

Review Article

Development in Tear Film Osmolarity Assessments: A Review

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Abstract

The aim of this article is to review the development in the assessment of tear film with tear osmolarity techniques. Also, to find out the average score for tear osmolarity in normal and keratoconjunctivitis sicca (KCS) patients based on the published work.

The use of tear osmolarity techniques started about 50 years ago. Over the last years a number of researchers have investigated the human tear osmolarity. The tear osmolarity assessment techniques have two procedures. The first procedure which is old, that involves the detection of freezing point depression and vapour pressure. The second one which is more advanced and known as the electronic method, that involves the use of electrical impedance technique.

All techniques used to assess tear osmolarity are good, accurate, reliable and provide comparable data. Based on the published work reviewed in this article, the average tear osmolarity for normal subjects is 303.2 ± 7.0 compared to 322.7 ± 15.9 in subjects with KCS.

Key word: Dry eye, Tear film, Osmolarity, TearLab.

Introduction

Dry eye

Dry eye disease affects up to 20% of the population in North America, 17% in Japan, 16% in Australia and 11% in Denmark (Moss, Klein, and Klein, 2000). The prevalence of dry eye might be even higher in hot and dry climate countries. Over the last 20 years, a significant progress in understanding the cause

and symptoms of dry eye has been made. However, the relation between disease status and symptoms, such as ocular inflammation or surface damage, remains subtle. Even though many dry eye questionnaires have been developed for the diagnosis or screening of dry eye, however, none of these questionnaires is capable of accurately monitoring the change in ocular dryness.

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In 1995, dry eye was defined by the Dry Eye workshop (DEWS) as “Dry eye is a disorder of the tear film due to either tear deficiency or excessive evaporation, which causes damage to the inter-palpebral ocular surface and is associated with symptoms of ocular discomfort”(Lemp,1995). In 2007, the DEWS committee updated the definition of dry eye to take into account the role of tear film hyperosmolarity, ocular surface inflammation and visual function affected due to dryness (Gipson,2007).

In 2007, the first TFOS DEWS definition of dry eye was as the following: “Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface” (Gipson, 2007).

The current TFOS DEWS II definition of dry eye is: “Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles” (Craig et al, 2017).

Dry eye definitions are often based on dry eye symptoms used to define dryness prevalence in population-based studies. Clinically, dry eye is habitually described as a ‘symptom-based’ disease. However, patient-reported symptoms are not often correlated with clinical tests unless the dryness is severe or the diagnostic tests are firmly controlled (Nichols, 2006).

Tear film structure

Tear film conventionally was described to consist of three layers as the innermost mucus layer, the intermediate aqueous layer and the

superficial lipid layer. Studies reported that in human’s tear film, the mucus, aqueous and protein components combine to form a pre-corneal hydrated gel. This mucus gel comprises a mixture of soluble and gel-forming mucins. The lacrimal glands, conjunctival goblet cells, and stratified ocular surface epithelium are responsible for mucus gel production. It is essential to preserve the integrity of the mucus gel to maintain ocular comfort and high vision quality (Pflugfelder, Solomon and Stern, 2000).

The tear fluid is a biochemical mixture of mucins, antimicrobial proteins (lysozyme and lactoferrin), immunoglobulins, and growth factors (transforming growth factor-alpha, hepatocyte growth factor, and epidermal growth factor). The role of such factors has not been well established, but it might contribute to maintaining homeostasis of the ocular surface, and might help in wound healing after ocular surface trauma (Pflugfelder, Solomon and Stern, 2000).

Dry eye diagnostic tests

Methods for dry eye diagnosis vary widely. It has been suggested that dry eyes can be used to diagnose by symptoms only. However, symptoms alone are insufficient to accurately determine ocular dryness, as there are some conditions, other than tear film disorders, can lead to similar symptoms (Khanal et al, 2008).

