**Original Research Article**

**The Role of PTEN Gene Deletion In Prostate Cancer In Relation To Proliferative Capability of Malignant Cells**

Nabeel Tawfeeq Kammuna*  Kaswr Mosa Al-Taraihi  Asaad Abdullhamza Al-janabi  
Al-Diwanya Teaching Hospital, Al-Diwanya, IRAQ

*E-mail:nabeelkamwhite@yahoo.com

Accepted 28 September, 2016

**Abstract**

PTEN gene is a tumor suppressor gene located in 10q23,3 that encode dual- specificity protein and lipid phosphatase with tensinhomologe. The PTEN protein signals adjust cell splitting up and express cells to enter normal cell death way. defeat of PTEN lead to over –foundation of AKt, which in circle , is related with unrestrained cell production. In this cross section study, we examined 50 paraffin-embedded blocks belongs to 50 patients with proved prostate cancer, All slides subjected to IHC KI67 Ab ( which is a parameter of proliferative activity of malignant cells), and to 1 molecular study of PTEN gene in all tissues by using CISH technique.  

The association between PTEN gene status examined by CISH technique with KI67 score performed by IHC technique , is highly significant, in that we get 71.43% (25/35) of no deletion cases had KI67 score ≤10% of cells, while in cases with PTEN gene deletion , we had 73.33% (11/15) of cases carry KI67 score >10% of cells.

**Key words**: PTEN- phosphatase and tension,Akt- Antigen Kinase Thymoma,CISH- chromogenic in situ hybridization, FISH- Flurescent in situ hybridization.

**Introduction**

Prostatic cancer is still one of the major health problems all over the world ,and is one of the leader causes of cancer deathin men. It forms a significant percentage of hidden malignancies existing with secondary metastasis. prostate cancers exhibit a uneven variety of clinical and behavioral forms, ranging from sluggish-mounting tumors of slight clinical consequence to extremely aggressive metastatic and deadly disease [1]. The PTEN gene, is a tumor suppressor gene located on chromosome
digoxigenin and can be
CISH probes are
microscopes used in FISH.
expensive and complicated fluorescence
microscopes rather than the more
laboratories because it uses bright
CISH is
, as perform in FISH technique. though,
attendance
CISH
immunohistochemistry
colored
Chromogenic
of tumor cells.
expression with the proliferative capacity
prognosis through linking its level of
enable us to predict
expression in
staining
of malignant
distinct
teachnique
blocks
Mostly, Ki
ambiguous
synthesis. the protein has an
in the polymerase ribosomal RNA
have
a
by a German
The Ki
metastatic types
frequent in prostate cancer,
angiogenesis
production
in turn
leads to over
of PI3K signaling
into
dissection
PTEN
prostate cancer
determine the aggressive behavior of
tumor cells.
by the genes and thoughtful the pathways
concerned in disease succession, its
forceful performance and treatment unwilling.
PTEN is thought to be deleted in variety
of tumors, particularly prostatic cancer, We
hope in this study to clarify the genetic
alteration of PTEN gene in patients with
prostatic cancer in relation to Ki-67 which
is a protein present in the nucleus and
linked with ribosomal RNA combination,
and the cell cycle progression (CCP) gain,
to assess the proliferative capability of
tumor cells.

Materials and Methods
Samples collection
In this retrospective study, we used 50
paraffin embedded prostate cancer sections
from the records of branch of pathology in
Al-Sader teaching hospital and the private
laboratories with reference guide to
the patients files from the Middle Euphrates
center for oncology. tissue samples were
taken from patients went to physicians to
be display a variety of prostate cancer
symptoms such as frequent and
urgenturination, difficulty in voiding and
hematuria, and their prostate will be
examined by one or more of these
following procedures: digital rectal
examination, PSA level assay, computed
tomography, and MRI. We collect 50
sample for those with total prostatectomy
and the trucut biopsies that proved as
prostate carcinoma, after we exclude all
the specimens that lacking the clinical date,
full investigations, or those with a
controversy about their diagnosis. All the
clinical data were collected from patient
file: age, ultrasonographic reports,
preoperative serum PSA.

Sectioning
Sectioning of paraffin embedded blocks
done by using the Leica rotary microtome
in department of pathology/Al-Kufa
Collage of Medicine, using extremely fine
steel blades, Paraffin sections are usually
cut at a thickness 5µm ensuring that only a
single layer of cells makes up the section. Sections are now “floated out” on the surface of warm water in a flotation bath to flatten them and then picked up onto microscope slides.

