Original Article

Dopamine levels in human tear fluid

Niyati Seshagiri Sharma, Suraj Kumar Acharya*, Archana Padmanabhan Nair, Jyoti Matalia[1], Rohit Shetty[1], Arkasubhra Ghosh, Swaminathan Sethu*

Purpose: To determine the levels of dopamine in tear fluid and demonstrate the use of tear fluid as a non-invasive source for dopamine measurements in humans. **Methods:** The study cohort included 30 clinically healthy individuals without any pre-existing ocular or systemic conditions. Matched tear fluid (using Schirmer's strips and capillary tubes) and plasma were collected from the subjects. Dopamine levels were evaluated using direct competitive chemiluminescent enzyme-linked immunosorbent assay (ELISA), dopamine kit (Cloud Clone Corp, TX, USA). **Results:** Significantly higher dopamine levels were found in the tear fluid compared to plasma in the study subjects. The level of dopamine was 97.2 ± 11.80 pg/ml (mean ± SEM), 279 ± 14.8 pg/ml (mean ± SEM), and 470.4 ± 37.64 pg/ml (mean ± SEM) in the plasma and in the tears collected using Schirmer's strips and capillary tubes, respectively. **Conclusion:** Dopamine was detectable in all the tear fluid samples tested and was also found to be at a higher concentration than in plasma samples. Tear fluid can be used as a non-invasive sample source to monitor dopamine levels.

Key words: Dopamine, ELISA, plasma, tear fluid

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Dopamine is a neurotransmitter known to be associated with the regulation of a range of behavioral, developmental and neurological functions.[1] Studies have also demonstrated that dopamine plays a role in maintaining renal, intestinal, ocular health and homeostasis.[2–4] Dopamine is implicated to play a role in regulating eye growth and development, as well as in the molecular transport mechanisms involved in light perception and vision.[4–6] Dopamine has been measured in various biological fluids including blood plasma, cerebrospinal fluid (CSF) and urine.[7] Tear fluid can be used as a non-invasive sample source to monitor dopamine levels. However, reports detailing the detection of dopamine in human tear fluid are scarce.[8,9] Tear fluid can be easily obtained using well-established methods of collection through Schirmer's strips and capillary tubes.[10–12] Moreover, tears are a non-invasive source of sampling as opposed to blood collection for plasma, which could improve donor compliance during sampling, particularly for longitudinal follow-up monitoring and in pediatric subjects. Thus, optimizing an ELISA-based method for the detection of dopamine from tear fluid would serve as an additional tool for monitoring local dopamine status.

Methods

Study design and clinical examination

The observational cohort study approved by the Narayana Nethralaya Institutional Review Board was conducted in adherence to the Indian Council for Medical Research (ICMR) guidelines and tenets of the Declaration of Helsinki. A total of 30 healthy volunteers were selected (after obtaining informed written consent) for the study through a questionnaire-based investigation at the Narayana Nethralaya Eye Hospital, Bangalore, India. The questionnaire was formulated to rule out the influence of known dietary and lifestyle habits on dopamine levels as well as any ongoing or recent ocular and/or systemic co-morbidity.

Plasma sample collection

Peripheral venous blood was collected aseptically by venipuncture into a sterile ethylenediaminetetraacetic acid (EDTA) containing BD Vacutainer® tube. The blood was immediately centrifuged at 2500 rpm at 4°C for 5 min. The supernatant plasma was separated carefully and stored at − 80°C until further use.

Tear sample collection

Tear fluid was collected using Schirmer's strip and capillary tubes as described earlier.[11,12] Schirmer's strip-based tear fluid collection was followed as it was reported to be suitable and comparable with capillary tube-based tear fluid collection for downstream analysis.[13] Briefly, tear fluid from the subjects was collected using sterile Schirmer's strips (5 × 35 mm^2; Tear Strips, Contacare Opthalmics and Diagnostics, India) according to the Schirmer's test I procedure. Tear fluid was extracted

GROW Research Laboratory, Narayana Nethralaya Foundation, [1]Narayana Nethralaya Eye Hospital, Bengaluru, Karnataka, India

*The first two authors have contributed equally to this work

Correspondence to: Dr. Swaminathan Sethu, No 258/A Hosur Road, Narayana Health City, Bommasandra, Bengaluru - 560 099, Karnataka, India. E-mail: swaminathansethu@narayananethralaya.com

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from Schirmer's strips by agitating small cut pieces of these strips in 300 µl phosphate-buffered saline (PBS) solution in a sterile 1.5 ml microcentrifuge tube at 4°C for 1.5 h. Tear fluid was then eluted by centrifugation and stored at − 80°C until further use. Tears collected from subjects using capillary tubes were suctioned out using a pipette and diluted with 300 µl PBS solution and stored at − 80°C in a sterile microcentrifuge tube until further use.

