CHAPTER 4

Salivary enzyme in serum
SUMMARY

Aim - Organ damage that directly results from an autoimmune attack in Sjögren’s syndrome (SS) can hypothetically be demonstrated by an increase of organ-specific enzymes in serum, as an alternative to currently practised techniques that demonstrate loss of function or change in architecture. This assumption could be true for the salivary glands, containing large amounts of amylase, and almost invariably involved in SS. The objective of this study is to determine the clinical value of measurement of serum isoamylase activity as a clinical parameter in SS.

Methods - In a group of 100 consecutive patients referred for diagnostics of SS serum activity of salivary (S) and pancreatic (P) isoamylase were assessed. The patients were either diagnosed as positive or negative for SS according to the revised European criteria.

Results - SS patients showed significantly higher serum activities for salivary- and total (salivary and pancreatic) amylase compared to non-SS patients. The optimum threshold for detecting SS, selected from a Receiver-Operating Characteristic (ROC) curve, had a specificity of 89%, but a limited sensitivity of 35%. Further data analysis revealed that in SS patients S-type isoamylase serum activity had a biphasic course (increase-decrease) related to the duration of oral complaints, explaining the limited sensitivity. In addition, S-type isoamylase serum activity correlated positively with the sialochemical variables sodium and chloride concentration, which both are known to be related to inflammation of the salivary glands.

Conclusions - This prospective clinical study shows that measurement of isoamylases in serum has limited diagnostic value for SS, but does have potential use for assessing disease progression.
THE PERFORMANCE OF SERUM SALIVARY ISOAMYLASE AS A CLINICAL PARAMETER IN SJÖGREN’S SYNDROME

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INTRODUCTION

Sjögren’s syndrome (SS) is considered a systemic autoimmune disease with the exocrine glands as main target-organs. The diagnosis of SS is based on both subjective and objective findings that mainly result from damage to the lacrimal and salivary glands due to chronic inflammation. Since none of these objective findings is pathognomonic for SS, new diagnostic tests that are more accurate or less invasive than the tests currently applied, are widely evaluated.

In general, organ damage can be demonstrated by loss of function, change of architecture, and by increase of organ-specific enzymes which are released in serum. The first two of these three general approaches are currently used for diagnosing SS. Loss of function is assessed by measuring tear- and saliva secretion (Schirmer tear test and sialometry, respectively). Change of architecture is assessed by imaging techniques and salivary gland biopsy.

So far, measurement of serum enzymes as an estimate for organ damage is not routinely used in patients suffering SS. This seems rather surprising considering that the major salivary glands contain large amounts of amylase while these glands are almost invariably involved in the disease process of SS.\(^1,5\) Besides the pancreatic gland, the salivary glands are the only human organs that contain large amounts of the digestive enzyme amylase. Consequently, increase of serum amylase activity is highly suggestive for either pancreatic or salivary gland disorders. By determining the particular isoenzymes pancreatic (P) and salivary (S) isoamylase it is possible to differentiate between pancreatic and salivary gland disorders. It has been suggested that hyperamylasemia reflects (initial) salivary gland damage, e.g. immediately after exposure to ionising radiation, whereas decreased activity of S-type isoamylase may indicate progressive salivary gland destruction.\(^6\)
The objective of this study is to determine the clinical value of serum measurement of isoamylases in SS.

**Patients and Methods**

Patients

One hundred consecutive patients referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen in the period from January 1998 until January 2000 were included in this study. Patients suspected of Sjögren’s syndrome (SS) were referred by rheumatologists, internists, neurologists, ophthalmologists, ENT-specialists, general practitioners and dentists. Reasons for referral included mouth-dryness, eye-dryness, swelling of the salivary glands, arthralgia and fatigue. The diagnostic work-up for SS was carried out in all patients and included the following aspects: subjective complaints of oral and ocular dryness (table 3.1.1), sialometry and sialochemistry, sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal staining and Schirmer tear test). In addition to these diagnostic tests, serum isoamylases were measured. Also, the duration of oral symptoms, defined as the time from first complaints induced by or related to oral dryness until referral, was assessed.

In this study, the revised European classification criteria for Sjögren’s syndrome were used as reference standard for the diagnosis of SS, categorising patients as primary and secondary SS and non-SS patients.

Serum isoamylase assessment

None of the patients had clinical evidence of acute pancreatitis, gall bladder disease, or acute parotitis for at least six weeks prior to the blood collection, and none were known to suffer from alcohol abuse. These conditions are considered exclusion criteria for this study.

