Contact Lens and Anterior Eye xxx (xxxx) xxx

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Tear film stability in children: Age and sex associations explored through non-invasive tear break-up time

Filipe Da Silva [a,*], João M.M. Linhares [a], Jorge Jorge [a], Madalena Lira [a]

[a] Physics Center of Minho and Porto Universities (CF-UM-UP), School of Sciences, University of Minho, Braga, Portugal

ARTICLE INFO

Keywords:
NIBUT
Tear film
Tear film stability
Children
Middle childhood

ABSTRACT

Purpose: The stability of the tear film is crucial for maintaining good ocular health. Its assessment provides valuable insights into paediatric eye conditions. This study investigates the stability of the tear film in children and its association with predictive factors such as age and sex, for a better understanding of tear film dynamics in a paediatric population.

Methods: A cross-sectional study was conducted in a semi-urban area in northern Portugal. Tear film stability was evaluated using non-invasive tear break-up time (NIBUT) measured with Tearscope Plus. The evaluation was conducted subjectively, with measurements obtained exclusively in the right eye. The mean of three consecutive measures of the NIBUT was considered in the statistical analysis.

Results: A total of 2094 children (1072 females, 1022 males), with a mean age of 8.6 ± 1.3 years, were included. It was found a mean NIBUT of 14.8 ± 5.4 s (s), with no statistically significant difference between females (14.9 ± 5.4 s) and males (14.7 ± 5.4 s) ($p = 0.407$). Around 20 % of the children had a NIBUT < 10 s, and about 7 % had a NIBUT ≤ 5 s. A significant increase in NIBUT with age during the middle childhood stage (6 to 11 years old) was also found ($p = 0.019$), with NIBUT increasing from 14.1 ± 5.7 s in 6-year-olds to 16.3 ± 4.8 s in 11-year-olds ($p = 0.006$). However, when results were separated by the participants' sex, only the male group showed statistically significant differences ($p = 0.021$).

Conclusions: A significant increase in NIBUT was found during the middle childhood stage, primarily driven by male participants. These findings underscore the importance of considering age and sex in assessing tear film stability in paediatric populations.

1. Introduction

Research on the ocular surface and tear film has been predominantly centred on adults, with relatively less emphasis on children [1]. Nevertheless, it is paramount to expand such knowledge to children, as changes that affect a particular developmental stage may have future unforeseen impact. Dry eye is a multifactorial disease that typically occurs in the presence of an abnormality of the tear film combined with symptoms related to the ocular surface [2]. However, children are less likely to report symptoms than adults, which makes it even more important to assess the characteristics of the tear film at this age stage [3]. Additionally, children being increasingly exposed to the use of video display terminals (such as smartphones and computers), was associated with a decrease in the frequency of blinking and can lead to the development of dry eye symptoms [4–6].

The ocular surface is complex and composed of various epithelial and glandular structures and tissues. The lacrimal glands produce tear film that, after distribution, lubricates and protects the ocular surface, enabling better visual health and clear vision. The tear film stability promotes the integrity of the ocular surface and the visual optical quality [7,8], and is an important marker of tear film homeostasis in the diagnosis of dry eye [2].

The stability of the tear film can be assessed by measuring the fluorescein tear film break-up time (FBUT), a technique that can induce a reduction in the tear film stability and may not provide an accurate representation of its condition [9]. As such, the non-invasive measurement of the tear film break-up time (NIBUT) is a more recommended method [2]. Furthermore, invasive procedures may cause anxiety and fear in children, [10] making non-invasive techniques preferable in paediatric populations [11].

* Corresponding author.
E-mail addresses: filipe.silva.opt@gmail.com (F.D. Silva), jlinhares@fisica.uminho.pt (J.M.M. Linhares), jorge@fisica.uminho.pt (J. Jorge), mlira@fisica.uminho.pt (M. Lira).

https://doi.org/10.1016/j.clae.2024.102351
Received 23 July 2024; Received in revised form 6 December 2024; Accepted 6 December 2024
1367-0484/© 2024 The Author(s). Published by Elsevier Ltd on behalf of British Contact Lens Association. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

In the adult population, there is a decline in tear film function with advancing age, with reduced tear film stability being observed in older adults [12,13]. Additionally, tear film stability appears to be similar between males and females, even when comparing different age groups [12,14]. However, in the paediatric population, available studies are limited, limiting definitive conclusions.

The aim of this cross-sectional study was to analyse the tear film stability in children and examine its association with predictive factors such as age and sex.

