IL-1β, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>21.24 ± 6.12</td>
<td>5.12 ± 1.47</td>
</tr>
<tr>
<td>* RG1_1, RG1_5, RG1_10, RG2_2, RG2_5, RG2_10</td>
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<td></td>
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<tr>
<td>Research group 1 (RG1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (RG1_1)</td>
<td>363.98 ± 98.94</td>
<td>21.02 ± 6.01</td>
</tr>
<tr>
<td>* C, RG1_5, RG1_10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 day (RG1_5)</td>
<td>227.98 ± 68.46</td>
<td>15.33 ± 3.8</td>
</tr>
<tr>
<td>* C, RG1_1, RG1_10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ RG2_5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 day (RG1_10)</td>
<td>57.12 ± 13.23</td>
<td>9.36 ± 3.1</td>
</tr>
<tr>
<td>* C, RG1_1, RG1_5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ RG2_10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research group 2 (RG2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (RG2_1)</td>
<td>345.24 ± 84.71</td>
<td>21.17 ± 6.08</td>
</tr>
<tr>
<td>* C, RG2_5, RG2_10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 day (RG2_5)</td>
<td>187.55 ± 52.49</td>
<td>11.83 ± 3.4</td>
</tr>
<tr>
<td>* C, RG2_1, RG2_10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ RG1_5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 day (RG2_10)</td>
<td>48.74 ± 12.97</td>
<td>7.5 ± 1.58</td>
</tr>
<tr>
<td>* C, RG2_1, RG2_5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ RG1_10</td>
<td></td>
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</tbody>
</table>

Note. A statistically significant difference for these groups with the corresponding number: Δ – $P<0.01$; * – $P<0.001$. 

109
Regarding the correlations between TNF-α and IL-1β levels, a strong positive relationship (rs = 0.87; P<0.001) was demonstrated.

Indicators of quality of life in patients of our study are shown in Table 2.

At the beginning of the study, the values of quality of life on the Emotional subscale in experimental groups 1 and 2 significantly exceeded the control group in 3.9 and 4.0 times, respectively, and did not differ significantly (P=0.28). Throughout the study, there was a progressive decrease in the levels of this component of quality of life. Thus, on the 5th day, this indicator was 2.9 times lower than the initial values in experimental group 1 and 4.0 times lower in experimental group 2 and differed significantly (P<0.001). On the 10th day of the study, the levels of the studied indicator were lower than the initial values by 3.3 and 3.4 times for the experimental groups 1 and 2, respectively. It should be noted that on the 5th day of the study, the values of quality of life on the Emotional subscale in both study groups did not differ significantly (P=0.19) and from the values of the control group (P=0.28 and P=0.83 for the study groups 1 and 2, respectively).

At the beginning of the study, the values of quality of life on the Functional subscale in experimental groups 1 and 2 significantly exceeded the control group in 3.8 and 3.9 times, respectively, and did not differ significantly (P=0.45). Throughout the study, there was a progressive decrease in the levels of this component of quality of life. Thus, on the 5th day, this indicator was 3.2 times lower than the initial values in the experimental group 1 and 3.4 times lower in the experimental group 2 and differed significantly from each other (P=0.46). On the 10th day of the study, the levels of the studied indicator were lower than the initial values by 3.7 and 3.9 times, and on the 5th day by 1.2 and 1.1 times for the experimental groups 1 and 2, respectively. It should be noted that on the 10th day of the study, as in the previous study period, the values of quality of life on the Functional subscale in both study groups did not differ significantly (P=0.62) and the values of the control group (P=0.47 and P=0.84 for experimental groups 1 and 2, respectively).

