The possible meaning of transferrin and its soluble receptors in seminal plasma as markers of the seminiferous epithelium

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Transferrin (Tf) and soluble transferrin receptors (S-Tf-R) were measured by enzyme immunoassay in seminal plasma of 130 semen samples. The mean concentration of S-Tf-R in cases with normozoospermia was 10.4 IU/ml (95% confidence interval: 9.5-113) and it was significantly lower in patients with oligozoospermia (6.6, 95% CI: 5.8-7.5, P < 0.001), asthenozoospermia (8.5, 95% CI: 5.5-10.7, P < 0.05), azoospermia of primary testicular origin (7.9, 95% CI: 6.1-9.6, P < 0.05) and post-vasectomy samples (5.9, 95% CI: 5.4-6.9, P < 0.001). The concentration of S-Tf-R in post-vasectomy samples was lower than that in patients with azoospermia of primary testicular origin (P < 0.05; positive likelihood ratio = 7 at value of 83 IU/ml). S-Tf-R was positively correlated with motile sperm concentration (r = 0.50, P < 0.0001), percentage motility (r = 0.38, P < 0.001), percentage of normal forms (r = 0.43, P < 0.001), sperm linear velocity (r = 0.42, P < 0.001), and ATP concentration (r = 0.67, P < 0.0001). Follicle stimulating hormone (FSH) was found to be negatively correlated with the concentrations of both Tf (r = -0.31, P < 0.05) and of S-Tf-R (r = -0.45, P < 0.01). The mean concentration of Tf in seminal plasma was 50.4 µg/ml (35.9-67.2) in samples with normozoospermia (n = 22), and the concentration was significantly lower in patients with oligozoospermia (P < 0.05), azoospermia of testicular origin (P < 0.001), and post-vasectomy samples (P < 0.001). Seminal Tf was correlated with motile sperm concentration (r = 0.36, P < 0.001), percentage of motile spermatozoa (r = 0.25, P < 0.05), linear velocity (r = 0.24, P < 0.05) and ATP concentration (r = 0.44, P < 0.001). The concentration of Tf was positively correlated with that of S-Tf-R both in cases with spermatozoa present (r = 0.66, P < 0.001), and in cases with azoospermia of testicular origin (r = 0.51, P < 0.05) but not in vasectomy cases. It is concluded that S-Tf-R in seminal plasma is a marker of spermatogenesis and may give information on the presence or absence of spermatogenetic cells in cases with azoospermia. Further investigations are needed to assess its usefulness for clinical practice.

Key words: male infertility/semen analysis/soluble transferrin receptors/transferrin

Introduction

Rapidly dividing cells require iron for their growth and metabolism (Aisen and Listowsky, 1980), and the transferrin (Tf) receptor plays an essential role in cellular iron uptake across the cell membranes. In fact, the number of receptors reflects the potential for cell proliferation (Kohgo et al., 1985). Transferrin receptor is a transmembrane glycoprotein composed of two identical disulphide-linked subunits of 90 kDa each. The expression of transferrin receptor is inversely related with the iron concentration, both at the level of transcription and translation (Sylvester and Griswold, 1994). Pan and Johnstone (1983) reported externalization of the Tf receptor into culture medium from reticulocytes in vitro. A soluble form of the Tf receptors has also been isolated directly from rat and human plasma, and it has been suggested as a useful tool for assessing erythropoiesis (Chitambar et al., 1991) and as a sensitive index of iron status and total mass of tissue receptors (Cook et al., 1993).

As far as testicular cells are concerned, Holmes et al. (1983) have demonstrated that Sertoli cells, and early as well as mid-pachytene spermatocytes, contained Tf-binding sites. Vannelli et al. (1986) found Tf receptors to be present only in spermatocytes and early spermatids. Hence, Tf secreted by Sertoli cells may serve as a growth stimulant for pachytene spermatocytes by supplying iron for haem proteins or non-haem metalloproteins. However, no data are available on the levels of soluble Tf receptor (S-Tf-R) in seminal plasma.

Transferrin was found to be positively correlated with sperm concentration and motility (Ber et al., 1990). In addition, Orlando et al. (1985) and Barthelemy et al. (1988) found the level of Tf in azoospermic and severely oligozoospermic men to be low in comparison with those of fertile men. Seminal plasma Tf levels have been proposed as a functional parameter of Sertoli cells (Cek et al., 1992; Irisawa et al., 1993), though Chan et al. (1986) found Tf in seminal plasma not to be a useful marker for Sertoli cell or seminiferous tubular function.

The purpose of this study was to investigate the concentrations of S-Tf-R and Tf in seminal plasma, and their correlation with parameters of spermatogenesis, and blood hormones.