2. Methods

A cross-sectional study was conducted in a semi-urban area in northern Portugal. Only one eye was considered during the measurement of the NIBUT, in the interest of the time of the screening performed. For convenience, only the right eye was used and considered in this analysis. Non-invasive tear break-up time (NIBUT) was assessed using the Tearscope Plus (Keeler, Windsor, UK). The Tearscope Plus used a cold catheter light, to minimizing the eye fatigue during the procedure and reduce the influence of the device over the measurement. The NIBUT was performed as usual by projecting a fine geometric and repetitive grid onto the corneal surface. NIBUT measurements were performed by a single observer using the handheld version of the Tearscope without the use of magnification. With the projection grid properly centred with the central cornea, and focused, the patient was asked to blink three times and to keep their eyes open for as long as possible afterwards. The Tearscope Plus stopwatch was used to measure the time from the last blink to the first distortion of the image of the projected grid perceived by the researcher. The NIBUT was measured three times, with the grid adjustment procedures repeated before each measurement to reduce observational and procedures mistakes and single occurring events that could maximize or minimize the NIBUT. The mean taken across the three measures was considered in the statistical analysis.

2.1. Inclusion/exclusion criteria

All children attending grade 1 (normally starting at the age of 6 years old) through 4 (normally leaving this school grade at the age of 10 years old) in schools within the Paredes Municipality, a semi-urban municipality in the north of Portugal, were included in the study presented, after parental consent was obtained. No inclusion or exclusion criteria was put in place to select the participating schools. All schools under the supervision of the political authority that govern the region were contacted. The ones that accepted the contact were the ones selected to draw the students used in this study. Most children in grade 4 are typically 10 years old. However, Portuguese legislation enforces a later year school start for some children, leading to the inclusion of children 11 years old. Factors such as ocular surgical interventions, developmental issues, and neurological problems were exclusion criteria. No history of ocular allergies, contact lens use, or other factors that may impact the tear film stability was collected. Nevertheless, individual isolated cases that might exist are expected to be diluted within the sample size analysed.

The study protocol was reviewed and approved by the Ethics Subcommittee for Life and Health Sciences of University of Minho with reference number CEICVS 020/2014. After being fully informed about the study purpose and methods, the parents of each child signed an informed consent form in accordance with the Declaration of Helsinki principles.

2.2. Statistical analysis

According to the Dry Eye Workshop II (DEWS II) from the Tear Film and Ocular Surface Society (TFOS), the minimum sample size required to detect a clinical difference of 5 s in NIBUT using the Tearscope Plus, based on a 2-sample *t*-test comparison with 80 % power and a significance level of $p < 0.05$, is 33 participants. Furthermore, if the dry eye metric deviates from a normal distribution, as is the case with NIBUT, the authors recommend increasing the sample size by 10 % to compensate, resulting in a total of 37 participants.[2] Based on these recommendations, the minimum sample size for this study is 222 subjects, with at least 37 participants per age group.

Statistical analysis was performed using IBM® SPSS® Statistics software (version 28.0, SPSS Inc, Chicago, IL). The normality of the frequency of the distribution of the variables was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney *U* test was used to analyse statistically significancy when comparing age and NIBUT (continuous variables) between sexes (binary variable). The Kruskal-Wallis H test was used to analyse the statistically significance differences in NIBUT across age groups (ordinal variable). The chi-square test was used to assess the independence of NIBUT groups frequencies (ordinal variable) between age groups. Correlations were determined using the Spearman correlation.

3. Results

Two thousand and ninety-four Caucasian children (2094 eyes) were included (1072 females, 1022 males), of which 128 were 6-year-olds, 380 were 7-year-olds, 417 were 8-year-olds, 578 were 9-year-olds, 526 were 10-year-olds and 65 were 11-year-olds. Mean age was 8.6 ± 1.3 years, with females having 8.5 ± 1.3 years and males 8.6 ± 1.3 years. Differences in age between male and female groups were not statistically significant ($p = 0.894$). Fig. 1 represents the relative frequency (%) of the NIBUT for the entire sample ($n = 2094$), measured in seconds (s) and binned into 5-s intervals.

It was found that the mean NIBUT was of 14.8 ± 5.4 s (ranging from 1 to 28 s), with females having a NIBUT of 14.9 ± 5.4 s (ranging from 1 to 28 s) and males 14.7 ± 5.4 s (ranging from 1 to 25 s), with the difference between means having no statistical significance ($p = 0.407$). Furthermore, differences with statistical significance were not found between the sexes when the age groups were examined independently (Table 1).

Table 1 shows the mean NIBUT values for different age groups separated by sex. Differences between the mean NIBUT values estimated across age groups and considering all participants were found to be statistically significant ($p = 0.019$). It was also found that children in the 11-year age group had a higher NIBUT value than those in the 6-year age group ($p = 0.006$), 7-year age group ($p = 0.005$) and 8-year age group ($p = 0.012$), differences with statistical significance. The 11-year-old age group did not, however, differ with statistical significance from the 9-year-old age group ($p = 0.051$) or the 10-year-old age group ($p = 0.073$).