**Staining the slides**

Before staining, the slides put in an electric oven at 60°C to remove the paraffin from them, in about 30 minutes. The usual stain Hematoxylin and Eosin (H&E) is used to provide essential structural information about the specimens. After staining, the sections are covered with a glass coverslip. Interpretation of slides done by authors to grading them according to last updated Gleason score.

**Immunohistochemistry**

Detection system: DakoEnVision+ system-HRP(AEC) code K4004

**Staining Procedure**

1. **relate Peroxidase** building block, sufficient diluted primary antibody or negative control reagent to coatblock, put adequate peroxidase connected Polymer, put adequate of the ready-to-use AEC+ substrate-chromogen, then put hematoxylin counterstain; then slides mounted and cover-slipped with an aqueous-based rising medium such as Glycerol Mounting Medium.

**KI-67 Antibody Kit**

Dilution: the dilution range of 1:100 when useful on paraffin sections. By take 20 minutes heating epitope recovery in Target Retrieval Solution, Low pH, and incubate at 20 min. with the primary antibody [9].

**Evaluation of Staining Results**

In evaluation of staining results for ki-67 expression we estimate the proportion of Ki-67-positive malignant cells and divide the number by the total number of malignant cells within one HPF, and were categorised into four degrees: grade 0 (<1% of malignant cells), grade 1 (1-10% of malignant cells), grade 2 (11-30% of malignant cells), grade 3 (>30% of malignant cells) [10]. For statistical analysis, the KI67 score categorized into dichotomous variable (≤10%, >10%) as used by Cuzick J et al in 2012 [13].

**The Zytodot2C CISH Implantation Kit Protocol**

(ZytoDot 2C SPEC PTEN/CEN 10 Probe, code-3053) Molecular study of PTEN gene in which, Dual-color CISH was carried out on tissue sections, to map the PTEN gene on chromosome 10q23.3 region, the 5 μm tissue sections were deparaffinized, proteolyses by pepsin solution, put in Pretreatment Solution EDTA, Heat in a covered staining jar standing in a boiling water bath to 37°C, 50°C, then to at 95°C. Dehydration: in 70%, 90%, and 100% ethanol, each for 1 min.

Air dry sections, denature the slides at 50°C for 5 min. then 78-80°C for 5 min., e.g. on a hot plate. Transfer the slides to a humidity chamber and hybridize overnight at 37°C (e.g. in a hybridization oven), remove the cover slips, immerse slides in 1x Wash Buffer TBS (prepared using WB5) and drain off or blot off the 1x Wash Buffer TBS. Apply Anti-DIG/DNP-Mix (AB14) dropwise (3-4 drops per slide) to the slides and incubate for 20 min. at 37°C in a humidity chamber. Then in the same manner apply HRP/AP-Polymer-Mix, AP-Red Solution, Apply HRP-Green Solution, counterstain and dehydrate the slides (modified from ZytoVision and ZytoDot of ZytoVision GmbH catalogue 2015).

Then we counted chromogenic signals in 100 non-overlapping interphase nuclei for each sample, so PTEN gene deletion was defined in tumor nuclei that contain one or no 10q23.3 locus signal.

**Interpretation of CISH Results**

The PTEN probe is labeled with digoxigenin (DIG), that results in permanent dark-green signals, the probe also labeled with dinitrophenyl (DNP) that results in permanent bright-red signals by using AP-Red solution. By this procedure we can detect 2 green and 2 red distinct dot-shaped signals with rounded edges in each nucleus in normal diploid cells, but in mitotic cells additional signals may be visible (ZytoDot 2C CISH Implementation Kit catalogue 2015).
Total lake of probe spots (green spots) in ≥60% of malignant nuclei of the tissue specimens, considered as homozygous deletion of PTEN gene, with presence of one or two green signals in the remaining nuclei, while absence of one probe signal (green signal) in ≥60% of tumor nuclei of tissue spot, was defined as heterozygous deletion of PTEN gene. [10]

**Results**

The mean age of patients participating in the current study was 68.18±8.72 years and their ages ranged from 48–87 years. The division of patients according to 10 years intervals was outlined in Table 1.

**Table 1:** Distribution of patients according to 10 years age intervals

<table>
<thead>
<tr>
<th>Age interval</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 years</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>50-59 years</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>60-69 years</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>70-79 years</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>80-87 years</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The mean of total prostatic specific antigen (PSA) serum level of the study population (preoperative measure) was 31.57±39.45 ng/ml and a median of 31.57 ng/ml. The range of PSA was (0.9 up to >100 ng/ml). The patients were classified into four groups according to the serum level of PSA as follows (Table-2).