Materials and methods

Semen samples of 130 men attending the andrology outpatient clinic, and split ejaculate samples from four fertile semen donors were analysed. The samples were grouped into normozoospermia (n = 22), oligozoospermia (n = 26), asthenozoospermia (n = 17), azoospermia due to primary idiopathic testicular failure (n = 25), and post-vasectomy samples (n = 40) according to the criteria of WHO (Rowe

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Serum analysis

Hormone (FSH) and prolactin were measured by immunoradiometric assay kits (Medenrix, Fleurus, Belgium) and RIA-gnost Prolactin (Behring, Marburg, Germany) respectively. Serum testosterone was measured by radioimmunoassay using a kit from Medgenix (Fleurus, Belgium).

**Statistics**

The statistical analysis was performed using the MedCalc program (MedCalc Software, Mariakerke, Belgium) (Schoonjans et al., 1995). The significance of differences was assessed by Student’s t-test. Square root transformation was used to transform the skewness of the data into normal distribution whenever required. Correlation coefficients were calculated to detect the relation between two variables, when the distribution was normal, and multiple regression analysis with stepwise elimination was also performed with Tf or S-Tf-R as dependent variable. In addition, receiver operating characteristic (ROC) curve analysis was performed to estimate the power of S-Tf-R to discriminate between cases with azoospermia of primary testicular origin from those after vasectomy.

**Results**

Table III shows the mean and the 95% confidence interval of S-Tf-R and Tf concentrations in seminal plasma of the different groups. The concentration of S-Tf-R was significantly lower in samples with oligozoospermia (P < 0.001), asthenozoospermia (P < 0.05), azoospermia (P < 0.05), and post-vasectomy (P < 0.001) than with normozoospermia. The concentration of Tf was significantly lower in samples with oligozoospermia (P < 0.05), asthenozoospermia (P < 0.05), and post-vasectomy (P < 0.001) than in those with normozoospermia. In addition, we found the concentration of S-Tf-R in post-vasectomy samples to be significantly lower (P < 0.05) than in samples with azoospermia of primary testicular origin, but Tf concentration was not different between these two groups (P = 0.39).

ROC curve analysis indicates the S-Tf-R to have a moderate capacity to discriminate between cases with azoospermia of testicular origin or post-vasectomy, with area under the curve equal to 0.63, sensitivity of 68% and specificity of 60% at the selected criterion value of 6.3 IU/ml. When the S-Tf-R concentration is 8.3 IU/ml or more, the positive likelihood ratio of the sample to belong to a patient with primary testicular failure rather than vasectomy is 7, with specificity of 96% and sensitivity of 28%.

S-Tf-R and Tf concentrations in seminal plasma were positively correlated with motile sperm concentration, percentage of motile spermatozoa, percentage of normal forms, linear...
Table IV. Correlation of seminal plasma transferrin (Tf) and soluble transferrin (S-Tf-R) receptor concentrations with sperm characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TF (µg/ml)</th>
<th>S-Tf-R (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore sperm concentration (10^9/ml)</td>
<td>0.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Grade (A+B) motility (%)</td>
<td>0.25</td>
<td>0.38</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>0.20</td>
<td>0.43</td>
</tr>
<tr>
<td>Linear velocity (µm/s)</td>
<td>0.24</td>
<td>0.42</td>
</tr>
<tr>
<td>ATP (Hg/ml)</td>
<td>0.44</td>
<td>0.67</td>
</tr>
</tbody>
</table>

$r = \text{correlation coefficient.}$

Table V. Correlation of the total amount of seminal plasma transferrin (Tf) and soluble transferrin (S-Tf-R) receptors with blood hormones

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TF (µg/ejaculate)</th>
<th>S-Tf-R (IU/ejaculate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/ml)</td>
<td>-0.31</td>
<td>-0.45</td>
</tr>
<tr>
<td>LH (IU/ml)</td>
<td>-0.01</td>
<td>-0.16</td>
</tr>
<tr>
<td>Testosterone (ng%)</td>
<td>0.00</td>
<td>0.14</td>
</tr>
</tbody>
</table>

$r = \text{correlation coefficient.}$

Table VI. The level of soluble transferrin (S-Tf-R) receptor and transferrin (Tf) in the first and second fractions of split ejaculates (median, range) (n = 4)

<table>
<thead>
<tr>
<th></th>
<th>1st fraction</th>
<th>2nd fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Tf-R (IU/ml)</td>
<td>10.7 (9.1-15.9)</td>
<td>5.6 (1.2-7.0)</td>
</tr>
<tr>
<td>TF (µg/ml)</td>
<td>115.8 (71.9-196.9)</td>
<td>34.2 (19.3-44.0)</td>
</tr>
</tbody>
</table>

velocity, and ATP concentration (Table IV). Among semen samples with spermatozoa present, S-Tf-R and Tf concentrations were positively correlated (n = 65, r = 0.66, P < 0.001). The same occurred among samples with azoospermia due to testicular failure (n = 25, r = 0.51, P < 0.05), but there was no correlation between S-Tf-R and Tf in the post-vasectomy samples (n = 40, r = 0.25, P = 0.12).