Table 2. Indicators of quality of life in the studied contingent

<table>
<thead>
<tr>
<th>The term of the study</th>
<th>Emotional condition</th>
<th>State of functional activity</th>
<th>Physical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group (C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>5.21 ± 1.54</td>
<td>4.71 ± 1.6</td>
<td>5.71 ± 1.64</td>
</tr>
<tr>
<td>* RG1_1, RG1_5, RG2_1</td>
<td>* RG1_5, RG2_5</td>
<td>* RG1_1, RG1_5, RG1_10, RG2_2, RG2_5</td>
<td></td>
</tr>
<tr>
<td><strong>Research group 1 (RG1)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (RG1_1)</td>
<td>20.12 ± 2.71</td>
<td>18.1 ± 2.57</td>
<td>22.14 ± 3.06</td>
</tr>
<tr>
<td>* C, RG1_5, RG1_10</td>
<td>* C, RG1_5, RG1_10</td>
<td>* C, RG1_5, RG1_10</td>
<td></td>
</tr>
<tr>
<td>5 day (RG1_5)</td>
<td>6.98 ± 1.6</td>
<td>5.74 ± 2.19</td>
<td>19.79 ± 2.35</td>
</tr>
<tr>
<td>* C, RG1_1, RG1_10</td>
<td>* C, RG1_1, RG1_10</td>
<td>* C, RG1_1, RG1_10</td>
<td></td>
</tr>
<tr>
<td>* RG2_5</td>
<td>* RG1_1</td>
<td>* RG2_5</td>
<td></td>
</tr>
<tr>
<td>10 day (RG1_10)</td>
<td>5.57 ± 1.47</td>
<td>4.95 ± 1.38</td>
<td>10.33 ± 1.18</td>
</tr>
<tr>
<td>* RG1_1, RG1_5</td>
<td>* RG1_1</td>
<td>* RG1_1</td>
<td></td>
</tr>
<tr>
<td><strong>Research group 2 (RG2)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (RG2_1)</td>
<td>20.76 ± 3.37</td>
<td>19.55 ± 2.91</td>
<td>22.86 ± 3.57</td>
</tr>
<tr>
<td>* C, RG2_5, RG2_10</td>
<td>* C, RG2_5, RG2_10</td>
<td>* C, RG2_5, RG2_10</td>
<td></td>
</tr>
<tr>
<td>5 day (RG2_5)</td>
<td>5.21 ± 1.37</td>
<td>5.43 ± 1.55</td>
<td>17.02 ± 1.89</td>
</tr>
<tr>
<td>* RG2_1</td>
<td>* C, RG2_1</td>
<td>* RG1_1</td>
<td></td>
</tr>
<tr>
<td>10 day (RG2_10)</td>
<td>5.14 ± 1.56</td>
<td>4.79 ± 1.65</td>
<td>8.38 ± 1.5</td>
</tr>
<tr>
<td>* RG2_1</td>
<td>* RG2_1</td>
<td>* RG1_1</td>
<td></td>
</tr>
</tbody>
</table>

Note. A statistically significant difference for these groups with the corresponding number: # – P<0.05; Δ – P<0.01; * – P<0.001
At the beginning of the study, the values of quality of life on the Physical subscale in experimental groups 1 and 2 significantly exceeded the control group in 3.9 and 4.0 times, respectively, and did not differ significantly ($P=0.32$). Throughout the study, there was a progressive decrease in the levels of this component of quality of life. Thus, on the 5th day, this indicator was 1.1 times lower than the initial values in experimental group 1 and 1.3 times lower in experimental group 2 and differed significantly ($P<0.001$). On the 10th day of the study, the levels of the studied indicator were lower than the initial values by 2.1 and 2.7 times, and by the values on the 5th day by 1.9 and 2.0 times for the experimental groups 1 and 2, respectively. In addition, on the 10th day of the study, the values of quality of life on the Physical subscale in both study groups differed significantly ($P<0.001$) and exceeded the control values by 1.8 times in experimental group 1 and 1.5 times in the experimental group 2.

There was a strong positive correlation between quality of life values for Emotional and Functional subscales ($rs=0.84; P<0.001$) and for Emotional and Physical subscales ($rs=0.72; P<0.001$). For the pair Functional and Physical subscales, a positive correlation of moderate strength was proved ($rs=0.69; P<0.001$).

Correlations between the levels of inflammatory mediators studied and the subscales of the quality of life questionnaire are shown in Table 3.

As can be seen from Table 3, both inflammatory mediators studied were directly correlated with moderate strength with the Emotional and Functional subscales of the quality of life questionnaire. At the same time, the correlations between both TNF-α and IL-1β were positively strong.

### 4. DISCUSSION

The results of the analysis of the dynamics and relationships of local levels of TNF-α and IL-1β and the emotional, functional and physical components of quality of life allowed us to establish a number of patterns.