When groups were analysed by sex, only males showed differences between the age groups with statistical significance ($p = 0.021$) (Table 1). It was also found that males in the 6-year age group had a NIBUT value lower than those in the 10-year age group ($p = 0.045$) and 11-year age group ($p = 0.003$), differences with statistical significance. Additionally, males in the 11-year age group had a NIBUT value higher than those in the 7-year age group ($p = 0.005$), 8-year age group ($p = 0.007$) and 9-year age group ($p = 0.022$), differences with statistical significance. Correlations with statistical significance were found when considering all participants as a group ($r = 0.072$, $p < 0.001$, Spearman) and in the group of male participants ($r = 0.102$, $p = 0.001$, Spearman). However, in the group of female participants, the correlation was not significant ($p = 0.149$). Fig. 2 represents the increasing NIBUT with increasing age, considering the group with all the participants (left panel) and with the male participants (right panel). Continuous line represents the median, the symbol x represents the mean and the box plot the interquartile ranges (Fig. 2).

Around 20 % of the participants had a NIBUT of < 10 s, and around 7 % had a NIBUT of ≤ 5 s. About 40 % of the participants had a NIBUT between 10 and 20 s, while 40 % had a NIBUT of 20 s, with only 0.1 % of the participants having a NIBUT of more than 20 s. The frequency of

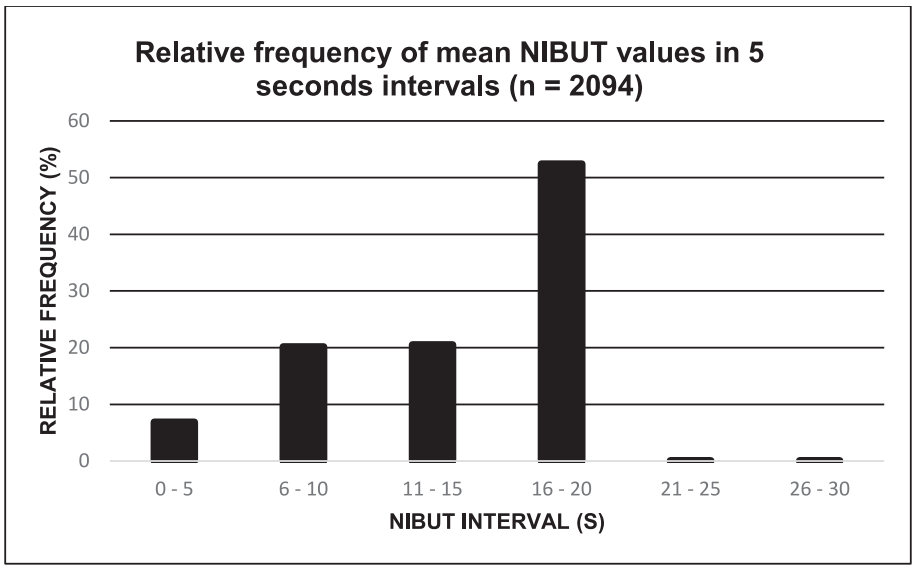

Fig. 1. Relative frequency of non-invasive tear break-up time (NIBUT), measured in seconds, binned into 5-s intervals (n = 2094).

Table 1
Mean NIBUT across age groups (n=2094) and age groups divided by sex (nFemale = 1072 and nMale = 1022). *p*- values indicate the statistical significance of the difference found across groups.

		6 years	7 years	8 years	9 years	10 years	11 years	*p*
NIBUT(s)	All sample	14.1 ± 5.7	14.4 ± 5.4	14.6 ± 5.5	15.0 ± 5.3	15.1 ± 5.4	16.3 ± 4.8	*0.019**
	Male	13.7 ± 5.6	14.3 ± 5.5	14.4 ± 5.4	14.8 ± 5.4	15.2 ± 5.2	16.6 ± 5.0	*0.021**
	Female	14.4 ± 5.8	14.5 ± 5.4	14.8 ± 5.7	15.2 ± 5.2	15.0 ± 5.8	15.7 ± 4.5	*0.688*
p		*0.376*	*0.755*	*0.393*	*0.323*	*0.779*	*0.293*	

*Statistically significant.

