Significance of S100A4 expression in prostatic adenocarcinoma

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Abstract:

- **Introduction:** S100A4 is a marker that has been proposed to be implicated in tumorogenesis, invasion and metastasis of many malignant tumors. The aim of this study was to evaluate the expression of S100A4 in benign and premalignant prostatic lesions in addition to prostate adenocarcinoma and to compare these results with the clinicopathological data trying to assess the role of S100A4 in prostate adenocarcinoma.

- **Materials and methods:** This study was carried out on 34 cases of prostatic adenocarcinoma, 28 cases of PIN (12 cases were high grade and 16 were low grade PIN), in addition to 8 cases of benign prostatic hyperplasia used as control. The cases were collected during the period from 1997 to 2009. The patients had not received radiation, hormonal therapy, or chemotherapy before surgery. Representative sections were stained immunohistochemically with antibodies against S100A4, and assessed semiquantitatively. The Gleason score and other clinicopathological data including five years survival were recorded and the relationship between the expression of S100A4 protein and those clinicopathological parameters was studied.

**Results:**

A highly statistically significant positive correlation was found between Gleason grade of the carcinoma and the presence of high grade PIN and lymph node metastasis ($P < 0.01$). Also there is a statistically significant positive correlation between Gleason grade and presence of capsular invasion, perineural invasion and serum PSA level before resection of the tumor ($P < 0.05$). A statistically significant difference in the expression of S100A4 in different prostatic lesions was reported ($P < 0.05$). A highly significant correlation between S100A4 expression and grade of the carcinoma ($P < 0.01$). Also there is a significant correlation between S100A4 expression and presence of capsular invasion, L.N. metastasis, as well as serum PSA before resection of the tumor ($P < 0.05$).

- **Conclusion:** S100A4 might be involved in prostate development and progression. S100A4 may also play a role in tumor invasion and metastasis.

**Key words:** S100A4, prostatic adenocarcinoma, high grade PIN, immunohistochemistry (IHC).
Introduction

Worldwide, the prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% of the total new cancer cases and 6% of the total cancer deaths in males in 2008 [1].

In Egypt, according to National Cancer Institute, Cairo University, it accounts for 85% of the total malignancies being the fifth most common male cancer after urinary cancer, liver cancer, Non-Hodgkin lymphoma and lung cancer [2]. However according to [3], prostate cancer formed the majority of male genital cancer (60.7%) in Egypt in the last 10 years with median age 72.8 years. According to El Gharbia population based cancer registry, prostate cancer is uncommon. It represents 2% of all incident cancers, ranking seventh in males and the majority of cases (79%) present with advanced disease [4].

Complete understanding of the causes of prostate cancer remains elusive (Hasing et al., 2006). The primary risk factors are obesity, age and family history. It is very uncommon in men younger than 45 year, but become more common with the advancing age. The average age at time of diagnosis is 70 [5]. Men who have first degree family members with prostate cancer appear to have doubled the risk of getting the disease compared to men without prostate cancer in the family [6].

Being the most frequently diagnosed cancer in males, prostate cancer is a major health problem. Despite the fact that its mortality rate has been decreasing by about 4% per year since 1992, this cancer still kills 30,000 man annually in the US alone [7].

Prostate cancer presents in 2 distinct forms. One is a latent form and the second is an aggressive form. The disadvantages of the frequently used prostate-specific antigen (PSA) serum test are that this test detects indiscriminately both types of prostate cancer and that PSA can also be increased in nonmalignant prostatic diseases, such as benign prostatic hyperplasia and prostatitis leading to overdiagnosis (of latent forms) followed by overtreatment, with the associated side
effects. So, markers that can differentiate aggressive from non aggressive disease are urgently needed [8].

S100A4 proteins are involved in a variety of intracellular and extracellular functions including cell growth, cell to cell communication. Energy metabolism and intracellular signal transduction [9,10]. S100A4, a member of S100 protein family is overexpressed frequently in normal cells such as macrophages, neutrophils, and T lymphocytes [11]. It is also overexpressed in various cancer types such as those of the breast, ovary and colon and is mainly associated with the invasion and metastasis of malignant tumors [12, 13, 14].