The S-isoamylase activity was calculated from the total amylase and P-isoamylase activities. Total amylase and P-isoamylase were determined according to the instructions of the manufacturer (Roche, prod.nr. 1660675). P-isoamylase activity was determined after inhibition of S-isoamylase activity with two different monoclonal antibodies (Roche, prod.nr. 1660764).
Statistical analysis
Data were submitted for statistical analysis using MedCalc version 5.0 in order to calculate Receiver-Operating Characteristic (ROC) curves and the Statistical Package for the Social Sciences (SPSS) version 9.0 for the remaining statistical procedures. These included independent sample T-test, one way analysis of variance (ANOVA) and Pearson’s correlation test. A significance level of 0.05 was pre-defined in all cases.

Results

Studied group
By applying the revised European classification criteria for Sjögren’s syndrome (SS), 37 patients were categorised as SS (22 primary SS and 15 secondary SS; male/female ratio: 1/18, mean age 57 years, SD 13, range 24 to 84) and 63 patients as non-SS (male/female ratio: 1/31, mean age 54 years, SD 12, range 20 to 84)(table 4.1.1). The latter were, based upon additional clinical and laboratory tests, diagnosed as having sialoadenosis (n=13), sodium retention dysfunction syndrome (n=18), medication induced xerostomia (n=13), or as having no alternative disease directly related to salivary gland pathology (n=19).

Mean isoamylase concentrations
The mean total amylase activity in the group of 37 SS patients (184 ± 92 U/L) significantly exceeded the corresponding activity for the 63 non-SS patients (146 ±
58 U/L). This difference was mainly due to an increase in S-type isoamylase activity (table 4.1.2). With regard to the P-type isoamylase, no significant difference was observed between SS- and non-SS patients.

Table 4.1.2. Serum activity of amylase iso-enzymes in SS- and non-SS patients. Significant differences of mean amylase iso-enzyme activity between SS- and non-SS patients are marked with * (independent T-test).

<table>
<thead>
<tr>
<th>N</th>
<th>SS</th>
<th>non-SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean total amylase (U/L)</td>
<td>184* (SD 92)</td>
<td>146 (SD 58)</td>
</tr>
<tr>
<td>Mean S-type isoamylase (U/L)</td>
<td>97* (SD 88)</td>
<td>70 (SD 45)</td>
</tr>
<tr>
<td>Mean P-type isoamylase (U/L)</td>
<td>87 (SD 29)</td>
<td>76 (SD 32)</td>
</tr>
</tbody>
</table>

Patients with S-type isoamylase level above normal (N ≤ 105U/L) 13 (35%) 9 (14%)
Patients with S-type isoamylase level below mean-SD 8 (21%) 18 (28%)
Patients with P-type isoamylase level above normal (N ≤ 115U/L) 5 (14%) 8 (13%)

Reference value for amylase iso-enzymes
When applying the normal reference values (thresholds) for the isoamylases (according to our hospital laboratory), 35% of the SS patients showed S-type activity above the reference range compared to 14% of the non-SS patients (N: ≤ 105U/L) (table 4.1.2). P-type isoamylase was above reference range in 14% of the SS patients and in 13% of the non-SS patients (N: ≤ 115U/L).

Through analysis with a Receiver-Oeering Characteristic (ROC) curve of the S-type isoamylase activity in SS- and non-SS patients, the optimum threshold for differentiating SS from non-SS was found to be similar to the normal threshold at 105 U/L (figure 4.1.1). In our studied population, this threshold has a likelihood ratio of 3.2 with a specificity of 89% and a sensitivity of 35% for a diagnosis of SS (table 4.1.3). The total and P-type amylase activities were also evaluated by ROC-curve analysis (figure 4.1.1). These variables proved less specific but more sensitive for SS than S-type isoamylase, however, with rather poor likelihood ratios (table 4.1.3).
Isoamylase activity versus duration of oral symptoms in SS
Mean duration of oral symptoms before referral was 29 months for both SS- and non-SS patients (range: SS 0-156, non-SS 0-240 months). In SS patients, serum S-type isoamylase activity and duration of oral symptoms related inversely ($r_{\text{pearson}}$ -0.33, $p=0.05$). The SS patients with high S-type isoamylase activity (above normal range) had a much shorter duration of oral symptoms (mean 11 months, range 0-30), than the SS patients with normal S-type isoamylase activity (mean 35 months, range 0-156), whereas the SS patients with low S-type isoamylase activity (below mean minus SD) had a much longer duration of oral symptoms (mean 49 months, range 12-108) (figure 4.1.2). These observed differences proved statistically significant by a one-way analysis of variance (ANOVA).
Isoamylase activity versus other salivary gland diseases
Two-thirds of the non-SS patients were diagnosed with another condition affecting the salivary glands, including sialoadenosis, sodium-retention dysfunction syndrome or medication induced hyposalivation. Patients with the non-inflammatory salivary gland diseases sialoadenosis or sodium-retention dysfunction syndrome had a tendency to a higher mean S-type isoamylase activities in serum (84 and 81 U/L, respectively) than patients with medication induced hyposalivation or without another condition affecting the salivary glands (63 and 54 U/L, respectively). These differences, however, did not reach significance probably due to rather low numbers.