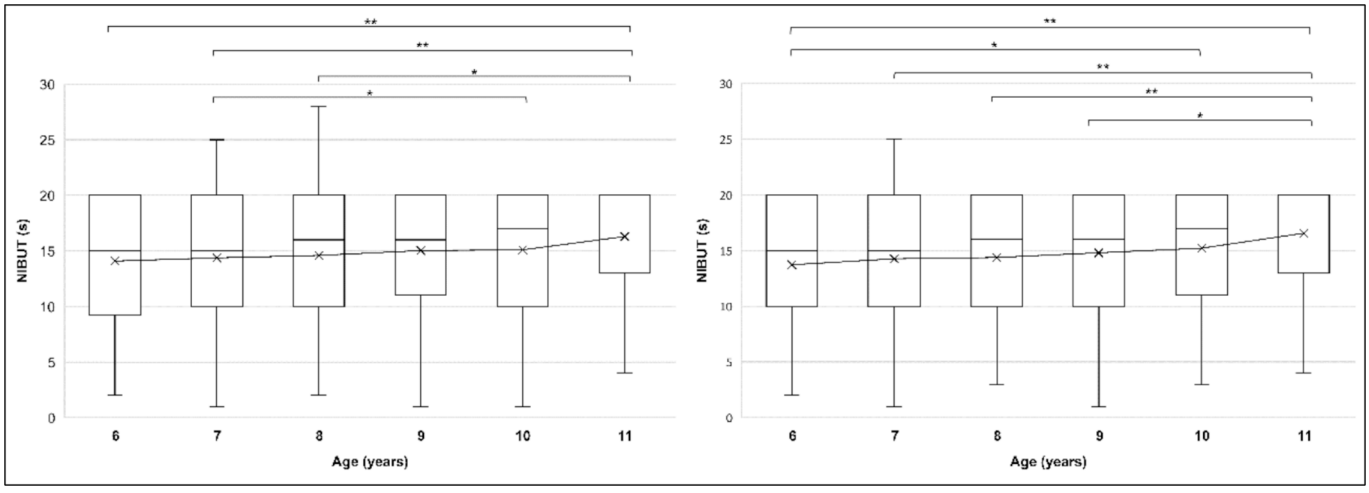

Fig. 2. Boxplot with medians (solid line), means (x) and interquartile ranges for NIBUT measurements between age groups, considering all participants in a group (left panel) and only male participants (right panel). *$p < 0.05$; **$p < 0.01$*

participants with a NIBUT of less than 10 s did not differ by sex (p = 0.921) and showed no statistically significant change with age (p = 0.175).

Thus, participants were grouped into three degrees of NIBUT: NIBUT of less than 10 s (n = 426), NIBUT between 10 and 20 s (n = 833) and NIBUT of 20 s or more (n = 835) (Table 2).

When comparing the frequency of these degrees of NIBUT between the sexes, no statistically significant difference was found across all the participants (p = 0.292) or when evaluating the age groups separately (Table 2). Furthermore, no statistically significant difference was found when comparing the frequency of the NIBUT between the age groups (p = 0.066) (Table 2). However, when analysing by sex, differences with statistical significance were found in the frequency of the NIBUT across groups, but only when considering male participants (p = 0.034). Correlations with statistical significance were found between the NIBUT and the age groups when considering the entire sample (r = 0.070, p = 0.001, Spearman) and in the group of the male participants (r = 0.102, p = 0.001, Spearman). However, in the group of the female participants, the correlation was not statistically significant (p = 0.195).

Table 2
Frequency of the NIBUT across age groups (n=2094), and age groups divided by sex (nFemale = 1072 and nMale = 1022).

NIBUT		Age of the participants in years						*p*
		6	7	8	9	10	11	
Allsample	< 10 s	25%	20%	23%	19%	19%	12%	*0.066*
	≥ 10 s and < 20 s	41%	44%	39%	40%	38%	32%	
	≥ 20 s	34%	36%	38%	41%	43%	56%	
Males	< 10 s	22%	21%	23%	20%	18%	12%	*0.034**
	≥ 10 s and < 20 s	52%	45%	41%	41%	40%	25%	
	≥ 20 s	26%	34%	36%	39%	42%	63%	
Females	< 10 s	28%	20%	23%	18%	21%	14%	*0.637*
	≥ 10 s and < 20 s	31%	42%	37%	40%	36%	45%	
	≥ 20 s	41%	38%	40%	42%	43%	41%	
p		*0.054*	*0.738*	*0.590*	*0.687*	*0.640*	*0.214*	

*Statistically significant.

4. Discussion

The study aimed to explore the NIBUT across a population of children, considering a segmentation of the population over of the sex and the age of the children. In a normal population, the NIBUT cut-off value for the loss of tear film homeostasis is of about 10 s [2]. In addition, there appear to be no differences in NIBUT measured subjectively with various instruments available on the market [15].

It was found that the mean NIBUT value found here of 14.8 ± 5.4 s increased by 2 s with increasing age. The mean NIBUT values found in this study was significantly lower than the findings reported by Jones and Nischa (21.8 ± 4.1 s) considering a population of children with no known ocular surface disease or anterior segment abnormality [11]. The study presented focused on children aged between 6 and 11, corresponding to the middle childhood stage [16]. Jones and Nischa assessed a population with a broader age range (2 to 15 years), spanning early childhood, middle childhood, and early adolescence. The authors did not provide a distribution of children by age stage hindering a comprehensive and direct comparison of the NIBUT values found [11]. Considering a population with a broader range of age could potentially contribute to the differences in the mean NIBUT values found. Jones and Nischa used a population comprising 72 % Caucasians, 25 % Asians, and 3 % Afro-Caribbeans, while the population in our study consisted solely of Caucasians [11]. These ethnic differences may also have contributed to differences when comparing the mean NIBUT results across both studies. Moreover, variations in the mean NIBUT found in the mentioned paper might also be attributed to a smaller population size (n = 36) considered [11]. On the other hand, Zhao et al. found a median NIBUT (10.1 s; 6.8 to 16.3 s) closer to that observed in this study, although slightly lower, while studying a Chinese paediatric population with complaints of blurred vision. Furthermore, the authors also observed a trend of increasing NIBUT with advancing age, with NIBUT showing a positive correlation with age [17].