**Aim of the work**

This study aimed to evaluate the expression of S100A4 in benign prostatic hyperplasia PIN, and prostatic adenocarcinoma and to compare it with the clinicopathological data trying to assess the role of S100A4 in prostate carcinoma.

**Materials and methods**

**Clinical investigations and tissue samples:** The present study was retrospective study based on formalin fixed paraffine embedded biopsy specimens from 70 Egyptians patients with different types of prostatic lesions. The cases included 8 cases of benign prostatic hyperplasia were taken as control, 28 cases of PIN and 34 cases were prostatic adenocarcinoma. Cases were collected in the period 1997-2009. They were selected from files of pathology department, Faculty of Medicine- Benha University. Cases were selected according the availability of clinical and follow-up data for 5 years. Only patients with primary prostatic adenocarcinoma who had not undergone previous irradiation or chemotherapeutic treatment were included in the study.

Grading was carried out according to the Gleason (1992) into well, moderately and poorly differentiated tumors. 13 cases were of low grade, 11 cases were intermediate grade with Gleason score 7, and 10 cases were high grade adenocarcinoma [15].

Formalin-fixed, paraffin-embedded gastric tissues were used. Three sections of 4 micron thickness were obtained from each case. One section was H & E stained
for diagnosis and grading. Two experienced pathologists blindly and independently confirmed the histological diagnosis of each gastric lesion and agreed on the grading. Other sections were mounted on positively-charged slides, immunohistochemically stained with antibodies against S100A4 using the Ultra Vision Detection System (Anti-polyvalent, HRP/DAB, ready-to-use, Lab Vision corporation).

**Immunohistochemical staining:** tissue sections mounted on positively-charged slides, were heated at 60°C for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. After antigen retrieval with microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. #: AP-9003), pH 6.0, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. Sections were washed 3 times with cold 0.01 M phosphate buffered saline (PBS). After blocking with 10% normal rabbit serum, sections were incubated with polyclonal antibody against pre-diluted ready to use S100A4 (Thermo Scientific, Fremont, CA 94538, USA). Both were incubated overnight at 4°C. The prepared DAB-substrate-chromogen solution was applied and incubated for 5-15 minutes until color intensity has been reached. Lastly, sections were counterstained with Mayer's Hematoxylin. A tissue section of tonsil was used as external positive control for S100A4. Negative controls were performed by omitting the primary antibody step.

**Interpretation and evaluation of S100A4 immunohistochemical staining:** sections were screened on low power. S100A4 was stained yellow-brown in the cytoplasm and nucleus. The degree of immunostaining based on the staining intensity and percentage of positive cells. The intensity grading scale was according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining yellow-brown) and 3 (strong staining, brown). The extent of staining was based on the percentage of positive tumor cells in the two most prominent Gleason grades: 0 (less than 5%), 1(6%-25%), 2(26%-50%) and 3 (51%-100%). The final score was assessed by multiplying the results of intensity and the extent of staining. The cases were considered negative if the final score was 0 or 1(9-) and positive if the score was 2 or 3(+), 4 or 5 (++), 6 or 7 (+++) or 8 or 9(++++) [16].

**Statistical analysis:** Statistical analysis was performed using the SPSS (version 16.0 for windows) software package according to Sperman's correlation coefficient. Correlation between several variables was computed using Fisher's exact test. P value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.
Results

A: Histopathological results:
The present study was carried upon 8 cases of prostatic hyperplasia, 28 cases of PIN (12 cases of them were of high grade and 16 cases were of low grade PIN). From prostatic adenocarcinoma cases 13 cases (38.2%) were of grade I, 11 cases (32.4%) were of grade II and 10 cases (29.4%) were of grade III. Out of 13 cases of Grade I (Gleason score 4-6) adenocarcinoma there were 3 cases (23.1%) showed capsular invasion by the tumor all of them were scored as 5 on Gleason grading system. Out of 11 cases of Grade II adenocarcinoma (Gleason score 7 (intermediate grade)) there were 3 cases (27%) with extracapsular extension of the tumor which were scored as 7 on Gleason grading system. However all the 10 cases (100%) of Grade III adenocarcinoma (Gleason score 8-10 (High grade)) were associated with capsular invasion by the tumor; four of them were scored 8 and six of them were scored as 9 on gleason scoring system.