The Schirmer test is one of the most common diagnostic tests for dry eye objectively. It has been in use for about one century, and nevertheless, it is considered inaccurate and unreliable due to the test’s invasive nature which stimulates the tear reflex (Khanal et al, 2008). Additionally, it overlooks the tear evaporation and measures only the tear production. However, the ease of strip application along with its low cost has led to the test to be commonly used in clinics particularly for lacrimal secretory function in dry eye patients (Khanal et al, 2008).

Tear break-up time (TBUT) assessment with fluorescein is one of the techniques that is widely used by practitioners in clinics. This test is more reliable than the Schirmer test, as it is minimally invasive. However, tear film assessment with such technique might be affected by the installation of fluorescein which can destabilize the tear film. Non-invasive tear break-up time (NIBUT) can overcome such problems, but both TBUT and NIBUT cannot provide direct information on ocular dryness due to tear evaporation (Khanal et al, 2008).

Also, for dry eye identification ocular surface staining with rose Bengal, lissamine green and fluorescein can be used. The weakness of such techniques is that dry eye is identified by measuring the degree of ocular surface damage, and does not differentiate dry eye from ocular surface staining which may occur as a result of other conditions. Therefore, it does not necessarily detect the dry eye condition early on (Khanal et al, 2008; Messmer, Bulgen and Kampik, 2010).

Tear film dynamic stabilization requires adequate tear production, tear distribution, turnover rate, drainage, and evaporation. The tear physiology tests can also be applied to diagnose dry eyes. Tear osmolarity is believed to be an attractive guide for ocular dryness detection as it detects the end products of tear dynamics change (Khanal et al, 2008).

Osmolarity is a measure of the number of solute particles per litre of solution versus osmolality, which is the number of solute particles per kilogram. Tear osmolarity is a function of the balance between rate of tear production and tear loss from the eye.

In 1882, a French chemist Raolt introduced the term Osmolarity. He observed the freezing point of a water solution based on its osmolarity. There is a negative correlation between the quantity of solute and the freezing point. For example, pure water freezing point by definition is 0 °C; the values for normal

human serum are in the range of – 0.532 to – 0.539 °C, which equates to osmolarity values of 285 to 290 mOsm/l (Murube 2006). Freezing point depression (Gilbard and Farris, 1979a; Benjamin and Hill, 1983; Farris et al, 1983; Farris, Stuchell and Mandel, 1986; Gilbard, 1994; Craig and Tomlinson, 1995; Iskeleli et al, 2002) and vapour pressure techniques had been used to measure tear film osmolarity, until the TearLab Osmometer (OcuSense, Inc, San Diego, CA) (Benelli et al. 2010; Tomlinson, McCann and Pearce, 2010) was developed. Electrical impedance has been used in recent work, which offers an accurate and quick assessment of osmolarity using a small sample of liquid (less than 0.5 µl). (McCann 2009) The Tear Lab Osmometer (OcuSense Inc. San Diego, CA, USA) measures the tear film osmolarity by average of impedance spectroscopy technique (Sullivan and Donsky, 2007).

The purpose of this article is to review the development in dry eye assessment using different tear film osmolarity techniques.

Development in tear film osmolarity assessment techniques

1. Freezing point depression

Freezing point depression technique can be used to measure tear film osmolarity using a Clifton osmometer (Figure 1) (Clifton Technical Physics, Hartford, NY, USA). A glass microcapillary tube (Bilbate Ltd, Northants, UK) is used to collect a sample of tears from the inferior tear meniscus. The contact with the ocular surface should be avoided to ensure avoidance of reflex tearing. A syringe and a micropipette are used to inject the sample into a vessel containing heavy oil, to avoid evaporation before analysis took place (McCann, 2009).

In two separate vessels, samples of nanopure water and 290 standards (Clinitrol 290, Advanced Instruments Inc.) are placed. An

injection for calibration is made to control the variation in environment conditions.

