GLOBAL JOURNAL OF MEDICAL RESEARCH: F DISEASES CANCER, OPHTHALMOLOGY & PEDIATRIC
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<td>Director, EP Laboratories, Philadelphia VA Medical Center Cardiovascular Medicine - Cardiac Arrhythmia Univ of Penn School of Medicine Web: pennmedicine.org/wagform/MainPage.aspx?</td>
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2. Severe Hypernatremic Dehydration in a Neonate. 9-12
Polymorphism of Rs1800629 Gene of TNF-A in the Pathogenesis of Acne Vulgaris

By Nilufar N. Malikova, Khamid Y. Karimov, Saidkasim S. Arifov & Kodirzhon T. Boboev

Tashkent State Dental Institute

Abstract- Background: The study of the association between acne vulgaris and the TNF- gene polymorphism rs1800629 in the Uzbek population is discussed in this article.

Methodology: The research was conducted on 165 patients with various clinical forms of acne vulgaris and 161 healthy donors.

Findings: During this research, allele frequencies and genotypes of rs1800629 polymorphism of TNF-α gene were found with statistically significant differences between the control group and subgroups of patients with acne vulgaris. As a result of the investigation, it was discovered that heterozygous genotype G/A should be a genetic marker for an increased risk of acne development, whereas homozygous genotype G/G should be a marker for a reduced risk of acne formation.

Keywords: acne vulgaris; TNF-α; gene polymorphism.

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Polymorphism ofRs1800629 Gene ofTNF-A in thePathogenesis ofAcneVulgaris

Nilufar N. Malikova, Khamid Y. Karimov, Saidkasim S. Arifov & Kodirzhan T. Boboev

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I. Introduction

Acne vulgaris (AV) is a widespread disease that causes damage to the sebaceous glands and associated hair follicles due to several genetic and environmental factors [13]. Epidemiological studies have shown that people between the ages of 12 and 24 are more susceptible to acne, with about 85% of the population suffering from it. Almost 80% of first-degree relatives suffer from acne and the disease occurs earlier and is more severe in people with a positive family history. In 15-20% of acne vulgaris patients, the disease is moderate to severe (Bhate K., Williams H.C., 2013; Mahto A. 2017; Janani, S., Sureshkumar, R. A., 2019; Heng AHS, Chew FT. 2020). It has been established that both endogenous and exogenous factors, such as antimicrobial peptides, in particular the expression of cathelicidin (LL-37), defensin-2, cytokines (IL-1α, TNF-α, IL-1β, IL-8, IL-10, etc.), vitamin D and matrix metalloproteinases, interferon γ, P. Acnes, St. Aurous, et al. (Dréno B., 2017; Ibrahim AA et all, 2018; Xu H. Li H. 2019; Ebrahimi A et all. 2019; Swelam MM et all 2019; Ragab M, 2019; Ayeingoz IE et al. 2021).

Along with exogenous and endogenous factors, genetic predisposition plays a leading role in the formation of the disease. At present, enough data have been accumulated on the involvement of various candidate genes, the protein products of which are directly or indirectly involved in the regulation of the immune response during inflammation, in the formation of a predisposition to acne. Despite this, the molecular genetic basis of acne production has not been well investigated.

Tumor necrosis factor-α (TNF-α) is a powerful pro-inflammatory cytokine, a mediator of inflammatory processes plays a central role in the initiation and regulation of the cytokine cascade during the inflammatory and immune response.

The TNF-α gene encoding this cytokine is located on the short arm of chromosome 6 (locus 6p21.1-6p21.3). To date, several functional polymorphic loci have been identified, among which the G-308A variant (replacement of guanine for adenine at nucleotide position -308, international code: rs1800629) is the most common polymorphism. The influence of this locus on the synthesis and level of concentration in the body of the cytokine TNF-α has been proven [Kroeger KM, 1997; Wilson AG 2003].

In studies of hereditary predisposition to various multifactorial diseases, namely the 308A allele and the G/A and A/A genotypes of the rs1800629 polymorphism of the TNF-α gene are considered as a risk factor for the development of various pathological processes associated with an impaired immune response.

The research aims to study the association of rs1800629 polymorphism of the TNF-α gene with the development and clinical course of acne.