Relation of isoamylase activity with sialometry and sialochemistry in SS
The activity of S-type and total amylase in sera of SS patients correlated significantly with the sialochemical variables sodium and chloride (also after correction for salivary flow rates), which are related to inflammation of the salivary glands (table 4.1.4). Furthermore, the SS patients with S-type isoamylase activity above normal range had on average 30 to 50 percent higher salivary flow rates for all major salivary glands, compared to the SS patients with normal S-type isoamylase activity. So, there is a tendency of high S-type isoamylase activity in SS patients with (relatively) high salivary flow rates and high salivary sodium and chloride concentrations.

### Table 4.1.4. Relation between S-type and total amylase activity in serum and sialochemical variables in SS patients (n=37).

<table>
<thead>
<tr>
<th></th>
<th>S-type isoamylase</th>
<th>Total amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rspearman</td>
<td>P</td>
</tr>
<tr>
<td>Parotid saliva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0.46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SM/SL saliva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results from this prospective clinical study show that serum isoamylase-measurement has limited clinical value with regard to the diagnosis of Sjögren’s syndrome (SS). It does have, however, potential for monitoring disease progression.

Serum measurement of total amylase seems to lack sufficient specificity for detecting SS, as it includes both the salivary and pancreatic fractions of amylase. Measuring only salivary isoamylase results in better discrimination between SS- and non-SS patients. Since most recordings in SS-patients were still within reference
range, optimising the threshold of S-type isoamylase for the differentiation between SS and non-SS by analysing data with a ROC-plot was tried. However, the sensitivity (and the likelihood ratio) of the optimum threshold for SS remained too low to be of clinical value for diagnosing SS.

The observations that high serum S-type isoamylase activity in SS patients corresponded with a relatively short duration of oral symptoms (less than one year), and relatively high salivary flow rates (and high salivary sodium and chloride concentrations), confirm the assumption that (initial) salivary gland damage is reflected by high serum S-type isoamylase activity. In a progressed phase of oral manifestation the enzyme leakage in serum seems to extinguish, as low serum S-type isoamylase activity corresponded in SS patients to long duration of oral symptoms (on average four years) and decreased salivary flow rates. In a previous study it was demonstrated that sialometry and sialochemistry are useful to stage the oral manifestation of SS (§3.1). An early stage of SS was characterised by either normal (secretory) gland function with changed salivary composition or by selective dysfunction of the submandibular/sublingual (SM/SL) salivary glands, whereas a progressed stage was characterised by extreme dysfunction of the SM/SL glands or by extreme dysfunction of all major salivary glands. Such staging seems also possible with S-type isoamylase activity in serum. High activity may indicate an early stage and low activity a progressed stage.

The biphasic course, in terms of time, of serum S-type isoamylase in SS patients may well explain its low sensitivity for SS. Only the patients who recently developed oral complaints from SS seem likely to show increased serum activity of this salivary enzyme. The remaining SS patients may be beyond this initial phase of intracellular enzyme leakage and, therefore, cannot be recognised enzymatically anymore. Interestingly, leakage of salivary isoamylase in serum correlated significantly to the disturbance of the sialochemical variables sodium and chloride, which are characteristic for salivary gland inflammation in SS. As leakage into serum of intracellular enzymes is thought to result from increased cell death, it might be hypothesised that the amount of serum amylase leakage in SS corresponds with the inflammatory activity of the disease at the glandular level. Perhaps, salivary isoamylase in serum may even be informative regarding the prognosis of salivary gland function. High serum iso-amylase activity may indicate an active disease at the glandular level (thus relatively rapid deterioration of secretory functions), normal activity may indicate a more stable situation, whereas low activity an end situation with little change to be expected in secretory function.
In conclusion, measurement of S-type isoamylase in serum has limited diagnostic value for SS. Measuring S-type isoamylase activity may be very useful for assessing disease progression, rather than for diagnosing SS. A long-term prospective study is warranted in order to verify such considerations regarding outcome variables in SS.

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**REFERENCES**

classification suggested by the analysis of the receiver operating characteristic (ROC) curve of the criteria performance [abstract]. J Rheumatol 1997;24(Suppl 50):38.