Thus, the dynamics of local levels of inflammatory mediators is almost the same for TNF-α and IL-1β [10], reflects the nature of the inflammatory process in the nasal mucosa [11,12] and clearly correlate with the physical component of quality of life. To a lesser extent, the levels of the studied proinflammatory cytokines in the patients of our study correlated with the emotional and functional components of quality of life. In our opinion, this can be explained by the connection of the emotional component of quality of life not only with the physical condition of patients but also with the peculiarities of their psychoneurological status [13].

When studying the dynamics of changes in the components of quality of life, in the period between 1 and 5 days there was a significant jump in the indicators of emotional and functional components. This may be due to the fact that about 5 days of the disease with an uncomplicated course in most patients there is a regression of the main symptoms [4,14]. Thus, nasal congestion and rhinorrhea, which are the causes of the main discomfort in patients, are significantly reduced. And, although the residual manifestations of the disease in the form of slight oedema of the mucous membrane and moderate nasal discharge still persist (as evidenced by slightly different dynamics of the physical component of quality of life), they do not cause significant discomfort for the functional activity of patients and are not associated with extremely negative emotions.

Also in our study, it was found that the addition of traditional treatment with oxolin in the form of nasal ointment significantly reduced the duration of treatment in patients of experimental group 2. In addition, patients in this group were less likely to have bacterial microflora with the development of purulent complications, although statistically

### Table 3. Correlations between levels of inflammatory mediators studied and pooled quality of life indicators

<table>
<thead>
<tr>
<th>The term of the study</th>
<th>Quality of life indicator</th>
<th>Emotional condition</th>
<th>State of functional activity</th>
<th>Physical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td></td>
<td>$rs=0.69$;</td>
<td>$rs=0.69$;</td>
<td>$rs=0.89$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
<td>$rs=0.68$;</td>
<td>$rs=0.66$;</td>
<td>$rs=0.88$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
</tr>
</tbody>
</table>
significant differences could not be confirmed. Also, patients in experimental group 2 had significantly faster recovery of the studied indicators compared with experimental group 1.

In our opinion, the best results in the treatment of patients in study group 2 are related to the mechanism of action and effects of oxolin.

According to the scientific literature, oxolin has antiviral properties [15,16,17]. It has been suggested that the drug binds to guanine residues within the host cell [17,18]. This is consistent with the finding that oxolin inhibits RNA synthesis in infected and uninfected cells. The drug acts by reducing overall cellular metabolism and thus prevents replication of the virus [17,19].

On the other hand, the drug is ineffective against bacterial or fungal infections, except in extreme doses and has no effect on systemic administration [17,18]. Therefore, the lower incidence of bacterial complications in the group of patients who were prescribed oxolin, in our opinion, at least in part, can be explained by the peculiarities of the dosage form of the drug. Thus, the drug acts by reducing overall cellular metabolism and thus prevents replication of the virus [17,19].

Isolated adverse reactions with oxolin were not accompanied by significant changes in local levels of the studied mediators of inflammation and did not significantly affect the quality of life of patients, as evidenced by finding values between 25 and 75 percentiles at each time point. Such side effects have been described in the scientific literature before [20].

The regularities established by us give grounds for their further in-depth study in a larger contingent of patients and with the involvement of related specialists and additional methods of laboratory and instrumental research.

5. CONCLUSION

1. The dynamics of local levels of TNF-α and IL-1β reflects the nature of the inflammatory process in the nasal mucosa and clearly correlates with the physical component of quality of life.
2. In patients with acute viral rhinitis, only partial recovery of local levels of TNF-α and IL-1β and indicators of the physical component of quality of life is observed within 10 days of treatment.
3. In patients with acute viral rhinitis, the emotional and functional components of quality of life are more correlated than with the physical component and local levels of proinflammatory cytokines, which are objective indicators of the activity of the inflammatory process [21].
4. Complementing the traditional treatment regimen with 0.25% oxolin in the form of an ointment can reduce the duration of the disease, reduce the incidence of bacterial complications and in a short time to improve the quality of life of patients with acute viral rhinitis.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

As per international standard or university standard, patient’s written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
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