Salivary alpha amylase levels may correlate with central serous chorioretinopathy activity

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SALIVARY ALPHA-AMYLASE LEVELS MAY CORRELATE WITH CENTRAL SEROUS CHORIORETINOPATHY ACTIVITY

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Purpose: To investigate and compare the salivary alpha-amylase (sAA) activity as an indicator of the sympathetic activity and stress response in patients with central serous chorioretinopathy (CSC) and healthy control subjects.

Methods: Prospective multicenter case series, including 80 CSC patients and 88 healthy control subjects. Central serous chorioretinopathy status was classified as either active or inactive, depending on the presence of subretinal fluid on optical coherence tomography. Salivary samples were collected in the morning from patients and control subjects of the main cohort and at midnight for the additional cohort. Salivary alpha-amylase activity was determined in all patients and control subjects.

Results: Morning sAA activity was significantly higher in patients with active CSC compared with inactive CSC ($P = 0.049$) and to healthy control subjects ($P = 0.012$). There was no significant difference in sAA activity between patients with inactive CSC and control subjects ($P = 1.0$). Nocturnal sAA activity did not show any significant difference between patients with active CSC and either inactive CSC or control subjects ($P = 0.139$).

Conclusion: Morning sAA activity is increased in patients with active CSC, although diurnal rhythmicity is preserved. Measurement of sAA is easy to perform and might be an eligible tool to further investigate the relation between stress and CSC.

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Central serous chorioretinopathy (CSC) is a retinal disease that is characterized by detachment of the neurosensory retina, which most probably occurs as a result of choroidal abnormalities.[1] Even more than 150 years after its first description by Albrecht von Graefe,[2] the pathogenesis of CSC remains to be fully understood.

Currently available literature suggests that glucocorticoids and adrenergic hormones play an important role in the pathogenesis of CSC.[3–5] A dysfunctional steroid regulation may be an important factor bcause most known risk factors for CSC are associated with increased levels of endogenous or exogenous glucocorticoids, like psychological stress,[6,7] type A personality,[8,9] endogenous Cushing syndrome,[10] steroid use,[11,12] and pregnancy.[13]

The stress response is coordinated around two pillars: 1) activation of the hypothalamic–pituitary–adrenal axis and 2) activation of the sympathetic adrenomedullary system (SAM).[14] Although the contribution of the former to stress-related disease may be evident, the role of the sympathetic nervous system in CSC development is not clear at all.[15] However, sympathomimetic agents are under suspicion to trigger the disease,[16–18] and CSC patients show increased SAM activity.[19,20] Consequently, it is postulated that stress and adaption to stress are relevant factors in the

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origin of disease.[21] Yet, as emphasized in the review of Scarinci et al, there is a need for future studies investigating the role of SAM in the cause of CSC.

A choroidal vascular hyperpermeability is a common finding in CSC patients.[22,23] The choroid is controlled by the autonomic nervous system, and the autonomic nervous system is related to the stress response in the human body. Concluding, the activity of the autonomic nervous system could influence the choroidal blood flow and CSC development.[19] Moreover, it is hypothesized that an autonomic dysregulation might lead to choroidal vascular hyperpermeability.[24]

A very recently developed tool to investigate and quantify SAM activity is determine the salivary alpha-amylase (sAA; a-1, 4-a-D-glucan 4-glucanohydrolase) as an indicator for the sympathetic activity.[25] The activity of sAA follows a circadian rhythmicity,[26] and physiological regulations seem to play also a role in sAA secretion.[27] However, SAA may be an eligible parameter to measure a general autonomic dysregulation, which might be an important factor in CSC development. To date, there is only one study that measured diurnal sAA activity in a small group of CSC patients in comparison to control subjects.[28] This study presented that diurnal sAA activity was higher in CSC patients in comparison to control subjects.[28]

Salivary alpha-amylase could be helpful in monitoring treatment success of stress reduction interventions in eligible patients and could unravel a possible role for autonomic dysregulation in CSC. The aim of this current study was to investigate the role of sAA in a larger CSC cohort in comparison to control subjects and differentiate between active, inactive CSC patients, and control subjects.

Patients and Methods

This study followed the tenets of the Declaration of Helsinki and was performed in accordance with the regulations of the local ethics committee (Approval number University of Cologne: 16-383). Written informed consent was obtained from all participants, before inclusion to the study. No animal experiments were performed for this study.