II. Materials and Methods

The study was conducted on a sample of 165 acne patients and 161 conditionally healthy donors (control group). The clinical picture of acne vulgaris is characterized by the appearance of comedones, papules, pustules, nodes, cysts on areas of the facial skin (forehead, cheeks, chin), front and back of the back.
containing sebaceous glands (Tan A.U., Schlosser B.J., Paller A.S. 2018). The degree of activity of the process, the planned method of therapy is determined by the severity of the course of acne. Practical dermatology therefore often uses the classification proposed by the Global Alliance to Improve Acne Outcomes, which distinguishes between mild, moderate, or severe acne (Thiboutot D., Gollnick H., Bettoli V., et al; 2009.).

The analysis of associations was carried out by comparing two samples according to the "case-control" type. The sample "case" was formed from patients with different severity of acne (165 patients). 59 of them suffered from mild, 64 – moderate, and 42 - severe forms of acne. The "control" group included 97 conditionally healthy individuals without any dermatological or other diseases. To isolate DNA from peripheral blood, we used the standard phenol-chloroform extraction method and the RNA/DNA-sorb kit from InterLabService LLC (Russia) (Fig. 1). For genotyping rs1800629 of the TNF-α gene, test systems of NPF "Litekh" (Russia) were used according to the manufacturer's instructions. PCR detection was performed using a GeneAmp PCR-system 2720 thermal cycler (Applied Biosystems, USA).

Fig. 1: Electropherogram for TNFα gene polymorphism detection.

K- negative control; K+ positive control;
1,2,3,5,6,9,10,13,14,15 - wild genotype G/G;
4,7,8,11,12,16 - heterozygous genotype G/A;

Statistical processing of the obtained data was carried out using the statistical software package OpenEpi 2009, Version 2.3.

III. Results and Discussion

The frequency distribution of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene and their statistical difference among patients and the control sample are shown in Table 1, where statistically significant differences were found between the compared groups. As can be seen from Table 1, the frequency of distribution of G and A alleles in the combined group of patients and the control sample was 79.4% and 20.6% versus 86.6% and 13.3%, respectively. Rare allele A (20.6%) of this polymorphism was found to be statistically significantly more common in the combined patients’ group than in the comparison group (26.7%). According to the odds ratio for carriers of this genotype, the risk of developing acne is significantly increased by 1.7 times (χ²=5.4; p = 0.02; OR=1.7; CI95% 1.08-277). Along with this, no reliable association of the homozygous genotype (A / A) with the formation of acne was found. This genotype was detected only in the group of AV patients (1.2%), while in the control group this genotype was not detected (χ²=2.0; p=0.2).

OR=0.5; CI: 95% 0.342-0.87) and the proportion of carriers of heterozygote G/A was significantly more frequent in patients with AV (38.8%) than in the comparison group (26.7%). According to the odds ratio for carriers of this genotype, the risk of developing acne is significantly increased by 1.7 times (χ²=5.4; p = 0.02; OR=1.7; CI95% 1.08-277). Along with this, no reliable association of the homozygous genotype (A / A) with the formation of acne was found. This genotype was detected only in the group of AV patients (1.2%), while in the control group this genotype was not detected (χ²=2.0; p=0.2).
Table 1: Frequency distribution of alleles and genotypes of the rs1800629 (G308A) polymorphism of the TNF-α gene in the group and subgroups of patients with acne and the healthy control

<table>
<thead>
<tr>
<th>№</th>
<th>Group</th>
<th>n*</th>
<th>Allele frequency</th>
<th>Genotype distribution frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Main group n=165</td>
<td>330</td>
<td>262</td>
<td>79.4</td>
</tr>
<tr>
<td>a</td>
<td>Severe form n=42</td>
<td>84</td>
<td>61</td>
<td>72.6</td>
</tr>
<tr>
<td>b</td>
<td>Moderate severity n=64</td>
<td>128</td>
<td>97</td>
<td>75.8</td>
</tr>
<tr>
<td>c</td>
<td>Mild severity n=59</td>
<td>118</td>
<td>104</td>
<td>88.1</td>
</tr>
<tr>
<td>2</td>
<td>Control group n=161</td>
<td>322</td>
<td>279</td>
<td>86.6</td>
</tr>
</tbody>
</table>

In the next stage of the work, a comparative analysis of the distribution of the frequencies of alleles and genotypes of this locus, depending on the severity of acne, was carried out.