The Clifton Osmometer consists of a thermoelectric cooling module with a temperature control, which works on the principle of the Peltier effect. The formation of a heat change from an electric voltage occurs when a current is conceded through two semi-conductors connected at Peltier junctions (Craig,1995; Fletcher, Hew and Davies,2001). The current transfers heat from one link to another thus as one cools, the other heats. Cooling of the stage in the Clifton Osmometer is achieved with the use of the Peltier device inside the cooling stage. This is used in conjunction with a stand and clamp, an aquarium pump, a stereomicroscope and a fibre optic lighting system (Craig 1995; Fletcher, Hew and Davies, 2001). A small sample of water and a standard from each vessel is placed into a capillary tube that is attached to a syringe. Tear samples are loaded into a sample plate, containing eight cells, which is situated on the cooling module resting on a stand. The cooling module is cooled by a steady flow of water. A thin layer of immersion oil is placed on top of the sample plate on the cooling module along with zinc oxide. It is placed around the edge of the sample plate to ensure an even temperature of the sample (Craig, 1995). The plate is illuminated from below and the sample plate is manoeuvred around so all eight holes are evenly illuminated. Once all samples are loaded, the temperature is lowered until all samples are frozen (Craig, 1995).

The temperature increases slowly, and the process is viewed through a stereo microscope. The point at which the last crystals of each sample melts is noted as the freezing point of the sample. The test is repeated three times and the average is calculated. The osmolarity of the tear samples are determined using Equation (1).

$$\text{Osmolarity} = [P(\frac{290}{S} - Z)] - Z \quad (1)$$

where Z is nanopure water; S is sodium chloride (NaCl) and P is tears.

2. Electrical impedance technique

In 2009, a new device for tear film osmolarity assessment was permitted by the US Food and Drug Administration (FDA). Such a device is known as TearLab Osmometer (Figure 2). TearLab osmolarity system is anticipated to measure the osmolarity of human tears to help in the identification of dry eye disease, in conjunction with other methods of clinical assessment. The electrical impedance technique used in such devices offers quick and precise measurement of the osmolarity by a single trial using a small sample of liquid. The TearLab Osmometer (OcuSense Inc. San Diego, CA, USA) measures the osmolarity of tear film by means of a patented impedance spectroscopy technique (Sullivan and Donsky, 2007).

A test chip (disposable single-use) made of high-density injection-molded polycarbonate covered with a gold conductive layer is mounted on the collection pen. A small sample of tear fluid is obtained by passive capillary action from the tear meniscus, inferior temporal. The pen monitors the collection procedure and affords an audible and chromatic signal when the tear sample has been collected. Once the fluid sample is obtained, the pen is put back on its base and electrical signals are passed through the sample fluid. The energy impedance is detected and converted to an output signal that indicates the osmolarity of the tear sample.

In 2009, Tomlinson et al (2010), compared the new TearLab Osmometer that depends on the electrical impedance “lab-on-a-chip” nanoliter technology, with the freezing point depression (Clifton Technical Physics, Hartford, NY).

They reported a significant correlation between the results obtained from TearLab and Clifton Osmometers.

In December 2017, the FDA approved a new tear film osmolarity assessment device which is known as the i-Pen (Figure 2). The i-Pen osmolarity system is a diagnostic testing device for the quantitative measurement of osmolarity of ocular tissues in normal and dry eye subjects (Nolfi and Caffery, 2017). The i-Pen consists of a single-use sensor inserted into a handheld pen which analyses tear osmolarity by gently touching the lower retracted conjunctiva. According to the manufacturer, it measures the impedance of the extracellular fluid contained in the eyelid tissue. The i-Pen system is designed to assess the human tear film osmolarity, and it has the ability to measure osmolarity ranges between 275 and 380 mOsmol/L (< 290 Normal, 290–310 for marginal dry eye, 310–330 for mild dryness, 330–350 for moderate dryness, and > 350 for severe dryness) (Chan et al, 2018).