All CSC patients had been diagnosed by means of a routine clinical ophthalmological examination and retinal imaging including fundus biomicroscopy, spectral-domain optical coherence tomography (SD-OCT), fluorescein angiography, and indocyanine green angiography. Patients had to show characteristic findings of CSC (e.g., serous macular neurosensory detachment possibly associated with pigment epithelial detachment in OCT, single or multiple leakage points in fluorescein angiography, areas of choroidal hyperpermeability in indocyanine green angiography, areas of retinal pigment epithelium atrophy, atrophic retinal pigment epithelium tracts) at any visit before study inclusion. Subjects with any other retinal pathology were excluded.

We included two different cohorts in this study, the difference between the cohorts was the time point the salivary samples were collected: in the first cohort, samples were collected in the morning and in the second cohort, they were collected at midnight. Additionally, the two cohort came from two different departments. In both cohorts, patients with active CSC and inactive CSC as well as healthy volunteers without CSC serving as a control group were recruited.

For the first cohort, salivary samples were collected at the Department of Ophthalmology at the University Hospital of Cologne (Germany) in the morning. Even so multiple salivary samples at one day would lead to more reliable results, we decided to choose only one time point to reduce patient burden in this pilot study. The morning measurement appeared to be most convenient because the highest number of patients could be reached at this time of day.

Considering that sAA secretion follows a circadian rhythm, the main study (Cohort 1) was extended by including an additional cohort (Cohort 2). For this second cohort, salivary samples were collected at midnight from patients with active CSC, inactive CSC, and healthy non-CSC volunteers serving as control from the Department of Ophthalmology, Leiden, the Netherlands.

Inclusion criteria for both cohorts were prior diagnosis of CSC on multimodal imagining and availability of SD-OCT images of all CSC patients within 30 days before or after sample collection. Disease activity of CSC patients was based on subretinal fluid on SD-OCT images. Active CSC was defined as the presence of subretinal fluid on SD-OCT, graded by a masked investigator. Healthy control subjects were randomly recruited from hospital employees for both cohorts.

Alpha-Amylase Collection and Assessment

Saliva samples of approximately 500 μL were collected between 10 and 12 AM in all Cologne subjects and between 11 PM and midnight in the Leiden subjects. Subjects were not allowed either to eat, drink, chew gum, smoke, or brush teeth for 30 minutes before sample collection. Samples were not collected when oral diseases, inflammation, or lesions were

present. The saliva samples were frozen immediately at −80°C, until analysis.

The sAA activity was measured using the Alpha-Amylase Saliva Assay (IBL International GmbH, RE80111, Hamburg, Germany). After thawing, samples were suspended and centrifuged at 2,000*g* to 3,000*gg* for 10 minutes, to remove particles. Ten microliters of the enclosed standard and positive control samples and the collected patient and healthy control sample were pipetted in duplicates into the respective wells of a 96-well plate. The standard samples were used to calibrate the sAA activity, whereas the enclosed control samples with known activity should serve as positive control subjects. Two hundred microliters of the substrate solution were added to each well with an eight-channel micropipettor to initiate the reaction.

After an incubation period of 3 minutes at room temperature, photometric measurement of the color intensity was performed in a microplate reader at a wavelength of 450 nm in room temperature (reference wavelength, 630 nm; Max Microplate Reader MRX Revelation; Dynex Technologies GmbH, Denkendorf, Germany). After further 5 minutes of incubation (total incubation time of 8 minutes), a second measurement was performed at room temperature (at 405 nm, reference 630 nm). The provided control samples and standard samples were measured and met the absorbance range stated in the kit.

The delta optical density for each standard, control, and sample was obtained as the difference in absorbance between first and second measurement. The obtained delta optical density of the standards samples was plotted against their concentration using automatic GraphPad Prism (Version 6.07; GraphPad Software Inc, La Jolla, CA). A cubic, third-order polynomial formula $y = B0 + B1 \times x + B2 \times x^2 + B3 \times x^3$ was applied to determine the nonlinear regression. The sAA activity of each sample and the provided control was interpolated using the calculated standard curve. Because control and patient samples were measured in duplicates, mean sAA activity was calculated and used for further statistical analysis.