The number of studies evaluating NIBUT in pediatric populations is limited, however, a reasonable amount of research has focused on FBUT [4,18–24]. Chidi-Egboka et al. conducted a *meta*-analysis and found that the combined mean from these eight studies was 14.6 s [1]. Although FBUT is a less ideal method compared to NIBUT, since it reduces tear film stability and may not accurately reflect its state, [2,9] the mean value reported by Chidi-Egboka et al. was almost identical to that found in this study (14.8 ± 5.4 s) [1].

Only 0.1 % of participants exhibited a NIBUT greater than 20 s, a percentage lower than expected. This result may be influenced by the significantly shorter attention span (the duration an individual can maintain an optimal attentional state) of children compared to adults. A study reported an average attention span of approximately 30 s in children aged 7–13 years (9.6 ± 1.6 years) [25]. Considering that the children assessed in the present study were younger (8.6 ± 1.3 years), it is reasonable to assume that their attention span was around or below 30 s. This low frequency of values of NIBUT > 20 s may be attributed to blinking induced by a loss of concentration, as all data were collected by a single observer, ensuring consistent application of observation criteria across the entire population.

The mean NIBUT found is still higher than the mentioned cut-off value to maintain the tear film homeostasis, but this study found a considerable number of children with a NIBUT < 10 s, which was not found by the Jones and Nischa [11]. On the other hand, Zhao et al. found that approximately 48 % of children had a NIBUT < 10, while in this study, the frequency was around 20 %. This discrepancy may be attributed to the higher percentage of myopic children in their sample (approximately 90 %), as myopia has been associated with lower NIBUT values compared to non-myopes [17]. Additionally, the frequency of NIBUT < 10 s found in this study (20 %) is quite similar to that reported in adults (27 %) [26].

Isenberg et al. reported a significantly higher NIBUT in new-borns compared to this study. The authors also found a thicker and more stable lipid layer in new-borns compared to children, suggesting that this may be the mechanism of resistance to tear film evaporation [27,28]. In addition, Lawrenson et al. evaluated neonates and infants and found a low rate of spontaneous blinking and prolonged periods of eye opening [29]. The low rate of spontaneous blinking in neonates and infants may be due to the immaturity of their dopaminergic system. While the relationship between central dopaminergic activity and the rate of spontaneous blinking is well established in adults, [30] it is not fully understood in early ages. Dopaminergic neurons appear early in human development, and there is significant postnatal change in the neuronal organization of the basal ganglia, altered levels of endogenous dopamine, and changes in the expression of dopamine receptor subtypes [31,32]. It is possible that the thicker lipid layer and high stability of the tear film may be a compensatory mechanism for the low frequency of blinking and the prolonged periods of eye opening between blinks in neonates and infants. This improves the quality of the visual information obtained, which is extremely important for the child development at this stage of life [33].

In the first year of life, there is approximately a 50 % increase in the mean ocular surface area from birth to 36–53 weeks of age, which would also increase the available area for tear evaporation [29]. Concurrent with this growth, there is a reduction in the thickness of the lipid layer of the tear film and a gradual increase in the spontaneous blink rate. The decrease in the thickness of the lipid layer may be due the increase in the area of the ocular surface, leading to a decrease in tear film stability. This instability may be offset by an increase in the frequency of blinking.

Lavezzo et al. assessed eye blinking in new-borns and children aged between 4 and 6 years. The researchers discovered that eyelid opening time approximately halved from birth to 4 years of age. Additionally, they observed a decline in eyelid opening time from 4 to 5 years of age, however, there was a slight increase from 5 to 6 years [34]. Given that eyelid opening time is positively correlated with tear film stability, it is plausible to theorize that tear film stability and lipid layer thickness decrease from birth to 5 years of age and then begins to increase around

6 years old, corresponding to middle childhood (6–11 years).

The mean NIBUT values found in this study were lower than those reported in a previous study involving young adult participants (17.8 ± 20.4 s) [26]. Similarly, Wang et al. also reported a higher mean NIBUT values in adults than those found in our study (16.7 ± 7.6 s) [35]. These results suggest greater tear film stability in adults compared to children aged between 6 and 11. On the other hand, Rico-del-Viejo et al. evaluated subjects ranging from 19 to 88 years old and found a negative correlation between tear film stability and age.[36] A decrease in NIBUT is expected when comparing older to younger adults, as older age is associated with several factors that impair tear film stability, such as the increased prevalence of Meibomian Gland Dysfunction (MGD) [13].