**Table 2:** Classification of patients according to serum PSA level

<table>
<thead>
<tr>
<th>PSA</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4 nmol/ml</td>
<td>12</td>
<td>24.00</td>
</tr>
<tr>
<td>&gt;4-≤10 nmol/ml</td>
<td>14</td>
<td>28.00</td>
</tr>
<tr>
<td>&gt;10-≤20 nmol/ml</td>
<td>4</td>
<td>8.00</td>
</tr>
<tr>
<td>&gt;20 nmol/ml</td>
<td>20</td>
<td>40.00</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

According to Gleason score, patients were categorized into: 22 patients (44%) with <7 score, 8 patients (16%) with score of 7 and the rest of patients (40%) with score of >7 (table 3). Median Gleason score was 7 and the range was 3-9.
Table 3: Classification of patients according to Gleason score

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7</td>
<td>22</td>
<td>44.00</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>16.00</td>
</tr>
<tr>
<td>&gt;7</td>
<td>20</td>
<td>40.00</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.00</td>
</tr>
</tbody>
</table>

According to Ki67 score, 29 patients (58%) had score ≤10% of tumor cells, while 21 patients (42%) had score > 10% of tumor cells. ≤10% score included score 0 and score 1 and involved 16 patients (32%) and 13 patients (26%), respectively, whereas >10% score included score 2 and 3 and involved 14 patients (28%) and 7 patients (14%), respectively (Table 4).

Table 4: Distribution of patients according to ki67 score

<table>
<thead>
<tr>
<th>ki67 score</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10 % score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16</td>
<td>32.00</td>
<td>29</td>
<td>58.00</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>26.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 % score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>28.00</td>
<td>21</td>
<td>42.00</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>14.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.00</td>
<td>50</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 1: prostate cancer –KI67 score 0. (X400)
According to CISH method, patients were found to have the following: deletion was present in 15 patients (30%): 4 of them showed homozygous genotype (8%) and 11 showed heterozygous genotype (22%). The rest of patients (70%) had no gene deletion (figure 5).
**Figure 5**: Pie chart showing the results of CISH procedure.

**Figure 6**: Prostate cancer - CISH image show no PTEN deletion (40x400) oil immersion lens.

**Figure 7**: Prostate cancer-CISH image show heterozygous deletion of PTEN gene (480x361) oil immersion lens.
**Figure 8:** Prostatic cancer. CISH image show homozygous deletion of PTEN 9565x306) oil immersion lens.

The association between Gleason score and ki67 score is shown in table 5. Chi-square test was not valid as more than 20% of the cells possessed an expected count of less than 5.

**Table 5:** The association between Gleason score and ki67 score

<table>
<thead>
<tr>
<th></th>
<th>&lt;7</th>
<th></th>
<th>&gt;7</th>
<th></th>
<th>Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ki67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>36.36</td>
<td>2</td>
<td>25.00</td>
<td>6</td>
<td>30.00</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>31.82</td>
<td>2</td>
<td>25.00</td>
<td>4</td>
<td>20.00</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>18.18</td>
<td>3</td>
<td>37.50</td>
<td>7</td>
<td>35.00</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>13.64</td>
<td>1</td>
<td>12.50</td>
<td>3</td>
<td>15.00</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>100.00</td>
<td>8</td>
<td>100.00</td>
<td>20</td>
<td>100.00</td>
<td>50</td>
</tr>
</tbody>
</table>

Chi-square test is not valid because more than 20% of the cells had expected counts of less than 5.

**Association between PTEN gene status in CISH with ki67 score**

The association between CISH status of PTEN gene and ki67 score is shown in tables 6 and 7 and figure 9. It was proved to be highly significant (P<0.01).

**Table 6:** Association between CISH status and ki67 score

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th></th>
<th>1</th>
<th></th>
<th>2</th>
<th></th>
<th>3</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CISH</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>No deletion</td>
<td>13</td>
<td>37.14</td>
<td>12</td>
<td>34.29</td>
<td>6</td>
<td>17.14</td>
<td>4</td>
<td>11.43</td>
<td>35</td>
<td>100.00</td>
</tr>
<tr>
<td>Heterozygous deletion</td>
<td>3</td>
<td>27.27</td>
<td>1</td>
<td>9.09</td>
<td>6</td>
<td>54.55</td>
<td>1</td>
<td>9.09</td>
<td>11</td>
<td>100.00</td>
</tr>
<tr>
<td>Homozygous deletion</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td>2</td>
<td>50.00</td>
<td>2</td>
<td>50.00</td>
<td>4</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>32.00</td>
<td>13</td>
<td>26.00</td>
<td>14</td>
<td>28.00</td>
<td>7</td>
<td>14.00</td>
<td>50</td>
<td>100.00</td>
</tr>
</tbody>
</table>
**Table 7**: Association between CISH status and Ki67 score after rearrangement