Out of 11 cases of intermediate grade adenocarcinoma, 2 cases (18%) had lymph node metastasis and the all 10 cases of high grade adenocarcinoma (100%) had lymph node metastasis, however no cases of low grade adenocarcinoma showed lymph node metastasis. Six cases (46%) Out of 13 cases of low grade adenocarcinoma were associated with high grade PIN, 7 cases (64%) out of 11 cases of intermediate grade adenocarcinoma were associated with high grade PIN and 8 cases (80%) out of 10 cases of high grade adenocarcinoma were associated with high grade PIN.

All the 10 high grade cases (100%) showed perineural invasion, 10 cases (91%) out of the 11 cases of the intermediate grade adenocarcinoma showed perineural invasion and 8 cases (62%) out of the 13 cases of low grade adenocarcinoma showed perineural invasion.

Among the 11 cases of intermediate carcinoma, 5 cases (45%) had serum PSA before resection of the tumor below 20 ng/dl, another 5 cases (45%) had it between 21ng/dl & 40 ng/dl and only one case (9%) had the level 41-60 ng/dl. Out of the 10 high grade cases, 6 cases (60%) had 41-60 ng/dl PSA serum level and 4 cases (40%) had it >60 ng/dl. Table (1) showed the relationship between different clinicopathological variable of prostatic carcinoma cases studied.
**Table (1): Correlation between Gleason grade of the tumor and other clinicopathological parameters:**

<table>
<thead>
<tr>
<th>Clinicopathological variable</th>
<th>Total</th>
<th>Low grade</th>
<th>Intermediate grade</th>
<th>High grade</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capsular invasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>• Absent</td>
<td>18</td>
<td>10</td>
<td>8(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Present</td>
<td>16</td>
<td>3(%)</td>
<td>3(%)</td>
<td>10(%)</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>• Absent</td>
<td>22</td>
<td>13(09)</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Present</td>
<td>12</td>
<td>0</td>
<td>2(%)</td>
<td>10(%)</td>
<td></td>
</tr>
<tr>
<td><strong>High grade PIN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>• Absent</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>• Present</td>
<td>21</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Perineural invasion:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>• Negative</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>• Positive</td>
<td>28</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Serum PSA before resection of the tumor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>• 0-20ng/dl</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>• 21-40ng/dl</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>• 41-60ng/dl</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>• &gt;60ng/dl</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Stage of adenocarcinoma:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>• Stage I</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>• Stage II</td>
<td>19</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>• Stage III</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>5 years survival:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>• survived</td>
<td>25</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>• Did not survive</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**NB:** A highly statistically significant positive correlation was found between **Gleason grade** of the carcinoma and the presence of high grade PIN and lymph node metastasis (**P < 0.01**). Also there is a statistically significant positive correlation between **Gleason grade** and presence of capsular invasion, perineural invasion and serum PSA level before resection of the tumor (**P < 0.05**).
(B) Immunohistochemical results of S100A4 staining:

All of the 8 cases of benign prostatic hyperplasia (100%) showed scattered rare epithelial cells were S100A4 immunoreactive which were considered negative except for only 2 cases which showed low S100A4 positive expression score (+). Among the 16 cases of low grade PIN, 8 cases (50%) showed positive expression for S100A4 with different scores [6 cases (37.5%) showed + score, 2 cases (12.5%) showed ++ score] and the other 6 cases (50%) showed no expression of S100A4. Among 12 cases of high grade PIN, 9 cases (75%) showed positive expression of S100A4 with different scores [2 cases (16.7%) showed (+) score, 4 cases (33.4%) showed (++) score, 2 cases (16.6%) showed (+++) score, and one case (8.3%) showed (++++) score], while 33 cases (76.7%) of prostatic adenocarcinoma showed positive expression of S100A4 [8 cases were score +, 8 cases were score ++, 11 cases were ++++, and 6 cases were score ++++]. This difference was statistically significant (P<0.05).