Measurement of plasma and tear dopamine

Total dopamine levels in the plasma and tear fluid were quantified using a direct competitive chemiluminescent enzyme-linked immunosorbent assay (ELISA), dopamine kit (Cloud Clone Corp, TX, USA) according to the manufacturer's instructions. 50 µl each of diluted plasma (1:10 in 1 × PBS) and of tears extracted from Schirmer's strips and capillary tubes were also used for the ELISA analysis. Absolute values were obtained based on a standard curve. Values of samples within the detectable range were used for further analysis. The tear volume, tear wetting length, and buffer volume used were factored in as dilutions to determine the final dopamine concentration in the sample.

Statistical analysis

All statistical analyses were carried out in GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium). D'Agostino–Pearson omnibus normality test was used to check the normality of the data followed by *t* test to check for significant differences between the three sources for dopamine estimation. Wilcoxon matched-pairs signed-rank test was used to check for statistically significant differences between the three sources. Spearman's correlation analysis was used to check for any association between the tear fluid and plasma dopamine levels. The data are reported as mean ± SEM or as median with the range. $P < 0.05$ was considered to be statistically significant.

Results

The study cohort included 17 male and 13 female subjects of Indian origin. The age of subjects in the cohort ranged from 23 to 41 years (mean ± SEM, 30.2 ± 0.98 years; median 29.5 years).

Dopamine level in plasma

The measured plasma dopamine level ranged between 29.7 and 318.6 pg/ml (mean ± SEM, 97.2 ± 11.80 pg/ml; median, 74.5 pg/ml) as shown in Fig. 1. No significant difference was observed in the plasma dopamine level between male (mean ± SEM, 101 ± 16 pg/ml; median, 75.7 pg/ml) and female (mean ± SEM, 90.8 ± 17.5 pg/ml; median, 73.6 pg/ml) subjects in the study cohort [Fig. 2].

Dopamine level in tears

The dopamine level in tears extracted from Schirmer's strips was 279 ± 14.8 pg/ml (mean ± SEM) with values ranging between 152 and 519.1 pg/ml (median, 273.2 pg/ml) [Fig. 1]. Similar to plasma dopamine levels, a significant difference was not observed in the dopamine level in tears from Schirmer's strips between male (mean ± SEM, 284.8 ± 13.8 pg/ml; median, 280 pg/ml) and female (mean ± SEM, 291.5 ± 32 pg/ml; median, 274.6 pg/ml) subjects in the study cohort [Fig. 2]. The dopamine level in tears extracted from capillary tubes was 470.4 ± 37.6 pg/ml (mean ± SEM) with values ranging from 254.7 to 845 pg/ml (median, 428.4 pg/ml) [Fig. 1]. Unlike the dopamine level measured in the plasma and from Schirmer's strips, the dopamine level in tears extracted from capillary tubes showed a significant difference between male (mean ± SEM, 529.4 ± 64.38 pg/ml; median, 429.8 pg/ml)