Development in tear osmolarity research

There have been tremendous advances in tear osmolarity research over the last 10 years. Such increase in research and publications in this field started in the period between 2008 and 2010, with the invention of new techniques for the tear osmolarity assessment (impedance spectroscopy technique). The key words; tear osmolarity (Freezing point depression, Electrical impedance technique, TearLab and i-pen) were applied using three different research web search engines; Google Scholar, PubMed and Web of Science to show the increase in tear osmolarity publications (Figures 3–5). It was clear that the increase in the “tear osmolarity” search using the three

search engines was slow between 2000 and 2011. While, the increase was sharp between 2011 and 2018. For example, the increase was over 300, 375 and 400% in the 2018 compared with the search in the 2011 using Google Scholar (Figure 3), PubMed (Figure 4) and Web of Science (Figure 5), respectively. Table 1 shows a literature review of tear osmolarity assessment during the period 1970 to 2018 and revealed with the different techniques available. The results reported in Table 1 clearly indicated the use of freezing point depression technique was common between 1970 and 2005. While, the assessment of tear osmolarity through the determination of the vapour pressure of tears was rare and has been used in 1978.

There are many disadvantages associated with the use of freezing point depression and vapour pressure to assess tear osmolarity. Both techniques are dependent on the use of laboratory large equipment that require the use of relatively large volumes of tears. Also, there various problems that arise during tears collection, their evaporation and handling (Lemp et al, 2011). Moreover, for dry eye subjects, a relatively large volume of tears is not available in most cases. Therefore, such techniques have been replaced by more advanced techniques since 2010. The recent techniques for the assessment of tear osmolarity involve the use of TearLab™ osmolarity system and i-Pen. TearLab™ osmolarity system has been widely used since 2010 and is considered as a valuable test to be used in the diagnosis of dry eye. i-Pen has been introduced recently and has been used for the first time in the 2017. Such recent systems overcome most of the limitations that are associated with old techniques that involve measurement of freezing point depression and vapour pressure of tears (Korb, 2000).

Table 1: A review of the assessment of tear osmolarity between 1970 and 2018.

Author (reference)	Year	Tear osmolarity		Test
		N (KCS)±SD	N (normal)±SD	
Mishima <i>et al.</i> (Mishima, Kubota, and Farris 1970)	1970	6 (329 ± 4.7)	33 (304 ± 1.5)	Freezing-point depression
Gilbard <i>et al.</i> (Gilbard, Farris, and Santamaria 1978)	1978	30 (343 ± 32.3)	33 (304 ± 10.4)	Freezing-point depression
Terry & Hill. (Terry and Hill 1978)	1978	-	6 (310 ± 5.7)	Vapour pressure
Gilbard & Farris (Gilbard and Farris 1979b)	1979	20 (365 ± 77)	-	Freezing-point depression
Farris <i>et al.</i> (Farris, Stuchell, and Mandel 1981)	1981	123 (329.6 ± 17)	219 (304.4 ± 7.2)	Freezing-point depression
Benjamin and Hill (Benjamin and Hill 1983)	1983	-	6 (318 ± 31)	Freezing-point depression
Farris <i>et al.</i> (Farris et al. 1983)	1983	111 (326 ± 20)	180 (304 ± 8)	Freezing-point depression
Farris (Farris, Stuchell, and Mandel 1986)	1986	51 (324 ± 11)	51 (302 ± 5)	Freezing-point depression
Gilbard <i>et al.</i> (Gilbard et al. 1989)	1989	29 (317 ± 2.4)	-	Freezing-point depression
Gilbard (Gilbard 1994)	1994	31 (314 ± 1)	23 (304 ± 0.3)	Freezing-point depression
Craig & Tomlinson (Craig and Tomlinson 1995)	1995	-	100 (303.5 ± 13)	Freezing-point depression
Mathers <i>et al.</i> (Mathers et al. 1996)	1996	146 (313.7 ± 13)	72 (303 ± 10)	Freezing-point depression
Lester <i>et al.</i> (Lester et al. 2000)	2000	58 (349.5 ± 19)	-	Freezing-point depression
Isekei <i>et al.</i> (Iskeleli et al. 2002)	2002	-	56 (283.3 ± 11.3)	Freezing-point depression
Thai <i>et al.</i> (Thai, Tomlinson, and Pearce 2005)	2005	-	19 (302.1 ± 11.9)	Freezing-point depression
Messmer <i>et al.</i> (Messmer, Bulgen, and Kampik 2010)	2010	200 (308.9 ± 14)	200 (307.1 ± 11.3)	TearLab™
Sullivan <i>et al.</i> (Sullivan et al. 2010)	2010	224 (322.2 ± 18.8)	75(302.2 ± 8.3)	TearLab™
Versura <i>et al.</i> (Versura, Profazio, and Campos 2010)	2010	105 (304.9 ± 11.8)	25 (295.5 ± 9.8)	TearLab™
Tomlinson <i>et al.</i> (Tomlinson, McCann, and Pearce 2010)	2010	15 (321 ± 16.5)	21 (308 ± 6.2)	TearLab™
		15 (323 ± 14)	21 (310 ± 7)	Freezing-point depression
Utine <i>et al.</i> (Utine et al. 2011)	2011	10 (301.9 ± 11.4)	20 (294.8 ± 8.3)	TearLab™
Jacobi <i>et al.</i> (Jacobi et al. 2011)	2011	133 (320)	95 (301)	TearLab™