It was found that among patients with severe acne severity, the proportion of carriers of alleles G and A was 72.6% and 27.4%, and in the comparison group 86.6% and 13.3%, respectively. And the value of the relative risk, equal to 2.4 with the confidence interval CI95% 1.374 - 4.35 (χ²=9.6 and P=0.002), also confirms the significant association of the unfavorable allele A in the formation of acne (Table 2). The frequencies G/G, G/A and A/A in the studied subgroup of patients and the control sample were: 50.0%, 45.2% and 4.5% versus 73.3% and 26.7%, respectively. The heterozygous G/A genotype is a determinant of an increased risk of developing severe acne (45.2% versus 26.7%, χ²=5.4; p=0.02; OR=2.3; CI: 95% 1.12-4.56), while the favorable A/A genotype, on the contrary, a marker of a reduced risk of developing this form of the disease (50.0% versus 73.3%, respectively; χ²=8.4; p=0.004; OR=0.4; CI: 95% 0.181-0.73). The proportion of carriers of the homozygous genotype in the subgroup of patients was 4.5%, while in the healthy control this genotype was not detected (χ²=7.7; p=0.005) (Table 3).

Table 2: Differences in the frequency of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene in the study and control groups

<table>
<thead>
<tr>
<th>Alleles and genotypes</th>
<th>Main group</th>
<th>Control group</th>
<th>χ²</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>262</td>
<td>279</td>
<td>6.1</td>
<td>0.01</td>
<td>1.7</td>
<td>1.109-2.556</td>
</tr>
<tr>
<td>A</td>
<td>68</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>99</td>
<td>118</td>
<td>6.5</td>
<td>0.01</td>
<td>0.5</td>
<td>0.3424-0.8727</td>
</tr>
<tr>
<td>G/A</td>
<td>64</td>
<td>43</td>
<td>5.4</td>
<td>0.02</td>
<td>1.7</td>
<td>1.08-2.77</td>
</tr>
<tr>
<td>A/A</td>
<td>2</td>
<td>0</td>
<td>2.0</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
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Table 3: Differences in the frequency of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene in the subgroup of patients with mild acne and the control group

<table>
<thead>
<tr>
<th>Alleles and genotypes</th>
<th>Severe form</th>
<th>Control group</th>
<th>χ²</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>61</td>
<td>279</td>
<td>9.6</td>
<td>0.002</td>
<td>2.4</td>
<td>1.374-4.35</td>
</tr>
<tr>
<td>A</td>
<td>23</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>21</td>
<td>118</td>
<td>8.4</td>
<td>0.004</td>
<td>0.4</td>
<td>0.181-0.73</td>
</tr>
<tr>
<td>G/A</td>
<td>19</td>
<td>43</td>
<td>5.4</td>
<td>0.02</td>
<td>2.3</td>
<td>1.12-4.56</td>
</tr>
<tr>
<td>A/A</td>
<td>2</td>
<td>0</td>
<td>7.7</td>
<td>0.005</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
A similar pattern was detected when the allele and genotype frequencies of this locus were compared between a subgroup of patients with average acne severity and a population sample. The frequency of distribution of alleles and genotypes in the studied subgroups of patients with moderate severity of acne was: G=75.8% and A=24.2%, G/G=51.6%, G/A=48.4% and A/A=0.0. The risk of developing moderate acne is significantly higher in carriers of the functionally unfavorable allele A of genotype G/A 2.1 and 2.6 times than in carriers of other genotypes (2=7.9; p=0.005; OR=2.1; CI: 95% 1.237-3.475 and 2=9.8; p=0.002; OR=2.6; CI: 95% 1.412-4.706, respectively). At the same time, among patients with moderate severity, the carriage of the wild G/G genotype was significantly less frequent compared to the control group (P <0.05), i.e., on the contrary, this genotype is likely to have a protective effect on the formation and development of acne ($\chi^2=9.8; p=0.002; OR=0.4; CI: 95% 0.21-0.70$).