SPSS (IBM SPSS Statistics, Version 22; SPSS Inc, Chicago, IL) was used for the statistical analysis. Differences between all groups were assessed using the Kruskal–Wallis test. In case of significant differences, a post hoc analysis was performed.

Results

Salivary alpha-amylase activity was determined in saliva samples from 40 Cologne patients and 67 control subjects (main cohort) and 40 Leiden patients and 21 control subjects (second cohort) collected in the morning and around midnight (nocturnal), respectively.

A total of 107 saliva samples were taken in the morning for the first cohort. All samples for the first cohort came from Cologne, and the cohort consists of 27 patients with active CSC, 13 with inactive CSC, and 67 healthy volunteers serving as a control.

For the second cohort, saliva samples from Leiden patients were collected at midnight. This cohort consists of 35 patients with active CSC, 5 with inactive CSC, and 21 healthy control subjects.

To test our hypothesis of autonomic dysfunction in CSC patients, sAA levels were compared between patients with active CSC, inactive CSC, and healthy control subjects in two different cohorts. The CSC cohorts and control cohorts were balanced regarding gender and age; only the control cohort from Leiden was slightly younger compared with the CSC groups. Table 1 displays the detailed demographics of all participants in both cohorts.

In the main cohort from cologne in which the sAA activity was measured in the morning, the group of patients with active CSC (defined as presence of subretinal fluid on SD-OCT) showed a significantly higher sAA activity with a value of 218 ± 109 U/mL (mean ± SD) compared with the patients with inactive CSC (130 ± 90 U/mL; $P = 0.049$) and the control group (148 ± 101 U/mL; $P = 0.012$). No difference was seen between the inactive CSC group and the control group ($P = 1.00$). Figure 1 shows the sAA activity in the main cohort determined in the three groups.

In the second cohort, in which the samples were collected at midnight, the sAA activity was in general lower compared with the patients from whom samples were collected in the morning, and no differences could be detected between patients with active CSC (62 ± 61), inactive CSC (38 ± 21), or the control group ($P = 0.139$). Figure 2 displays the results for the second cohort.

Discussion

This study aimed to investigate the SAM activity in CSC patients via noninvasive measurement of salivary alpha-amylase activity in two independent cohorts from two different centers and with a different time point of sample collection. Each cohort included patients with active CSC, inactive CSC, and healthy control subjects. Both cohorts were analyzed separately. In our first cohort, salivary samples were collected in the morning. Morning sAA activity was

Table 1. Demographics of the Included Patients

	Gender	Age in Years (Mean ± SD)
Active CSC	Female: 11	52 ± 9
Cohort 1: morning sAA measurement (n = 27)	Male: 16	Range: 34–74
Inactive CSC	Female: 1	53 ± 10
Cohort 1: morning sAA measurement (n = 13)	Male: 12	Range: 37–73
Control Subjects	Female: 32	50 ± 8
Cohort 1: morning sAA measurement (n = 67)	Male: 35	Range: 27–68
Active CSC	Female: 5	50 ± 12
Cohort 2: midnight sAA measurement (n = 35)	Male: 30	Range: 33–77
Inactive CSC	Female: 0	49 ± 14
Cohort 2: midnight sAA measurement (n = 5)	Male: 5	Range: 36–72
Control Subjects	Female: 5	39 ± 13
Cohort 1: midnight sAA measurement (n = 21)	Male: 16	Range: 21–62

Cohort 1, sAA measured in the morning; Cohort 2, sAA measured at midnight.

increased in patients with active CSC when compared with patients with inactive CSC and control subjects.

Central serous chorioretinopathy has long been associated with increased stress levels.[6,7] However, measuring stress in humans is challenging and error prone. Consequently, detailed examination of the different stress system components has not been incorporated in the standard evaluation of CSC patients to date.