Szalai <i>et al.</i> (Szalai et al. 2012)	2012	41 (303.3 ± 17.2)	22 (303.5 ± 12.9)	TearLab™
Bunya <i>et al.</i> (Bunya et al. 2013)	2013	49 (314.5 ± 18)	-	TearLab™
Masmali <i>et al.</i> (Masmali et al. 2014)	2014	-	30 (299.1 ± 7.7)	TearLab™
Ashley <i>et al.</i> (Brissette et al. 2018)	2017	-	100 (293.4 ± 6.8)	TearLab™
Nolfi&Caffery(Nolfi and Caffery 2017)	2017	-	20 (295.4 ± 8.6)	TearLab™
		-	20 (319.4 ± 20.3)	i-Pen
Abusharha <i>et al.</i> (Abusharha et al. 2018)	2018	-	20 (308 ± 12)	TearLab™
Average ± SD for tear osmolarity based on the literature reviewed in this Table for subtype of KCS = 322.7 ± 15.9; normal = 303.2 ± 7.0				

Note: N is the number of subjects; KCS is keratoconjunctivitis sicca; SD is standard division



Figure 1: Clifton osmometer. (McCann, L.C. 2009)



Figure 2: TearLab (right) and i-Pen (left)

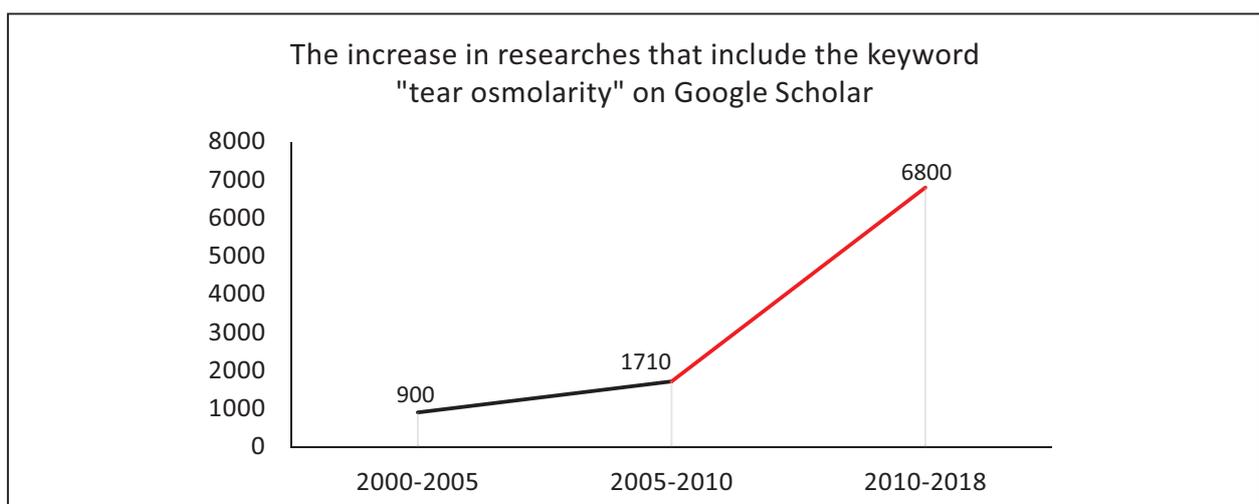


Figure 3: Line chart showing the increase in researches that include the keyword 'tear osmolarity' on Google Scholar between 2000–2018.