**Table 4:** Differences in the frequency of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene in the subgroup of patients with moderately severe acne and the control group

<table>
<thead>
<tr>
<th>Alleles and genotypes</th>
<th>Moderate severity</th>
<th>Control group</th>
<th>$\chi^2$</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>97</td>
<td>279</td>
<td>7.9</td>
<td>0.005</td>
<td>2.1</td>
<td>1.237- 3.475</td>
</tr>
<tr>
<td>A</td>
<td>31</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>33</td>
<td>118</td>
<td>9.8</td>
<td>0.002</td>
<td>0.4</td>
<td>0.21-0.70</td>
</tr>
<tr>
<td>G/A</td>
<td>31</td>
<td>43</td>
<td>9.8</td>
<td>0.002</td>
<td>2.6</td>
<td>1.412- 4.706</td>
</tr>
<tr>
<td>A/A</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

In a comparative analysis, no statistically significant differences were found between the samples of patients with mild acne and controls (Table 5, p>0.05). The frequency distribution of alleles and genotypes of this genetic marker in the studied group was: G=88.1% and A=23.7%, G/G=76.3%, G/A=23.7% and A/A=0.0% in the patient subgroup, G=86.6% and A=13.3%, G/G=73.3%, G/A=26.7% and A/A=0.0%, in the control group. The odds ratios were OR=0.9, OR=1.2 and OR=0.8, respectively.

**Table 5:** Differences in the frequency of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene in the subgroup of patients with severe AV

<table>
<thead>
<tr>
<th>Alleles and genotypes</th>
<th>Mild severity</th>
<th>Control group</th>
<th>$\chi^2$</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>G</td>
<td>104</td>
<td>279</td>
<td>0.2</td>
<td>0.7</td>
<td>0.9</td>
<td>0.4588- 1.663</td>
</tr>
<tr>
<td>A</td>
<td>14</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>45</td>
<td>118</td>
<td>0.2</td>
<td>0.6</td>
<td>1.2</td>
<td>0.58-2.345</td>
</tr>
<tr>
<td>G/A</td>
<td>14</td>
<td>43</td>
<td>0.2</td>
<td>0.6</td>
<td>0.8</td>
<td>0.42- 1.709</td>
</tr>
<tr>
<td>A/A</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of the frequencies of allelic and genotypic variations of the rs1800629 polymorphism of the TNF- gene in the subgroups of individuals with severe and mild AV demonstrated no significant differences.

Table 5 shows the distribution of the allele and genotype frequencies of the rs1800629 polymorphism of the acne TNF-α gene. In subgroups of patients with severe and mild forms of acne, a statistically significant difference in the frequency of occurrence of alleles and genotypes of this locus was revealed. So, the frequency of G and A alleles in the studied subgroups of patients corresponded to 72.6% and 27.4% versus 88.1% and 11.9%. The odds ratio was 2.7 (OR=2.7; 95% CI: 1.34-5.84) with $\chi^2=7.9; p=0.005$, indicating an association between the minor A allele and clinically severe acne.

The G/G, G/A and A/A genotypes occurred at 50.0%, 45.2% and 4.8% in the subgroup with severe acne, and 76.3%, 23.7% and 0.0% in the subgroup with a mild form of acne respectively. The frequency of the G/G genotype in the subgroup of patients with severe acne was significantly lower than in the subgroup of patients with mild acne (50.0% versus 78.3%, respectively). The odds ratio was 0.3 (OR=0.3; $\chi^2=7.4; p=0.006$; 95% CI: 0.13-0.72), which indicates a protective effect of this genotype against the development of severe acne.
Differences in the frequency of the heterozygous genotype were found between patients with severe and mild acne (45.2% versus 23.7%, respectively). The risk of developing a severe form with this genotype of acne was OR=2.6 ($\chi^2=5.2$; $p=0.02$; 95% CI: 1.13-6.23). These data also support the adverse effect of this genotype on the development of severe acne. The homozygous genotype A/A was found only in two patients with severe acne (4.8% versus 0.0%, with $\chi^2=2.9$; $p=0.09$).

When comparing the frequencies of alleles and genotypes between subgroups of patients with moderate and mild forms of diseases, significant differences were also revealed. The unfavourable allele A predominated in the moderately severe group compared with the mild acne group (24.2% versus 11.9%, respectively; $\chi^2=6.3$; $p=0.01$; RR=2.0; 95% CI: 1.14-3.64, OR=2.4; 95% CI: 1.19-4.73). Heterozygosity for the minor allele was markedly more frequent in patients with moderate acne than in the comparison group (48.4% and 23.7%, respectively; $\chi^2=3.84$; P=0.05; OR=0.3; 95% CI: 0.15-0.71). Also, the G/G genotype (51.6% vs 76.3%, respectively; $\chi^2=8.1$; P=0.004; 95% CI: 1.2-3.4, OR=3.0; 95% CI: 1.3-6.5) was protective against the formation of moderate acne.