<table>
<thead>
<tr>
<th>Ki67&gt;10%</th>
<th>≤10%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CISH</strong></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Deletion</td>
<td>11</td>
<td>73.33</td>
</tr>
<tr>
<td>No deletion</td>
<td>10</td>
<td>28.57</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>42.00</td>
</tr>
</tbody>
</table>

**Figure 9**: Association between CISH status and Ki67 score after rearrangement. (Ki67 negative= Ki67 ≤10%, Ki67 positive= Ki67 > 10%)

Ki67 has 73.3% sensitivity and 71.4% specificity to CISH status. The Odd Ratio is 6.8, 95% C.I = 1.76-26.76, Z statistic= 2.78, significant level P= 0.0054

**Discussion**

The age distribution of our patients reflects the association of prostate cancer with old age patients as proved by Maria Svensson et al [14], who concluded that the tumor become more common with advancing age and had the average age of his patients 70 years. Were 40% of our patients are in 70-79 years intervals, and in seldom we find patient under 50 years (1 patient).

In this study, there is high range of PSA level (0.9 - >100 ng/ml) and there is high percentage of patients 40% (20/50) have PSA serum level > 20 ng/mL. this display the relative association between PSA level and prostate cancer, so the benefit of applying the screening program of measuring PSA serum level annually to every man aged more than 50 years is highly significant to be depended in our country as it was agreed by FDA in U.S. [14].

In immunohistochemical study of Ki67 marker to all slides, we admix score 0+1 in one category (≤10% of tumor cells), and admix score 2+3 in other category (>10% of tumor cells), as it performed by Kazuhiro Tabata et al [10] and Cuzick et al [13] to get more advantageous analysis. So the proliferative capacity of 29 cases (58%) is low compared to high proliferative capacity of 21 cases (42%), that’s reflects the relative high percent of production in prostate malignant cells. So the proliferative capacity of 29 cases (58%) is low compared to high proliferative capacity of 21 cases (42%), that’s reflects the relative high percent of proliferation in prostate cancer cells.

In molecular study of PTEN gene status we used CISH technique as an alternative procedure to FISH, first because CISH is
much more practical in diagnostic laboratories since it uses bright-field microscopes rather than the more expensive and complicated fluorescence microscopes used in FISH\cite{13}. Second reason, is that we can get a permanent images to the slides, permits to further evaluation of them by authors.

Only a few studies have analyzed the frequency of PTEN deletion using FICH or CISH techniques, which are regarded as the gold standard for determination of gene copy numbers in tissue samples, or they used

In the previous studies PTEN deletion were reported from 17%-68% [12], Maria Svensson et al in 2015(14), they identified PTEN gene deletion in 37.4% (68/182) of cases by CISH technique, heterozygous PTEN gene deletion was seen in 19.2% (35/182), and homozygous deletion was seen in 18.1% (33/182) of cases, these results in general be in agreement with our results.

**Association between PTEN gene status in CISH with KI67**

The association between PTEN gene status examined by CISH technique with KI67 score performed by immunohistochemical technique, is highly significant in statistical analysis (P= 0.003), in that we get 71.43% (25/35) of no deletion cases had KI67 score ≤10% of cells, while in cases with PTEN gene deletion, we had 73.33% (11/15) of cases carry KI67 score >10% of cells. In addition we found that 100% of the homozygous PTEN gene deletion cases had KI67 score >10% of cells, while 63.63% of the heterozygous PTEN gene deletion cases had KI67 score >10% of cells.

So the sensitivity of KI67 scoring test to detect PTEN gene status in prostate cancer is 73.33%, with 71.43 % specificity. But this test had 100% sensitivity to detect homozygous deletion of PTEN gene. The Odd Ratio is 6.8, 95% C.I = 1.76- 26.76, Z statistic= 2.78, significant level P= 0.0054. Krohnnet al [12] found PTEN gene status and KI67 score data was significantly higher in PTEN deleted cases than in undeleted cancers (P=0.03).

**Conclusion**

The KI67 score in IHC can detect in sensitive way the genomic state of PTEN gene, so we expect to have gene deletion in advanced KI67 score.

**References**

711

Kammuna et al.  