The differences found in mean NIBUT between males and females had no statistical significance, even when comparisons were made between groups of children of the same age. These results were in line with those obtained in a previous studies with children (11 years (6–18)) and adults (24.4 ± 6.3 years), where differences with statistical significance were not found either [17,26]. However, even though there were no differences in the mean NIBUT values, there were differences in the correlation of the NIBUT values with age considering the sex of the children independently. In males, there was a statistically significant increase in the mean NIBUT with age ($p = 0.021$), but in females, this increase was not statistically significant ($p = 0.688$). Furthermore, when the children were grouped into NIBUT time group values (Table 2), differences with statistical significance were not found when comparing the frequency distributions in the total sample ($p = 0.066$) and in the group of females ($p = 0.637$). However, there were differences when considering the male population ($p = 0.034$). Table 2 shows that the number of male children with NIBUT < 10 s and children with NIBUT between ≥ 10 s and < 20 s decreases with age. On the other hand, the number of children with NIBUT ≥ 20 s increases with age, showing that most children tend to reach a NIBUT ≥ 20 s as age increases.

These differences between males and females may be due to genetic, hormonal, anatomical and environmental factors [37,38]. It is important to note that even though middle childhood (6–11 years) is an early stage of life, it is one of the main phases of human development, marked by the start of androgen secretion by the adrenal glands [39]. Also at this stage there is a small intermediate growth spurt, the differences between the sexes in body composition become more pronounced, and the sexual segregation of males and females reaches its peak during these years [40,41].

Currently, sex-related differences in the eye have already been identified, many of which are influenced by the effects of sex steroids [42]. These hormonal actions impact multiple structural and functional aspects of the eye, including aqueous tear production, lipid production, mucus secretion, tear film stability, and blink rate [43]. Additionally, several clinical studies report a relationship between higher oestrogen levels and a reduction in tear stability and meibomian gland secretion [43–46]. Since a relationship has already been found between hormones and the tear film, although at a later age, we may consider that hormonal oscillation is part of the mechanisms behind the change in NIBUT with increasing age in middle childhood. As the hormonal system in females is more complex and multifactorial, it is possible that the increase in NIBUT with age is not as linear as in males.

5. Conclusion

The work described here enabled insights into the dynamics of NIBUT concerning sex and age among a population of children. It would have been relevant to assess the symptoms and ocular surface damage, and to cross correlate the findings with this segmentation. It was found a statistically significant increase in NIBUT during middle childhood, primarily driven by male participants. Moreover, as children aged, it was found a consistent increasing in the mean NIBUT values, with older children exhibiting higher NIBUT levels, in particular children 11 years of age. Interestingly, no statistically significant difference was found in the comparison of NIBUT between sexes, even when considering age-matched comparisons. This study attempts to contributes to a better understanding of tear film dynamics in children, offering insights for clinical practice by providing expected results across a population of children from the north of Portugal. Further studies should be performed to compare the results found with a population from different regions of Portugal.

Funding

This work was supported by the Portuguese Foundation for Science and Technology (FCT) in the framework of the Strategic Funding UIDB/04650/2020 and UI/BD/154515/2023.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Chidi-Egboka NC, Briggs NE, Jalbert I, Golebiowski B. The ocular surface in children: A review of current knowledge and meta-analysis of tear film stability and tear secretion in children. Ocul Surf 2019;17:28–39. https://doi.org/10.1016/j.jtos.2018.09.006.

[2] Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II Diagnostic Methodology report. Ocul Surf 2017;15:539–74. https://doi.org/10.1016/j.jtos.2017.05.001.

[3] Han SB, Yang HK, Hyon JY, Hwang J-M. Children with dry eye type conditions may report less severe symptoms than adult patients. Graefes Arch Clin Exp Ophthalmol 2013;251:791–6. https://doi.org/10.1007/s00417-012-2097-2.

[4] Moon JH, Lee MY, Moon NJ. Association between video display terminal use and dry eye disease in school children. J Pediatr Ophthalmol Strabismus 2014;51:87–92. https://doi.org/10.3928/01913913-20140128-01.

[5] Yaginuma Y, Yamada H, Nagai H. Study of the relationship between lacrimation and blink in VDT work. Ergonomics 1990;33:799–808. https://doi.org/10.1080/00140139008927186.

[6] Moon JH, Kim KW, Moon NJ. Smartphone use is a risk factor for pediatric dry eye disease according to region and age: a case control study. BMC Ophthalmol 2016;16:188. https://doi.org/10.1186/s12886-016-0364-4.

[7] Montés-Micó R. Role of the tear film in the optical quality of the human eye. J Cataract Refract Surg 2007;33:1631–5. https://doi.org/10.1016/j.jcrs.2007.06.019.

[8] Montés-Micó R, Cerviño A, Ferrer-Blasco T, García-Lázaro S, Ortí-Navarro S. Optical quality after instillation of eyedrops in dry-eye syndrome. J Cataract Refract Surg 2010;36:935–40. https://doi.org/10.1016/j.jcrs.2009.12.044.