Thus, the data obtained at this stage of the study allow us to conclude that the carriage of the rs1800629 polymorphism of the TNF-α gene is correlated with a more severe course of acne (with severe and moderate variants). At the same time, the heterozygous G/A genotype is the molecular genetic marker of an increased risk of acne formation and development, while the homozygous G/G genotype is a marker of a reduced risk of diseases.

Interestingly, Turkish colleagues (Baz K., et all (2008)) reported no significant association (P<0.001) between this genotype and acne severity, although there was a significant increase in the frequency of carriers of the unfavorable G/A genotype compared to healthy controls (P>0.05).

The results of most studies on the contribution of the rs 1800629 polymorphism of the TNF-α gene to the development of acne in different populations are rather inconsistent. For example, two meta-analyses of the associations of the rs1800629 polymorphism of the TNF-α gene with acne risk, performed by Chinese authors, also did not give unambiguous results [Yang JK et all 2014, Wang and Y. 2018]. Nevertheless, in the world literature, the vast majority of case-control design studies confirm a significant association between unfavorable genotypes of the rs1800629 polymorphism of the TNF-α gene with acne formation [Al-Shobaili HA 2012, A. Agodi, 2012 Grech I 2014 Aisha NM 2016, Szabó, K. 2011, Heng AHS et all. 2021]. Our findings for the rs1800629 polymorphism of the TNF-α gene also do not contradict these global studies.

However, along with studies that have identified an association between acne and this polymorphic locus, there are studies that do not support this association. Thus, according to Sobjanek M, et al. (2009), Irawan A. Et all. (2013) and Yu J., et all. (2011) in the Polish, Indonesian and Chinese populations studied, no significant association of this polymorphism with acne formation was found. Perhaps, this is due to the fact that, according to most authors, this polymorphic locus has a high degree of heterogeneity in the frequencies of allelic and genotypic variants depending on the ethnicity or population affiliation of the studied groups [Parra-Rojas I., et al. 2006, Lee Y.H. 2007].

**IV. Conclusion**

Based on the findings and comparison results, it is possible to conclude that acne development and advancement are connected with the rs1800629 polymorphism of the TNF-α gene, depending on the patient’s ethnic background. Despite rather contradictory results obtained by researchers, there is no doubt that, at least in most European and Asian populations, unfavourable genotypic variants of the rs1800629 polymorphism of the TNF-α gene make a significant modifying and sometimes direct contribution to the predisposition to form various clinical forms of acne.

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**Author’s contributions**
Nilufar N. Malikova led the study design, data collection and planning, analysis plan and interpretation of findings and drafted the manuscript. Khamid Y. Karimov and Saidkarim S. Aritov were actively involved in the study conception, design and led the statistical analyses and interpretation. Kodirjon T. Boboev conceived the study, participated in its design, helped draft and revised the manuscript and led the team. The author(s) read and approved the final manuscripts.

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**Declarations**
**Ethics approval and consent to participate**
Ethics approval was obtained for the study from the Ethics Committee of Tashkent State Dental Institute. All participants provided written informed consent for the study. All methods were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Consent for publication**
Not applicable.
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Competing interests
The authors declare that they have no competing interests.

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Severe Hypernatremic Dehydration in a Neonate

By Aida Correia de Azevedo, Ana Sofia Rodrigues, Beatriz Parreira de Andrade, Raquel Cardoso & Susana Lopes

Abstract- Hypernatremia, a rare condition in newborns, is marked by elevated plasma sodium levels exceeding 150 mEq/L. It is more common in newborns who are exclusively breastfed or with excessive weight loss. Hypernatremic dehydration (HD) presents severe risks, including cerebral edema and other neurological complications.