In relation to the tumor grade, there was a statistically significant positive correlation between the expression of S100A4 and Gleason score of prostatic adenocarcinoma (P< 0.05). Among 13 cases of low grade adenocarcinoma, 12 cases (92%) showed positive expression of S100A4 with different scores [6 cases showed (+) score, 5 cases showed (++) score, and one case showed (+++) score], all the 11 cases of intermediate grade showed positive expression of S100A4 [2 cases showed (+) score, 3 cases showed (++) score, and 6 cases showed (+++) score]. All the 10 cases of high grade adenocarcinoma showed positive expression of S100A4 [4 cases showed (+++) score and 6 cases showed (++++) score of S100A4, cases showed high S100A4 expression. This relationship was statistically highly significant. (P<0.05).

- Concerning the state of lymph node, S100A4 was markedly expressed among cases with positive L.N. metastases. All of these cases (100%) showed high expression (6 cases were +++ score and 6 cases were ++++ score). In the 22 lymph node negative cases (8 cases showed + score, 8 cases showed ++ score and 5 cases showed +++ score, and one case did not express the marker. This relationship was statistically highly significant (P<0.01).
Among 17 cases with capsular invasion, 4 cases showed + score, 6 cases showed +++ score, 6 cases showed ++++ score and one case showed negative expression of S100A4. Among 18 cases without capsular invasion, 5 cases showed + score, 8 cases showed ++ score and 5 cases showed +++ score of S100A4. A statistically significant correlation was found between S100A4 expression and occurrence of capsular invasion (P < 0.05).

Regarding patient survival, the expression of S100A4 was not related to poor prognosis. There was a statistically insignificant relationship (P > 0.05) (Table 2).
Table (2): Correlation between S100A4 expression and clinicopathological data in examined cases:

<table>
<thead>
<tr>
<th>Clinicopathological variable</th>
<th>Total</th>
<th>S100A4 expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td><strong>Histopathological type:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>8</td>
<td>6(75%)</td>
<td>0</td>
</tr>
<tr>
<td>PIN:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Low grade</td>
<td>16</td>
<td>8(50%)</td>
<td>6(37.5%)</td>
</tr>
<tr>
<td>- High grade</td>
<td>12</td>
<td>3(25%)</td>
<td>2(16.6%)</td>
</tr>
<tr>
<td>Prostatic adenocarcinoma</td>
<td>34</td>
<td>1(2.9%)</td>
<td>8(23.6%)</td>
</tr>
<tr>
<td><strong>Gleason grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Low grade</td>
<td>13</td>
<td>1(8%)</td>
<td>6(46%)</td>
</tr>
<tr>
<td>- Intermediate</td>
<td>11</td>
<td>0</td>
<td>2(18%)</td>
</tr>
<tr>
<td>- High grade</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Capsular invasion</strong></td>
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<td></td>
<td></td>
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<tr>
<td>- Absent</td>
<td>18</td>
<td>0</td>
<td>5(28%)</td>
</tr>
<tr>
<td>- Present</td>
<td>16</td>
<td>1(6%)</td>
<td>3(19%)</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- Absent</td>
<td>22</td>
<td>1(8%)</td>
<td>8</td>
</tr>
<tr>
<td>- Present</td>
<td>12</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Serum PSA before resection of the tumor</strong></td>
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<td>- 0-20ng/dl</td>
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<tr>
<td>- 21-40ng/dl</td>
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<td>1</td>
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<tr>
<td>- 41-60ng/dl</td>
<td>8</td>
<td>0</td>
<td>2</td>
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<tr>
<td>- &gt;60ng/dl</td>
<td>4</td>
<td>0</td>
<td>0</td>
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<td><strong>Stage of adenocarcinoma:</strong></td>
<td></td>
<td></td>
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<tr>
<td>- Stage I</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>- Stage II</td>
<td>19</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>- Stage III</td>
<td>7</td>
<td>0</td>
<td>1</td>
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<tr>
<td><strong>Perineural invasion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Negative</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>- Positive</td>
<td>28</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>High grade PIN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Absent</td>
<td>13</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>- Present</td>
<td>21</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>5 years survival:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
- **survived**
  - 25
  - 9