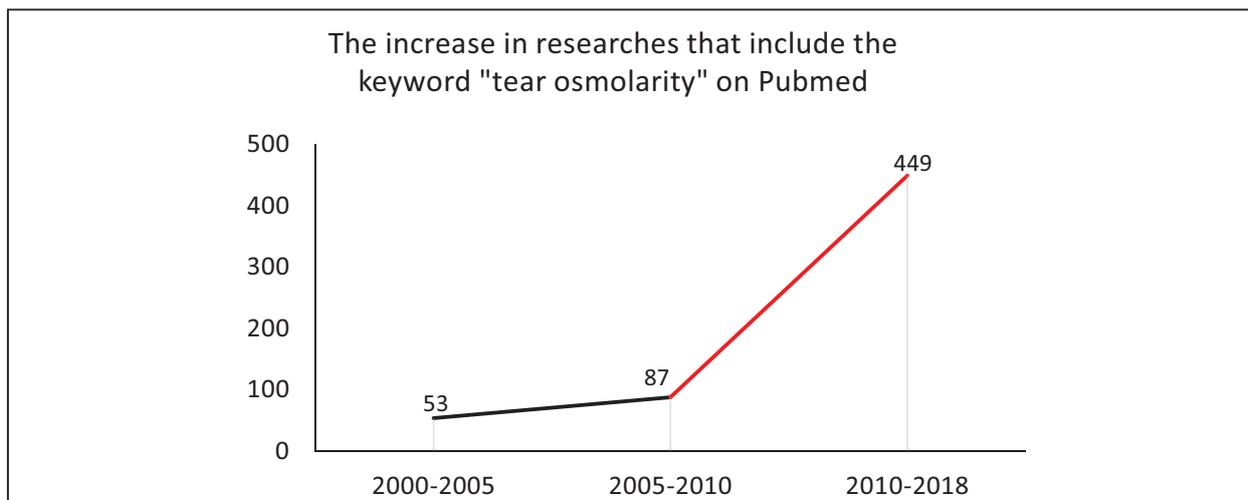


Figure 4: Line chart showing the increase in the research that includes the keyword ‘tear osmolarity’ on PubMed between 2000–2018.