A female newborn, 12 days old (birth weight: 2800g), was brought to the Emergency Department due to a 30% decrease in birth weight and reduced urine output. The newborn was solely breastfed every 2 hours, with a good notion of adequate reflexes and tolerance. Physical examination revealed a skeletal appearance, jaundice, sunken eyes and skin turgor. Blood pressure measurement was unsuccessful, while capillary blood glucose was 96g/dL. A saline bolus (10 mL/Kg) was administered. Venous blood gas analysis showed pH 7.39, lactate 4.3 mmol/L, HCO3- 21.8 mmol/L, and Na+ 180 mEq/L.

Keywords: breastmilk; exclusive breastfeeding; hypernatremia; hypernatremic dehydration; newborn.

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Severe Hypernatremic Dehydration in a Neonate

Aida Correia de Azevedo, Ana Sofia Rodrigues, Beatriz Parreira de Andrade, Raquel Cardoso, & Susana Lopes

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A female newborn, 12 days old (birth weight: 2800g), was brought to the Emergency Department due to a 30% decrease in birth weight and reduced urine output. The newborn was solely breastfed every 2 hours, with a good notion of adequate reflexes and tolerance. Physical examination revealed a skeletal appearance, jaundice, sunken eyes and skin turgor. Blood pressure measurement was unsuccessful, while capillary blood glucose was 96g/dL. A saline bolus (10 mL/Kg) was administered. Venous blood gas analysis showed pH 7.39, lactate 4.3 mmol/L, HCO3- 21.8 mmol/L, and Na+ 180 mEq/L. Treatment for dehydration began intravenously following the protocol. Laboratory tests showed normal blood count, Na+ 178 mEq/L, K+ 4.5 mEq/L, Cl- 143 mEq/L, urea 191 mg/dL, creatinine 1.39 mg/dL, and C-reactive protein 1.45 mg/dL. Formula milk was introduced, and the NB was admitted to the Neonatology unit for observation. The newborn remained stable, with gradual normalization of analytical parameters, along with sterile blood and urine cultures and a normal encephalic ultrasound. Post-discharge, the newborn underwent follow-up appointments, showing good weight evolution and normal neurodevelopment.

HD is potentially life-threatening, emphasizing the critical need for early diagnosis and appropriate treatment. It stands as a significant preventable cause of infant morbidity and mortality. Providing information and guidance on newborn feeding, as well as maintaining vigilant monitoring of risk factors for breastfeeding failure in an outpatient setting, is crucial for success, particularly in cases where the mother lacks necessary information upon discharge.

Keywords: breastmilk; exclusive breastfeeding; hypernatremia; hypernatremic dehydration; newborn.

1. INTRODUCTION

Hypernatremia consists of a serum sodium concentration exceeding 145 mEq/L, constituting a relatively infrequent condition in newborns [1-7]. The exact incidence of hypernatremia in newborns remains uncertain but is estimated to range between 1% to 5.6% [1,6,8]. In neonates, hypernatremic dehydration (HD) is more commonly observed in those exclusively breastfed or with excessive weight loss, with early discharge from the hospital being a significant risk factor. In these cases, HD is associated with ineffective lactation due to insufficient milk production or inadequate support [1-7,9-11].

Initial symptoms of HD are nonspecific, which can lead to delayed diagnosis and subsequent treatment. Consequently, many newborns with HD experience prolonged and severe hypernatremia, increasing the risk of serious complications such as seizures, thrombosis, intracranial hemorrhage, metabolic acidosis, acute kidney injury (AKI) and disseminated intravascular coagulation [1-11]. These complications are directly associated with hypernatremia and its treatment, and the severity of the symptoms does not correlate with blood osmolarity [1,2].

The management of HD involves identifying and addressing the underlying cause, as well as administering intravenous fluids to correct sodium levels.

This correction should occur at a controlled rate of 0.5-0.6 mEq/L per hour, aiming to prevent complications from a rapid decrease in sodium levels, particularly cerebral edema [1-3].

Despite the potential for severe consequences, most newborns recover fully without any lasting effects [1-3,9].

The authors have presented a case report of a newborn with severe HD. This report was previously showcased as an abstract at the 2021 European Academy of Pediatrics Meeting on April 25, 2021.

II. CASE PRESENTATION

A 12-day-old female newborn was referred by her family doctor to the Emergency Room due to significant weight loss and decreased urination. The infant was delivered at 39 weeks via cesarean section because of a pelvic presentation and the Apgar score was 9/10. Maternal serological tests showed negative results, and all prenatal ultrasounds reported normal findings.