- **Did not survive**
  - 0
  - 1

| 7 | 1 | 4 | 10 | 4 |

\[ P < 0.01 \]

\[ \text{NB:} \] A highly significant correlation between S100A4 expression and grade of the carcinoma \( (P < 0.01) \). Also there is a statistically significant positive correlation between S100A4 expression and histopathological type of prostatic lesion, presence of capsular invasion, L.N. metastasis, as well as serum PSA before resection of the tumor \( (P < 0.05) \).

**Figure (1):** Benign prostatic hyperplasia showing (-) score of S100A4 expression and prostatitis showing positive internal control (streptavidin-biotin x 100).
**Figure (2):** Low grade PIN showing (+) score of S100A4 expression with intensity score 1 x prevalence score 2 (streptavidin-biotin x 400).

**Figure (3):** High grade PIN showing (+++) score of S100A4 expression with intensity score 2 x prevalence score 3 (streptavidin-biotin x 400).
Figure (4): Grade I prostatic adenocarcinoma (Gleason score 6) showing (++) score of S100A4 expression with intensity score 2 x prevalence score 2 (streptavidin-biotin x 400).

Figure (4): Grade II prostatic adenocarcinoma (Gleason score 7) showing (+++) score of S100A4 expression with intensity score 2 x prevalence score 3 (streptavidin-biotin x 400).
Figure (5): Grade II prostatic adenocarcinoma (Gleason score 7) with (+++) score of S100A4 expression with intensity score 2 x prevalence score 3 showing perineural invasion (streptavidin-biotin x 400).

Figure (6): Grade III prostatic adenocarcinoma (Gleason score 8) showing (++++) score of S100A4 expression with intensity score 3 x prevalence score 3 (streptavidin-biotin x 400).
Figure (7): Grade III prostatic adenocarcinoma (Gleason score 9) showing (++++) score of S100A4 expression with intensity score 3 x prevalence score 3 (streptavidin-biotin x 400).

**Discussion**

Worldwide, prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males [17]. In addition to prostate specific antigen (PSA), other genes and proteins that may serve as biomarkers in prostate cancer have emerged, and some of these appear to play a direct role in the biology of the disease [18].

In the present study there was a statistically significant positive correlation between the Gleason grade of the tumor & the capsular invasion, the lymph node metastasis and perineural invasion (p value < 0.05). This result was in agreement with a study carried by Helpap et al., [19], who found a statistically significant positive correlation between the Gleason grade of the prostate adenocarcinoma & the capsular invasion. Ross et al., [20] found that prostate cancers with gleason grade ≤ 6 rarely metastasize to the lymph nodes.

Also, Lieberg et al., [21] found similar results suggesting that perineural invasion may predict occult, high-grade disease in otherwise low-risk patients.

The preoperative serum levels of PSA were higher in patients having tumors with higher gleason grades. Attard et al., [22] agreed with the current study regarding such correlation. In contrast to the current study, Hessels et al., [23]) found no significant correlation between the gleason grade and the preoperative serum level of PSA in prostate adenocarcinoma cases. The contrasting results may be due to the retrospective manner of the current study and differences in lab. kits used in measuring PSA level.

In the current work, a statistically significant positive correlation was found between the prostatic grade and and the presence of PIN which was in contrast to the study of Moussa et al., [24] who found no correlation between the Gleason grade of
the tumor and the presence of high grade PIN. These contrasting results may be due to the limited number of cases in the current study.

There were no statistical significant correlations between Gleason grade and prostatic carcinoma stage. This was in accordance with Hessels et al., [23].