Until recently, quantifying SAM activity was complicated and not suitable for clinical practice. Also, the attempt to measure catecholamines in saliva, based on the model of cortisol measurements in saliva, failed because of long duration of transfer from blood to saliva and the necessity to immediately handle the probes after sampling. Measuring sAA activity is a relatively new tool to noninvasively determine sympathetic activation (as a component of the stress response).[25,29] Salivary alpha-amylase, the predominant enzyme in saliva, is

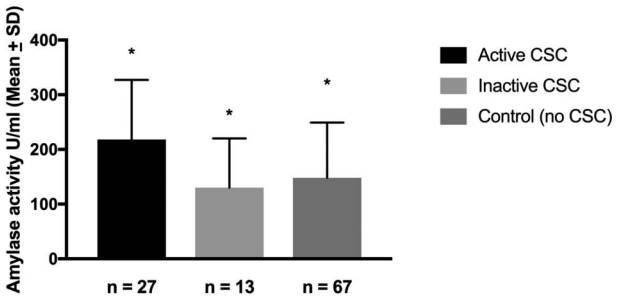

Fig. 1. Morning sAA in CSC patients and control subjects of the main cohort. *Kruskal–Wallis test (P = 0.008), adjusted P values after post hoc analysis: Group 1 versus Group 2 (P = 0.049), Group 1 versus Group 3 (P = 0.012), Group 2 versus Group 3 (P = 1.00).

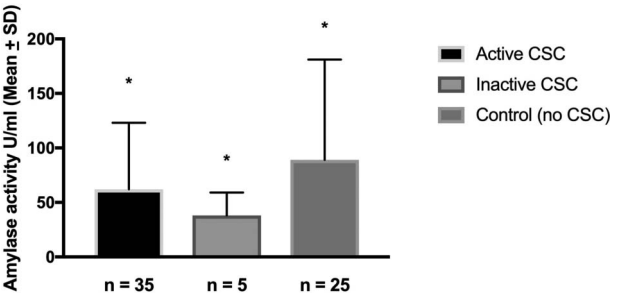

Fig. 2. Midnight sAA activity in CSC patients and control subjects of the additional cohort. *Kruskal–Wallis test (P = 0.139).

secreted from the salivary glands in a circadian rhythm.[26] Its secretion is influenced by the autonomic nervous system, and stress has been found to induce an sAA secretion.[29] Chatterton et al[30] first described increased levels of sAA in different stressful conditions. As a result of the easy extraction and sample handling, sAA has now become a popular biomarker for stress-related changes.[29] Nevertheless, one of the weaknesses of sAA secretion is it does not only reflect sympathetic but also parasympathetic effects, through the effect of PNS activity on saliva flow rate, and this should always kept in mind when interpreting sAA data.[27]

In this study, patients with active CSC showed increased sAA activity in the morning hours between 10 and 12 AM, both compared with patients with inactive CSC and control subjects. In line with our results, Scarinci et al[28] found also significantly different diurnal sAA levels in CSC patients in comparison to control subjects. In comparison to this study, Scarinci et al[28] collected three samples during the day (early morning 06:30–18:30- at midday at 13 hours and at evening at 20 hours) and found significantly altered sAA between CSC and control subjects in the groups in the second part of the day from 13 hours to 20 hours. Because of different sample collection time, a direct comparison between the studies is difficult, yet both studies give novel information about the different SAM activity in CSC patients. This study was not designed to answer the question which time point is the most sensitive one for sAA measurement in CSC and is limited as involves only 2 time points (10–12 PM and midnight) for the sAA activity. But because day time measurement between 10 and 20 PM showed the greatest differences between CSC patients and control subjects, a sample extraction during the day seems to be most convenient. Future studies should involve minimum of three samples taken at specific time points to be able to evaluate the sAA activity more accurately.

Increased sAA activity may reflect a SAM hyperactivity in CSC patients. This observation is in line with the findings of Bernasconi et al[20] and Tewari et al.[19] They

found increased SAM activity in CSC patients measuring the heart-rate (RR-interval) variability, a noninvasive method that reflects the balance of the sympathetic–vagal interaction. Tewari et al[19] additionally performed different tests for autonomic reactivity. Bernasconi et al not only distinguished between CSC patients and control subjects but also categorized the CSC patients in different groups, depending on the activity status of the disease (acute CSC, acute recurrent CSC, chronic persistent CSC, complete remission of CSC). They reported the highest SAM activity in acute and acute recurrent CSC, followed by chronic CSC. Lower levels were detected in patients with inactive CSC, whereas control subjects had the lowest levels. When salivary samples were collected in the morning between 10 and 12 AM, we also observed highest sAA activity in the active CSC group, but patients with inactive CSC showed lower sAA activity compared with control subjects without CSC, measured at the same time points. A possible explanation for this finding could be the selection of control subjects. We included control subjects with a general good health but without any examinations regarding stress levels or psychological disorders. Therefore, we cannot exclude the possibility that individuals with pathologic stress levels were included in the control group. Future studies should carefully match control patients considering individual stress levels or other factors that may affect sAA activity.