At birth, the newborn weighed 2800 grams (15th percentile; -2 < z-score <0) and measured 46 centimeters in length (3rd-15th percentile; -2 < z-score <0). Since delivery, she had been exclusively breastfed every two hours, exhibiting satisfactory suction reflexes and tolerance (the newborn was discharged home at the age of two days).
During the ER assessment, the newborn's weight was measured at 1940 grams, indicating a 30% weight loss compared to birth weight. She was emaciated, jaundiced and had sunken eyes (refer to Figure 1) and fontanelle. Capillary refill time was 3 seconds, heart rate was 123 bpm, blood pressure was immeasurable, and capillary blood glucose levels measured 96 mg/dL. A saline bolus (10 mL/kg) was administered with a good response in blood pressure. The newborn also appeared less responsive, with weak crying and mild global hypotonia; the remainder of the neurological examination was ordinary.

Venous blood gas analysis displayed the following values: pH 7.39, lactate 4.3 mmol/L, HCO3- 21.8 mmol/L, and Na+ 180 mEq/L. Following HD's approach guidelines, intravenous treatment for dehydration was initiated. Laboratory results indicated a normal blood count, with Na+ levels 178 mEq/L, K+ 4.5 mEq/L, Cl- 143 mEq/L, urea 191 mg/dL, creatinine 1.39 mg/dL, and C-reactive protein 1.45 mg/dL. The coagulation study yielded average results. Both blood and urine cultures returned negative results.

The protocol implemented to address the newborn's hypernatremia ensured a gradual reduction in sodium levels, aiming for a decrease of 0.5-0.6 mEq/L per hour. This process necessitated the adjustment of fluid infusion rates according to sodium levels, which were monitored every one to two hours during the initial phase of treatment. The infant remained stable, exhibiting a gradual normalization of analytical parameters, achieving complete sodium level normalization 60 hours after admission. Throughout the treatment period, formula milk was introduced and adjusted per newborn's tolerance.

An encephalic ultrasound was conducted to rule out any potential brain damage resulting from hypernatremia and its treatment, which revealed no abnormalities.

After a seven-day hospital stay, the newborn was discharged and continued follow-up care, showing positive weight gain and normal neurodevelopment.

*Figure 1:* Newborn with hypernatremic dehydration. Physical examination shows an emaciated appearance, jaundice, sunken eyes and skin turgor.
III. Discussion

HD is a severe condition defined by elevated sodium levels above 145-150 mEq/L, often stemming from excessive fluid loss or inadequate fluid intake, with the latter being a prevalent cause in neonates. While it occurs more frequently in premature infants due to feeding immaturity, it can also affect full-term healthy newborns, mainly due to breastfeeding issues in the initial weeks of life [1-3,5-7,9-11]. In our case, the newborn exhibited unmistakable signs of dehydration linked to insufficient milk intake, notably a 30% decrease in birth weight and a skeletal appearance. The likely cause for breastfeeding failure was inadequate support provided to the mother, compounded by limited guidance during the prevailing pandemic, where the mother did not receive adequate support from both family and healthcare providers. This situation allowed inadequate breastfeeding to persist until the first appointment for the newborn, which, within the Portuguese national healthcare system, can take place up to the fifteenth day of life.

Several risk factors for HD exist, including cesarean delivery, primiparity, breast anomalies, excessive pre-pregnancy maternal weight, delayed initiation of the first breastfeeding, and lack of prior breastfeeding experience [3,4,6]. In the case described, the mother was a primipara and had no previous experience or knowledge about breastfeeding. Additionally, the newborn was delivered via cesarean section. Neonates with HD often present well, as classic signs of dehydration, such as sunken eyes and depressed fontanelles, may not be initially evident. Parents may misinterpret certain signs, like the lack of crying or prolonged sleep, as indicators of satiety, leading to a delayed diagnosis [2,4,8-9]. Initial signs of HD are subtle and nonspecific, like lethargy, agitation, and irritability, progressing to severe symptoms including seizures, coma, and potentially death [1-5,8-9]. In our case, the parents perceived prolonged sleep and rare crying as positive signs, assuming the child's well-being.