The present work found no significant correlation between Gleason grade of prostate adenocarcinoma and 5 years survival of the patients. In contrast to this study carried out by Attard et al., [22], Epstein et al., [25] and Penney et al., [26] in which they found a significant positive correlation between the Gleason grade and the 5 years survival of prostate cancer. The contrasting findings between the current study and other studies may be referred to the different number of cases and follow up methods.

S100A4, a member of the S100 family of proteins, is involved in tumorigenesis and metastatic potential of multiple cancers as melanoma, pancreatic adenocarcinoma and osteosarcoma [27].

S100A4 was detected in the present study as cytoplasmic and occasionally nuclear brown granules with differences in the intensity and prevelance of expression in benign prostatic hyperplasia, PIN, and different Gleason grades of prostatic adenocarcinoma.

In benign prostatic hyperplasia (BPH), scattered rare glandular epithelial cells were S100A4 immunoreactive. These results were in agreement with Kwon et al., [28] and Shrawan et al., [29]. Also Yutaka et al., [30] observed that S100A4 expression was faint in the cytoplasm of the normal mucosa, but strong in the cytoplasm of lymphocyte and smooth muscle of gastric tissue specimens. Wang et al., [31] reported that S100A4 protein was detected in 12 of 92 (13.04%) human non tumorous gastric mucosa samples, and all samples expressed the protein at a low level.

In the current study, there was a statistically significant positive correlation between S100A4 expression and histopathological type of prostatic lesions examined, as the expression of S100A4 increased from benign prostatic hyperplasia to low grade PIN to high grade PIN to prostat carcinoma. Parallel to such results, Hermai et al., [32] reported that S100A8, S100A9 were up-regulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas, whereas benign tissue was negative or showed weak expression of the proteins. Also Andersen et al., [33] reported that positive S100A4 staining was not detected in the luminal epithelial cells of the ducts or lobules. In contrast Matsubara et al. [34] observed positive staining for S100A4 was in both the lymphocytes and fibroblasts used as internal controls in cases of lung denocarcinoma in which S100A4 positivity (> 30% tumor cells positive for S100A4) was found in only (20.2%) of lung adenocarcinomas although low levels of S100A4 expression were detected in (40.4%) cases.

In the present study there was a significant statistical positive correlation between Gleason score and S100A4 expression, as the score of expression increases with the progression from lower Gleason scores to higher scores (P value < 0.05).

These results were in agreement with results reported by Yong-Wook et al., [35] who noticed a progression in S100A4 immunoreactivity according to the pathologic grade (Gleason score). The same results were reported by Hifzur et al., [36] who found that S100A4 is not merely a metastatic protein but also an
oncoprotein that plays a critical role in the development of prostate cancer, as S100A4 showed progressively increasing expression in prostatic tissues with the advancement of the grade of prostate cancer. Cho et al., [37] found that S100A4 expression has been proved to be a highly significant and independent prognostic marker showing a significant correlation between over-expression of S100A4 and grade progression of colorectal cancer. This may indicate tumor cell aggressiveness.

Ran et al., [38] on his study on endometrium found that expression of S100A4 was expressed in the tumor cell cytoplasm of poorly differentiated tumors, but was not detected in normal endometrial glandular epithelium. Also, Hua et al., [39] showed a significant correlation between S100A4 overexpression and gastric carcinoma grade increase, indicating the significant role of S100A4 in tumor cell prognosis.

In contrast, Rud et al., [40] found in his work on non small cell lung cancer (NSCLC) that S100A4-positive tumors were smaller and more differentiated than S100A4 negative tumors. What might explain these unexpected results that S100A4 is associated with a non-aggressive phenotype in NSCLC, could be that in contrast to one of the most important biological functions contributing to S100A4-induced metastasis which is increased invasive capacity. However, induction of S100A4 has also been shown to decrease motility and invasiveness in some tumors such as in squamous cell carcinoma (Uozumi et al., [41]). Down-regulation of S100A4 in astrocytes was found to increase their migratory capacity in vitro (Fang et al., [42]).

Furthermore, certain lines of evidence suggest that S100A4 may have tumor suppressor functions in the lung. S100A4 knockout mice, that were otherwise phenotypically normal, were prone to spontaneous tumor development, and the most frequent tumor observed was carcinoma of the lung (El-Naaman et al., [43]).