The concentration of FSH in blood was negatively correlated with seminal plasma content of S-Tf-R and Tf per ejaculate; however, LH and testosterone were not correlated (Table V).

Table VI shows the concentrations of Tf and S-Tf-R in the two fractions of split ejaculates. In all four cases these concentrations were higher in the first than in the second fraction.

Multiple regression analysis indicated the coefficients of determination for semen Tf concentration and for S-Tf-R to be 0.81 and 0.76 respectively, when the following characteristics were used as independent variables: sperm concentration, progressive motility, linear velocity, ATP concentration, serum FSH, and S-Tf-R or Tf respectively. Stepwise elimination indicates that the Tf concentration mainly depends on progressive sperm motility, ATP content and S-Tf-R concentration with a coefficient of determination of 0.69. The determinants of S-Tf-R concentration are sperm concentration, progressive motility and Tf concentration with coefficient of determination equal to 0.66.

**Discussion**

Sertoli cells secrete many proteins that play a role in spermatogenesis. One of these proteins is transferrin, which is secreted in large amounts by the Sertoli cells, suggesting that iron transport may be an important function of these cells (Skinner and Griswold, 1982). On the other hand, Tf receptors have been found in spermatocytes and spermatids, and probably act in providing iron for cell proliferation and differentiation (Vannelli et al., 1986). A testicular iron shuttle has been proposed to play a role in Sertoli cells, moving iron through their cytoplasm, around the tight junctions, and eventually to the surface of the sequestered germ cells (Sylvester and Griswold, 1994). A soluble form of Tf receptor has been isolated from human plasma and this has been shown to be a cleavage product from Tf receptors of reticulocytes (Chitambar et al., 1991).

Our results showed that S-Tf-R is present in seminal plasma, and this can be explained by cleavage of this receptor from spermatocytes and spermatids. According to this hypothesis, the presence of S-Tf-R in seminal plasma may provide a useful guide to evaluate specific steps in spermatogenesis, namely the maturation of spermatocytes and spermatids into spermatozoa. Consistent with this hypothesis, we found S-Tf-R to be significantly lower in samples of patients with oligozoospermia, asthenozoospermia, azoospermia and post-vasectomy. The significantly lower concentration of S-Tf-R in samples after vasectomy, compared to those in patients with azoospermia of primary testicular origin, suggests this substance mainly to originate from spermatogentic cells. In addition, results obtained from split ejaculates, showing that the first fraction has the highest concentration of both Tf and S-Tf-R, suggest these substances to have a testicular origin.

On the other hand, Tf concentration in post-vasectomy samples is not different from that of patients with testicular azoospermia, and this may be due to additional contribution by the accessory sex glands (Holmes et al., 1982; Orlando et al., 1985).

The significant correlations of S-Tf-R and Tf with all sperm characteristics, but with the major determinants being progressive sperm motility, sperm concentration or ATP respectively, are compatible with the involvement of both substances in providing iron for the proliferation of germ cells. However, the coefficients of correlation with S-Tf-R are slightly higher than with Tf, which may suggest a closer relationship between S-Tf-R and spermatogenesis.

Our results show an inverse correlation between FSH on one hand, and Tf and S-Tf-R receptor on the other hand, suggesting that the secretions of Sertoli and germ cells regulate the secretion of inhibin which itself inhibits FSH secretion by the pituitary gland. It has been hypothesized that germ cells, in particular spermatids, control Sertoli cell function, probably via the transfer of germ cell materials (residual bodies) and germ cell soluble factors (Jégou, 1993). It is suggested that S-Tf-R may be one of the germ cell soluble factors involved in Sertoli cell control.

The findings of significantly decreased Tf levels in seminal plasma in cases of oligo- or azoospermia, and after vasectomy,
and the positive correlation between Tf and the concentrations of spermatozoa and ATP, correspond with those reported by Chan et al. (1986), Foresta et al. (1986), Cek et al. (1992), and Irisawa et al. (1993). Our results show no correlation between blood LH and testosterone on one hand and Tf on the other hand in agreement with Orlando et al. (1985), Chan et al. (1986) and Cek et al. (1992), but in contrast with Irisawa et al. (1993).

The relationship between Tf and S-Tf-R in seminal plasma, particularly in samples containing spermatozoa, suggests a combined role of the two factors in regulating spermatogenesis. The presence of such a correlation in cases of azoospermia due to testicular failure may permit the detection of a subgroup of azoospermic patients with spermatogenic arrest; however, this point needs further studies for clarification.

In conclusion, this study shows for the first time that S-Tf-R is present in seminal plasma. Assessment of this new variable may be helpful to study cases with azoospermia as it may identify men with primary testicular failure but spermatogenic activity present. Further investigations are needed to assess the relevance of this variable for the clinical management of couple infertility.

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