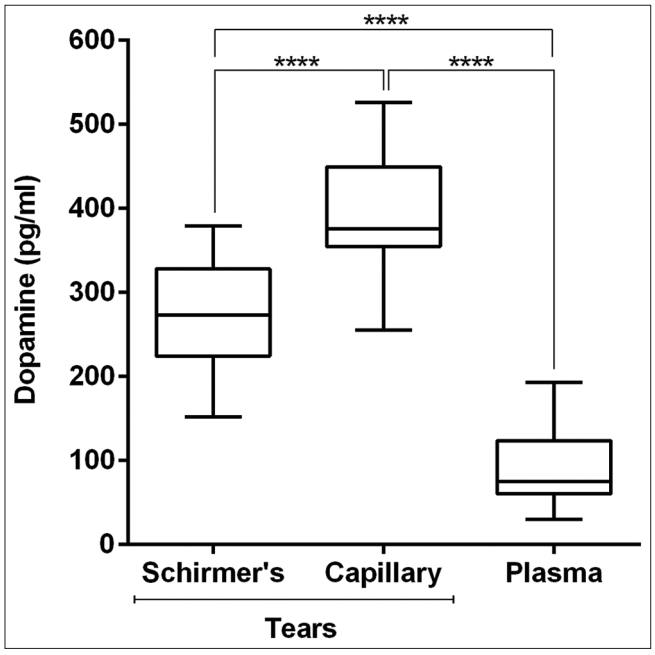

Figure 1: Dopamine levels in plasma and tear fluid of healthy human subjects. Box and whiskers plot shows matched dopamine levels in the study subjects. Schirmer's strips (n = 30); capillary tube (n = 20); plasma (n = 27); ****P < 0.0001, Wilcoxon-matched-pairs signed rank test

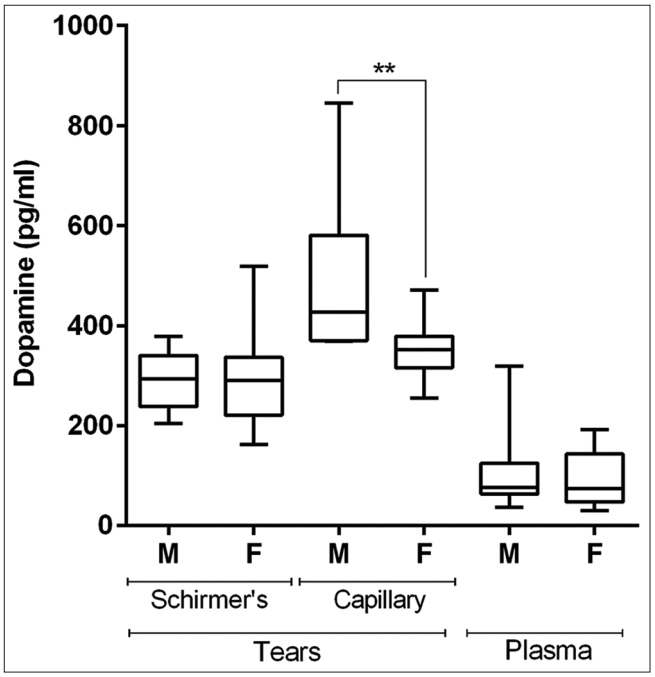

Figure 2: Dopamine levels in male and female subjects. Box and whiskers plot shows tears and plasma dopamine levels in the male (M) and female (F) study subjects. **P = 0.003, Mann–Whitney test

and female (mean ± SEM, 411.8 ± 43.6 pg/ml; median, 370 pg/ml) subjects in the study cohort [Fig. 2].

Significantly ($P < 0.0001$) higher dopamine level was observed in tears (both from Schirmer's strips and capillary tubes) compared to plasma [Fig. 1]. However, no correlation was observed between the levels of dopamine in plasma and the Schirmer's strip tears or between plasma and capillary tube tears. Tear dopamine levels were higher than those in the plasma for the majority of subjects. Dopamine levels were found to be 3.9 ± 0.48 (mean ± SEM) fold higher in tears from Schirmer's strips than plasma dopamine levels and 6.2 ± 0.85 (mean ± SEM) fold higher in tears from capillary tubes than plasma dopamine levels. In all, 83.3% (25/30) of the subjects had >1.5-fold higher dopamine levels in the tears from Schirmer's strips compared with plasma dopamine levels. Also, 80% of individuals had a >1.5-fold higher level of dopamine in tears from capillary tubes than in the plasma. A majority of individuals, i.e., 80% (16/20) had >1.2-fold dopamine levels in the capillary tube tears than the Schirmer's strip tears. Furthermore, though not significant, a positive association [($r = 0.368$, $P = 0.160$) Spearman's rank correlation coefficient] was observed between tear dopamine levels.