Taken together, these results indicate that the biological function of S100A4 is cell type-dependent, and possibly, S100A4 may not play a pro-metastatic role in all tumor types. One might also speculate that S100A4 could inhibit tumor progression in the early stages of NSCLC development, while promoting metastasis at later disease stages (Massague., [44]).

In the present study there was a significant statistical correlation between S100A4 score and the presence of capsular invasion and/or lymph node metastasis as the score of S100A4 increase with the presence of capsular invasion and/or lymph node metastasis. However there was no significant statistical correlation between the score of S100A4 and the presence of perineural invasion. In an agreement with the current study, a study carried out by Yong-Wook et al., [35] who observed high expression of S100A4 in invasive prostate cancer. These results indicate that S100A4 protein may be associated with invasion, and metastasis of cancer prostate.

Also Liu et al., [45] also found that there is a significant association between high S100A4 expression and the presence of lymph node metastasis in his study on colorectal carcinoma.

The increase of the expression of S100A4 with cancer progression and metastasis could be explained by the ability of S100A4 to stimulate motility of endothelial cells, promoting tumor-induced angiogenesis and plasmin formation, and stimulating remodeling of the extracellular matrix via production of extracellular-matrix-degrading enzymes from endothelial cells, through transcriptional activation of collagenase 3 (MMP-13) mRNA Wang et al., [31], Semov et al., [46], an Saleem et al., [47].
In contrast to the current work Wang et al., [31], kim et al., [48], and Kwak et al., [49] in their studies on colorectal carcinoma (CRC) and Ai et al., (2008) on his work on pancreatic carcinoma didn't find any correlation between S100A4 expression and either aggressiveness or lymph node metastasis. These discrepancies could arise from subtle variations in the number of patients, observation period, stage distribution, tissue fixation methods, use of antibodies, and cutoff of positivity.

In the present study there was a significant statistical correlation between the score of S100A4 expression and the preoperative serum level of PSA as the score of S100A4 increase with higher levels of serum PSA. However There was no significant statistical correlation between the score of S100A4 expression and the tumor stage of examined malignant cases. On the contrary, Ai et al., [50] in his work on pancreatic cancers found that pancreatic cancer with a large size and high TNM stage had a higher S100A4 expression.

Kwon et al., [28] observed that the PSA level of patients was significantly different among groups according to clinical stage, but not significantly different among groups according to pathologic grade. A significant progressive increase in S100A4 expression was observed in cancer specimens according to clinical stage and the pathologic grade (Gleason score). Liu et al., [45] found that there was a significant association between high S100A4 expression and advanced TNM stage in his work on colorectal carcinoma. Also Ran et al., [38] in his work on endometrial carcinoma found an increase of S100A4 expression with the stage being significantly higher in stage III and IV tumors compared with stage I.

There was no significant statistical correlation between the score of S100A4 expression and the 5 years survival of the patients in the current work (p value >0.05).

However Kwon et al., [28] found that during about 5 years of follow-up, 10 of 67 (14.9%) patients had a biochemical relapse based on PSA levels. He found that the length of biochemical relapse-free survival was shorter for those with higher expression of S100A4.

Also Kang et al., [51] found that S100A4 protein expression is an independent prognostic marker correlated with low overall survival in patients with Dukes stage A and B colorectal cancer. There was no significant statistical correlation between the score of S100A4 expression and the presence of associated high grade PIN in the current study.