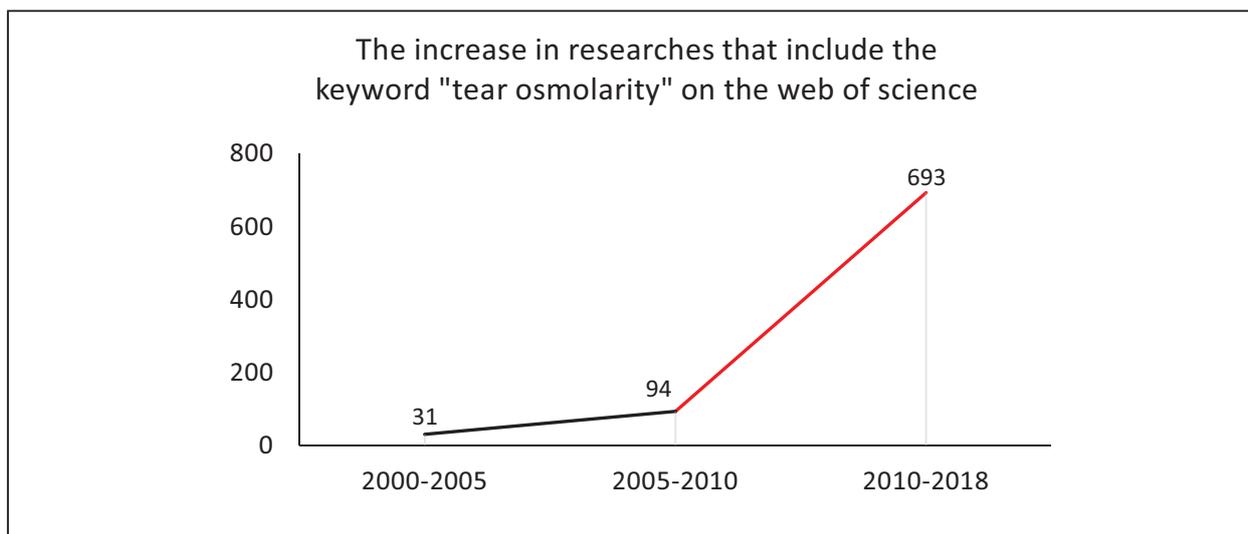


Figure 5: Line chart showing the increase in the research that includes the keywords ‘tear osmolarity’ on the Web of Science between 2000–2018.

Discussion

This study reviews the development in tear film osmolarity assessment techniques. The tear film osmolarity went through two phases, using three techniques. The first technique was the Freezing point depression method, which gets its name because the sample is super cooled to its freezing point (the freezing point of the solution “depressed”). The two advantages of this technique are: it requires only a 0.2 μl and it’s highly accurate. The problem with this

technique is that it’s expensive, technically challenging, and the equipment is only used by research labs. However, as a result of its accuracy it is the industry standard among the different techniques of osmolarity testing. The Vapour pressure osmometer is an alternative technique to measure tear osmolarity. The principle of this technique is that the vapour pressure of a solution is lower than the vapour pressure of the pure solvent at the same temperature and pressure. The more particles that are present in

the solution, the longer it takes to evaporate. The advantages of this technique is: it is quick and reliable. But, it does have a big drawback for measuring tear film as it requires a large sample (5 μ l) of tears. Also, it requires skill to collect the sample without inciting tear reflex.

These two techniques Freezing point depression and Vapour pressure were the only techniques available for tear osmolarity assessment. Since 1970 several studies have been published using freezing point technique. But only a few research studies have used the vapour pressure technique.

In the decade, the electrical impedance technique was used for the tear film osmolarity assessment. The Electrical impedance technique takes advantage of the electrical conductivity of fluids to measure tear osmolarity. It requires a very small tear sample 50 nl and the result can be obtained in a few seconds. On the downside, the instrument and the test cards are relatively expensive, particularly if the practitioner wants to use it in the clinic for the routine dry eye assessment. In addition, it can be only used up to 400 mOsmol/L. Such limitation inhibits the use of such technique in the measurement of the tear osmolarity in animals such as the camels whose tear osmolarity is higher than 400 mOsmol/L.

In 2009, Tomlinson et al (2010) compared the freezing point depression and electrical impedance techniques. Significant correlations were found between the two techniques. In 2012, Khanal and Millar (2012) reported that three sequential readings are required to attain a reliable result of tear osmolarity using the TearLab system. The dissimilarity in recording tear osmolarity makes it difficult to be widely used as a technique for the identification of mild dry eye (Khanal and Millar, 2012; Szczesna-Iskander, 2016).

The validity and reliability of the new handheld tear osmolarity system (electrical impedance, i-pen) has been investigated by Chan et al

(2018). The accuracy of i-pen was investigated by measuring the osmolarity of solutions with known osmolarity. They reported that the system is reliable and precise to measure the osmolarity of the solutions. Also, they indicated such an osmolarity device represents a quick and precise technique for tear osmolarity measurement (Chan et al, 2018).

Conclusion

Each of the techniques provides a numeric osmolarity measurement. The average tear osmolarity for normal subjects based on the published work reviewed in this article is 303.2 ± 7.0 compared to 322.7 ± 15.9 for subjects with KCS.

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