Discussion

Dysregulated dopamine levels have been causally linked to various conditions such as attention deficit hyperactivity disorder,[14] Parkinson's disease,[15] schizophrenia,[16] epilepsy,[17] Alzheimer's disease and senile dementia.[18] In the eye, dopamine has been associated with the development of myopia,[6] dry eye disease[19] and glaucoma.[20] Dopamine levels have been measured in various biological fluids such as blood and urine more commonly but not in tears. Tear fluid is increasingly being used as a source for detecting biomarkers in ocular and systemic conditions.[21] Very few studies have reported the levels of tear dopamine basally or following stimulation. Early studies on tear dopamine levels in humans have reported varying values such as 280×10^3 pg/ml in tears extracted from the conjunctival sac via micropipetting post ammonia gas stimulation using a fluorimetric trihydroxyindole method,[22] 0.68 pg/ml in tears using a microfluidic-based chromatographic method,[23] and 8.9×10^3 pg/ml in tears extracted through capillary tubes after trigeminal stimulation using high-performance liquid chromatography (HPLC) with electrochemical detection.[24] The mean dopamine levels in our study are 278.9 ± 14.8 pg/ml and 468.3 ± 38.8 pg/ml in tears extracted from the Schirmer's strips and capillary tubes, respectively. The difference in the values obtained in our study compared to those in earlier studies could be attributed to factors including age, ethnicity, variation difference in tear collection techniques, and dopamine measurement methods. The aforementioned studies have used stimulation to induce reflex tear production which may have influenced dopamine levels. Further, none of the studies have employed ELISA for the detection of dopamine and it is possible that the variation in values could be a result of the different techniques employed in dopamine measurement. In this study, we demonstrate that dopamine can be measured in human tears using a competitive chemiluminescent ELISA method. Here, we describe the use of tear fluid as a non-invasive source for monitoring dopamine levels and also compare tear levels with matched plasma levels in adults. We observed that dopamine level in tear fluid collected using capillary tubes is higher than the level in tears extracted from Schirmer's strips. The retention of biomolecules within the Schirmer's strip could be one of the factors,[25] leading to decreased levels of dopamine detected from the Schirmer's strip tears with respect to capillary tube tears. To date, this is the first study carrying out a comparative analysis between tear and plasma dopamine levels. In our study cohort, we observed an overall higher level of dopamine in tear fluid (mean, 278.9 pg/ml – Schirmer's strips; mean, 468.3 pg/ml – capillary tubes) compared to plasma (mean, 97.2 pg/ml). The higher levels of dopamine observed in the tear fluid than in the plasma could be attributed to the low level of plasma dopamine observed in circulation – derived from biosynthesis in the gastrointestinal tract[26] – or owing to the possibility of an ocular synthesis mechanism existing for dopamine as has been suggested in a very recent report.[27] The mean plasma dopamine level in the current study is 97.2 ± 11.80 pg/ml using ELISA which is comparable to range reported by other studies. Ambade *et al*. reported the average level of dopamine in plasma in their control study to be 21.8 pg/ml using HPLC with an electrochemical detection method.[28] In other studies the reported concentration of plasma dopamine varied from 15.3 pg/ml[26] to 70 pg/ml.[29] The difference in detected plasma levels of dopamine could perhaps be due to the selectivity and sensitivity of ELISA toward detecting dopamine and also due to differences in age and ethnicity of the subjects.

Our observation of dopamine levels in the tear fluid in adults indicates that tear fluid can be employed as a non-invasive source for monitoring dopamine levels, enabling relatively easy measurement during investigations involving multiple sampling procedures. The absence of normative values in large cohorts for tear dopamine might be a potential hurdle for its use as a non-invasive sample source for dopamine monitoring. However, relative dopamine levels estimated from tear fluid would still be useful in prospective studies with repeat measurements, as well as in case–control studies where the relative difference in dopamine levels would be informative even in the absence of absolute values. In particular, case–control studies will also help elucidate the influence of local vs systemic synthesis of dopamine on the measured values. Thus, there is a need to establish normative values for tear fluid dopamine level in a larger cohort and across different ethnicities in order to enable its routine use. The difference in tear level between the sexes in tear fluid by capillary but not in the Schirmer's strip indicates the need for larger cohort studies in determining these differences. Furthermore, tear cytokines and chemokines in human subjects have also shown diurnal variations. Hence, it would also be beneficial to study diurnal variations in tear dopamine levels which would aid in standardizing measurement and analysis.

Conclusion

The observations from the current study suggest that dopamine is present and can be measured in tear fluid. Tear fluid concentrations are higher than in the matching plasma samples in humans suggesting local synthesis. Tear fluid can serve as a non-invasive, yet reliable, source for monitoring dopamine levels, which would improve donor compliance and be useful in follow-up investigations with repeat sample collection. Furthermore, tear dopamine assessments can be used to study the role of dopamine in ocular health and disease.

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Conflicts of interest

There are no conflicts of interest.

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