Independently from the presence of CSC or disease activity, sAA activity was lower when samples were collected at midnight compared with measurements from the morning samples. Nater et al[26] reported diurnal profiles of sAA activity in line with these findings. Even if they took the last samples of each day at 8 PM, they found the highest sAA activity in the afternoon and lowest in the morning, so it can be assumed that sAA activity at midnight is also very low.

Although we did not find a significant difference in sAA activity in our small second cohort in which the salivary sample was collected at midnight, patients with active CSC and inactive CSC had tendentially lower sAA activity at midnight, compared with control subjects without CSC. A reason for this could be a more strongly marked diurnal profile in affected patients. Also, Nater et al[26] reported diurnal sAA profiles with higher sAA levels in patients with chronic stress. Becaue only one sample was collected from each patient, we cannot make a definite statement about whether sAA profiles differ between patients with CSC and non-CSC control subjects, although it is imaginable that a pathologic stress response in CSC patients is accompanied by a more variable sAA profile.

A great advantage of the sAA measurement, compared with the methods applied by Bernasconi et al and Tewari et al, is the practicability of sAA measurement. Samples can be taken easily during the whole day and night in different circumstances and can be stored at room temperature for sufficient time. Other methods to measure SAM activity are much more complicated, which has proven to be a great obstacle in clinical research.

This study should be seen and interpreted as a pilot study with the main goal to evaluate whether further research in this direction should be considered. A major limitation of this study is the lack of multiple time points for the saliva collection and small simple size. Further limitations include the lack of follow-up sAA measurements of the CSC patients and the very small number of patients with inactive CSC in the additional cohort. Therefore, the statistical difference of sAA activity between active and nonactive CSC patients should be interpreted carefully and should be validated in future studies. Furthermore, no OCTs or other ophthalmic examinations were performed in the control group; therefore, even though all control subjects denied having eye disease, we cannot completely rule out the possibility that there might be people in the control group with asymptomatic CSC. Additionally, the control subjects were not age matched, and in the active CSC group from Cohort 1, a relatively high number of female patients was included, which is not typical for CSC. Another important limitation is the lack of objective information about the possible influence of other factors on individual sAA activity, such as important life events, sleeping conditions, or psychological conditions. Moreover, even active decline in vision may contribute to an increased sAA activity and thus careful follow-up of the patients should be taken in consideration in future studies to further shed light on the relationship between SAA and CSC.

Despite the number of limitations which typically come with a pilot study, our preliminary findings should draw attention to the sAA measurement, which is in the field of ophthalmology not very well known yet but is convenient to perform.

Because the sAA secretion follows a circadian rhythm and physiological regulations play also a role in sAA secretion, the collection of multiple samples per day would also be desirable in future studies. Additionally, it would be interesting to distinguish between acute CSC and more chronic forms to analyze whether a pathologic sympathetic activation and stress response is more important in CSC development or also in the maintenance of the disease. We also included patients treated with photodynamic therapy or micropulse laser before study inclusion. Both treatments are known to be effective treatments for CSC,[31,32] but the treatment effect is only local and

does not influence the systemic findings responsible for the disease. So, a successful local treatment could mask a rather unchanged high activation of the sympathetic nervous system. It would be worthwhile to further investigate whether a high sAA activity is a risk factor for disease recurrence after a primarily successful local treatment.

Moreover, patient diaries would help to assess stress relevant events during everyday life. The combination of sAA profiles and patient diaries could avail to develop stress reduction strategies in general but also for individual patients, both for CSC and for other diseases in which stress could be of critical importance.

In conclusion, morning sAA activity was increased in patients with active CSC in this study, and in these patients, diurnal rhythmicity was preserved. This implies that future research toward further unraveling the role of the stress axis in the pathophysiology of CSC is warranted. In this respect, measurement of sAA is easy to perform and might be an eligible tool to further investigate the relation between stress, the response to stress, and CSC.

Key words: central serous chorioretinopathy, retinal diseases, salivary alpha-amylase, sympathetic activity, stress.

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