The diagnosis of HD is typically incidental during newborn weight checks, revealing a weight loss higher than 10% compared to birth weight, prompting comprehensive evaluation, including medical history, physical examination, and blood tests [1,2,5,7,11]. In our case, the family doctor immediately referred the infant to the ER upon finding a 30% weight loss for further investigation and treatment.

Treating HD involves identifying and addressing the underlying cause while administering intravenous fluids to restore sodium levels to normal values. The challenge lies in preventing rapid sodium level decreases, which can lead to severe problems such as intracranial hemorrhage, thrombosis, and cerebral edema, particularly within the first 24 hours of dehydration [1,2,5-7,10-11]. Sodium correction might take up to 48 hours or more, maintaining a decrease rate of 15 mEq/L in 24 hours or 0.5-0.6 mEq/L per hour [1-4,6-7]. In mild cases (sodium values below 160mEq/L), the infant may be given oral feeds at 1.5 times the maintenance with breast milk or formula milk [3,11]. For more severe cases, the fluid choice depends on the sodium level, aiming to prevent rapid changes. If the sodium levels are higher than 170 mEq/L, a customized solution aligning with the patient's osmolarity is recommended to avoid complications [1,2,5-7,9]. Regular monitoring and adjusted infusion rates prevented complications, resulting in normalized sodium levels 60 hours after admission with no evidence of cerebral edema.

While higher mortality rates are associated with sodium levels exceeding 160 mmol/L, newborns with HD typically make a full recovery without sequelae [2,3,8]. Indeed, in a cross-sectional study involving 46 infants diagnosed with HD, none exhibited central nervous system complications. Approximately half of the participants presented with AKI upon admission, which subsequently resolved entirely [3]. In a separate study encompassing 65 infants diagnosed with HD, over 12% of the neonates developed intracranial hemorrhage, while 12.8% experienced cerebral edema [8]. In our particular case, the newborn presented with AKI, which was resolved through appropriate correction of dehydration. Subsequently, the newborn showed complete recovery without cerebral complications, evidenced in follow-up appointments with normal encephalic ultrasounds and adequate psychomotor development.

It is crucial to maintain close follow-up in newborns who are exclusively breastfed to ensure adequate milk intake and to avoid serious conditions such as the one presented.

IV. Conclusions

HD carries the potential for significant complications, such as intracranial hemorrhage and disseminated intravascular coagulation. The primary recognized risk factor for HD is inadequate milk intake, primarily stemming from ineffective lactation and insufficient knowledge about breastfeeding practices.

Therefore, this case report draws attention to the severe consequences resulting from breastfeeding failure, underscoring the critical need to educate and support mothers in breastfeeding. Providing ample guidance and information on breastfeeding techniques, healthy practices, and identifying signs of breastfeeding failure is imperative for successful breastfeeding. Additionally, emphasizing the necessity for close monitoring of newborns during the initial weeks of life through regular weight assessments and
comprehensive physical examinations is essential to detect and manage conditions like HD promptly.

The benefits of breastfeeding are extensive and irreplaceable. Hence, breastfeeding promotion is crucial, requiring adequate support and guidance for mothers throughout this process.

Disclosures
Informed consent was obtained for the use of the newborn’s photo.

The authors don’t have any conflict of interest to declare.

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Appealing Decisions

Unless specified in the notification, the Editorial Board’s decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

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Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11"", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word “Abstract” in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper’s subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.
It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

**Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

**Author details**

The full postal address of any related author(s) must be specified.

**Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised not to include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

**Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

**Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

**Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

**Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

**Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.
Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Electronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.
6. **Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. **Revise what you wrote:** When you write anything, always read it, summarize it, and then finalize it.

8. **Make every effort:** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. **Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. **Use proper verb tense:** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. **Pick a good study spot:** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. **Know what you know:** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. **Use good grammar:** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. **Arrangement of information:** Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. **Never start at the last minute:** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. **Multitasking in research is not good:** Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. **Never copy others' work:** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. **Go to seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. **Refresh your mind after intervals:** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. **Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn’t be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

**Informal Guidelines of Research Paper Writing**

**Key points to remember:**
- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**
One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

**The introduction:** This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**
This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**
Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.
**Mistakes to avoid:**

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

**Title page:**

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

**Reason for writing the article**—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

**Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

**Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

### THE ADMINISTRATION RULES

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**Written material:** You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.
**CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)**

*BY GLOBAL JOURNALS*

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

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