In conclusion, our study on prostatic lesions samples found that S100A4 may not only be a metastatic protein but also an oncoprotein that may play a critical role in the development and progression of tumors. As S100A4 showed progressively increasing expression in prostatic tissues with the advancement of prostate adenocarcinoma and is associated significantly with capsular invasion and lymph node metastases. S100A4 expression is higher in patients with higher serum PSA level. S100A4 may be a useful marker to predict development, progression, and metastasis of prostate adenocarcinoma. Thus it could be used as a therapeutic target for cancer.
References


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الملخص العربي

مقدمة:

بعد سرطان البروستاتا من أكثر السرطانات تشخيصه في العالم و من أكثر أسباب الموت بين الذكور و يمثل 14% من كل حالات السرطان و 6% من حالات الوفاة بالسرطان بين الذكور في عام 2008.
اما في مصر، وفقاً لآخر إحصائيات المعهد القومي للأورام، جامعة القاهرة، فانه يمثل 8.5% من كل حالات الأورام السرطانية، وان أكثر السرطانات شيوعاً بين الرجال، وفقاً لاحصائيات مركز القدوة للإحصائيات، فإن سرطان البروستاتا يعد شيوعاً ادى 2% من كل الأورام السرطانية ويشمل المركز الساعب من أكثر الأورام السرطانية شيوعاً بين الذكور. ووجد ان معظم حالات سرطان البروستاتا تكشف في مرحلة مبكرة من المرض.

تهدف هذه الدراسة إلى تقييم دور تمرير S100A4 سواء في حالات التضخم الحميد أو سرطان البروستاتا والتشخيص يمكنها إجراء تناول النتائج الإحصائية في أورام البروستاتا السرطانية. وقد تم تحليل النتائج الإحصائية.

استُمِلت هذه الدراسة - و التي تم بائب رجعية - على 42 حالة من امراض البروستاتا حديثها وخبيثها. ضمن 8 حالات يعانون من التضخم الحميد للبروستاتا وقد استخدموا لغرض المقارنة، 13 حالة تم تشخيصهم كسرطان البروستاتا من الدرجة الأولى، و11 حالة من الدرجة الثانية و10 حالات من الدرجة الثالثة.

و بفضل الميكروسكوب المماثل للتحديات بقي وجود علاقات إيجابية إحصائية بين درجة سرطان البروستاتا وكلاً من اختراق الورم للكبسولة، انتقال الورم للغشاء المبطن، ووجود درجة داخلية للدورة، اختراق الورم للغشاء المبطن بال Ayrıca عبارة ونتيجة المستضد الخاص بالبروستاتا في الدم قبل استئصال الورم. في حين تبين عدم وجود أي علاقات ذات أهمية إحصائية بين درجة سرطان البروستاتا ومرحلته أو نسبة نجاح المرضى في سرطانات البروستاتا في خلال 5 سنوات.

و قد تم صياغة كل الحالات بالدالة المناعية S100A4 وتم مقارنتها بالبيانات الأكليمينية والباليولوجية للمرضى. وظهرت تلك الصياغة المناعية على هيئة حبيبات بنية اللون صبغة سلبية في هولى الخلايا ونسبة اقل في النواة. وقد اعطت نتائجها في 3 نقاط: 0-1-(4-6-7)-8-9++) و من هذه الدراسة تستنتج:

- إن الدخول العالي من سرطان البروستاتا طفا لتصنيف جليبسون لها علاقة باختراق الورم للكبسولة وانتشاره المبطن بالالعصاب.
- إن هناك علاقة طردية بين وجود ورم عالي الدرجة داخلية للدورة السرطانية ونتيجة المستضد الخاص بالبروستاتا في دم المرضى قبل استئصال الورم السرطاني و بين درجة سرطان البروستاتا في تصنيف جليبسون.
- احتمالية تورط الدالة المناعية S100A4 في مرحلة التضخم السرطانية.
- احتمالية وجود دور للدالة المناعية S100A4 في انتشار البروستاتا و اختراقه للانتشار.
وأخيراً نوصي بإجراء مزيد من الدراسات على الدلالة المناعية S100A4 باستخدام طرق تجريبية مختلفة لمزيد من الإيضاح لما يلي:

- دور الدلالة S100A4 في تسرطن البروستاتا.
- دور الدلالة S100A4 في تطور وانتشار أورام البروستاتا السرطانية.
- قيمة الدلالة S100A4 الالزامية كما تحدد نسبه انتظام أو نجاة المرضى من السرطان في خلال 5 سنوات.

وما قد يساعد على تطوير استراتيجيات جديدة لعلاج سرطان البروستاتا.