[9] Mooi JK, Wang MTM, Lim J, Müller A, Craig JP. Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability. Contact Lens Anterior Eye 2017;40:170–4. https://doi.org/10.1016/j.clae.2017.01.004.

[10] Bray L, Appleton V, Sharpe A. The information needs of children having clinical procedures in hospital: will it hurt? will I feel scared? what can I do to stay calm? Child Care Health Dev 2019;45:737–43. https://doi.org/10.1111/cch.12692.

[11] Jones SM, Nischal KK. The non-invasive tear film break-up time in normal children. Br J Ophthalmol 2013;97:1129–33. https://doi.org/10.1136/bjophthalmol-2013-303236.

[12] Ozdemir M, Temizdemir H. Age- and gender-related tear function changes in normal population. Eye 2010;24:79–83. https://doi.org/10.1038/eye.2009.21.

[13] Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, et al. TFOS DEWS II Epidemiology Report. Ocul Surf 2017;15:334–65. https://doi.org/10.1016/j.jtos.2017.05.003.

[14] Singh S, Srivastav S, Mohamed A, Basu S. Non-invasive Tear film assessment in normal population: effect of age, sex, and interparametric relationship. Front Med 2022;9:894184. https://doi.org/10.3389/fmed.2022.894184.

[15] Bandlitz S, Peter B, Pflugi T, Jaeger K, Anwar A, Bikhu P, et al. Agreement and repeatability of four different devices to measure non-invasive tear breakup time (NIBUT). Contact Lens Anterior Eye 2020;43:507–11. https://doi.org/10.1016/j.clae.2020.02.018.

[16] Williams K, Thomson D, Seto I, Contopoulos-Ioannidis DG, Ioannidis JPA, Curtis S, et al. Standard 6: age groups for pediatric trials. Pediatrics 2012;129:S153–60. https://doi.org/10.1542/peds.2012-0055I.

[17] Zhao G-H, Wang J-D, Liu M-R, Zhang L-J, Sun Y, Cao K. The Distribution and influence factors of non-invasive tear film break-up time in children. Clin Ophthalmol 2024;18:2697–704. https://doi.org/10.2147/OPTH.S475970.

[18] Akil H, Celik F, Ulas F, Kara I. Dry eye syndrome and allergic conjunctivitis in the pediatric population. Middle East Afr J Ophthalmol 2015;22:467. https://doi.org/10.4103/0974-9233.167814.

[19] Akinci A, Cetinkaya E, Aycan Z. Dry eye syndrome in diabetic children. Eur J Ophthalmol 2007;17:873–8. https://doi.org/10.1177/112067210701700601.
[20] Akinci A, Cakar N, Uncu N, Kara N, Acaroglu G. Keratoconjunctivitis Sicca in juvenile rheumatoid arthritis. Cornea 2007;26:941–4. https://doi.org/10.1097/ICO.0b013e3180de1f34.
[21] Akinci A, Cakar N, Kara N, Uncu N. Ocular findings in children with chronic renal failure. Cornea 2009;28:5–6. https://doi.org/10.1097/ICO.0b013e318181f9f0.
[22] Chen L, Pi L, Fang J, Chen X, Ke N, Liu Q. High incidence of dry eye in young children with allergic conjunctivitis in Southwest China. Acta Ophthalmol 2016;94:e727–30. https://doi.org/10.1111/aos.13093.
[23] Dogru M, Gunay M, Celik G, Aktas A. Evaluation of the tear film instability in children with allergic diseases. Cutan Ocul Toxicol 2016;35:49–52. https://doi.org/10.3109/15569527.2015.1010727.
[24] Gunay M, Celik G, Yildiz E, Bardak H, Koc N, Kirmizibekmez H, et al. Ocular surface characteristics in diabetic children. Curr Eye Res 2016;41:1526–31. https://doi.org/10.3109/02713683.2015.1136421.
[25] Simon AJ, Gallen CL, Ziegler DA, Mishra J, Marco EJ, Anguera JA, et al. Quantifying attention span across the lifespan. Front Cogn 2023;2:1207428. https://doi.org/10.3389/fcogn.2023.1207428.
[26] Da Silva F, Linhares JMM, Lira M. The influence of the tear film on the intraocular pressure and the corneal biomechanical properties analyzed with the Ocular Response Analyzer. J Optom 2024;17:100488. https://doi.org/10.1016/j.optom.2023.100488.
[27] Isenberg SJ, Del Signore M, Chen A, Wei J, Guillon J-P. The lipid layer and stability of the preocular tear film in newborns and infants. Ophthalmology 2003;110:1408–11. https://doi.org/10.1016/S0161-6420(03)00451-2.
[28] Zhao H, Wu S-N, Cheng Z, Xiao D, Shu H-Y, Ge Q-M, et al. Mean tear-film lipid layer thickness and video display terminal time as risk factors for abnormal blinking in children. Front Med 2021;8:785901. https://doi.org/10.3389/fmed.2021.785901.
[29] Lawrenson JG, Birhah R, Murphy PJ. Tear-film lipid layer morphology and corneal sensation in the development of blinking in neonates and infants. J Anat 2005;206:265–70. https://doi.org/10.1111/j.1469-7580.2005.00386.x.
[30] Karson CN. Spontaneous eye-blink rates and dopaminergic systems. Brain J Neurol 1983;106:643–53. https://doi.org/10.1093/brain/106.3.643.
[31] Meng SZ, Ozawa Y, Itoh M, Takashima S. Developmental and age-related changes of dopamine transporter, and dopamine D1 and D2 receptors in human basal ganglia. Brain Res 1999;843:136–44. https://doi.org/10.1016/S0006-8993(99)01933-2.
[32] Herlenius E, Lagercrantz H. Neurotransmitters and neuromodulators during early human development. Early Hum Dev 2001;65:21–37. https://doi.org/10.1016/S0378-3782(01)00189-X.
[33] Tutt R, Bradley A, Begley C, Thibos LN. Optical and visual impact of tear break-up in human eyes. Invest Ophthalmol vis Sci 2000;41:4117–23.
[34] Lavezzo MM, Schellini SA, Padovani CR, Hirai FE. Eye blink in newborn and preschool-age children. Acta Ophthalmol 2008;86:275–8. https://doi.org/10.1111/j.1600-0420.2007.00969.x.
[35] Wang J, Palakuru JR, Aquavella JV. Correlations among upper and lower tear menisci, noninvasive tear break-up time, and the schirmer test. Am J Ophthalmol 2008;145:795–800.e1. https://doi.org/10.1016/j.ajo.2007.12.035.
[36] Rico-del-Viejo L, Lorente-Velázquez A, Hernández-Verdejo JL, García-Mata R, Benítez-del-Castillo JM, Madrid-Costa D. The effect of ageing on the ocular surface parameters. Contact Lens Anterior Eye 2018;41:5–12. https://doi.org/10.1016/j.clae.2017.09.015.
[37] Danel DP, Wacewicz S, Kleisner K, Lewandowski Z, Kret ME, Żywiczyński P, et al. Sex differences in ocular morphology in Caucasian people: a dubious role of sexual selection in the evolution of sexual dimorphism of the human eye. Behav Ecol Sociobiol 2020;74:115. https://doi.org/10.1007/s00265-020-02894-1.
[38] Vehof J, Sillevis Smitt-Kamminga N, Nibourg SA, Hammond CJ. Sex differences in clinical characteristics of dry eye disease. Ocul Surf 2018;16:242–8. https://doi.org/10.1016/j.jtos.2018.01.001.
[39] Bogin B. Evolutionary hypotheses for human childhood. Am J Phys Anthropol 1997;104:63–89. https://doi.org/10.1002/(SICI)1096-8644(1997)25+%3C63::AID-AJPA3%3E3.0.CO;2-8.
[40] Del Giudice M, Angeleri R, Manera V. The juvenile transition: a developmental switch point in human life history. Dev Rev 2009;29:1–31. https://doi.org/10.1016/j.dr.2008.09.001.
[41] Wells JCK. Sexual dimorphism of body composition. Best Pract Res Clin Endocrinol Metab 2007;21:415–30. https://doi.org/10.1016/j.beem.2007.04.007.
[42] Da Silva F, Linhares JMM, Lira M. What intrinsic factors affect the central corneal thickness? Ophthalmic Physiol Opt, Published online November 4, 2024. Doi: 10.1111/opo.13414.
[43] Sullivan DA, Rocha EM, Aragona P, Clayton JA, Ding J, Golebiowski B, et al. TFOS DEWS II sex, gender, and hormones report. Ocul Surf 2017;15:284–333. https://doi.org/10.1016/j.jtos.2017.04.001.
[44] Versura P, Fresina M, Campos EC. Ocular surface changes over the menstrual cycle in women with and without dry eye. Gynecol Endocrinol 2007;23:385–90. https://doi.org/10.1080/09513590701350390.
[45] Gagliano C, Caruso S, Napolitano G, Malaguarnera G, Cicinelli MV, Amato R, et al. Low levels of 17-β-oestradiol, oestrone and testosterone correlate with severe evaporative dysfunctional tear syndrome in postmenopausal women: a case–control study. Br J Ophthalmol 2014;98:371–6. https://doi.org/10.1136/bjophthalmol-2012-302705.
[46] Golebiowski B, Badarudin N, Eden J, You J, Hampel U, Stapleton F. Does endogenous serum oestrogen play a role in meibomian gland dysfunction in postmenopausal women with dry eye? Br J Ophthalmol 2017;101:218–22. https://doi.org/10.1136/bjophthalmol-2016-308473.