Evaluation of correlation between expression of P53 and Malondialdehyde levels in prostate cancer patients

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Abstract
The analytical study was conducted at the National University of Sciences and Technology, Islamabad, Pakistan from Nov 2012 to Nov 2013 to find out, correlate and assess negative correlation of serum malondialdehyde (MDA) with expression of p53 gene, and comprised 32 samples. Expression of p53 and MDA levels were determined by real time quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) technique respectively. Mean value of MDA in prostate carcinoma (CaP) and control group were compared, and the difference was statistically significant (p=0.002). Mean cycle threshold (CT) value of CaP was compared with control group, and the difference was statistically significant (p<0.05).

Introduction
The p53 transcription factor is encoded by TP53 gene, located on chromosome 17q13. The p53 gene and its encoded protein play a central role in regulating cell cycle progression, deoxyribonucleic acid (DNA) repair, cellular growth and apoptosis.1

Cancer is a major public health problem in many parts of the world, including the United States of America (US). Currently, one of the two men in US will develop cancer in his life.2 The growing prevalence of cancer survivors was estimated to be over 28 million worldwide.3 Prostate cancer (CaP) is the second most common cancer in males in the US).4 CaP is also the second leading cause of cancer deaths in men in US5 with estimated 192,000 new cases and 27,000 deaths in 2009.1 It accounts for approximately one-third of cases reporting for prostate diseases.4

Metastatic CaP, by progressing to castration-resistant prostate cancer (CRPC), represents a major threat to life of American men, resulting in estimated 577,190 deaths from the disease.2 According to a study, it is the most common malignancy among American men.6 Estimated numbers of US cancer survivors by site in January 1, 2012, were 43%, while it will become 45% by January 1, 2022.2 CaP is the most commonly diagnosed solid malignancy and the second commonest cause of cancer-related deaths in men in developed countries.7 In the United Kingdom (UK), the incidence is likely to double over the next 20 years.7 CaP affects African men more than Caucasian men.8 CaP has highest incidence among urological tumour in Brazilian population.9 CaP remains the commonest non-dermatological and the second leading cause of cancer death in Western men due to its high prevalence and metastatic rate.10 In different geographical regions, morbidity and mortality rates of CaP are remarkably different in Asia.11 During 1998-2002, the age-standardised incidence rate in Karachi, Pakistan, was 10.1 per 100,000 men.12 Old age, especially more than 55 years, had almost 17-fold higher risk of developing CaP compared to age less than 55 years.11

Many studies have proven that cancer is a genetically-linked disease, which is characterised by mutation in several cancer-related genes. These include tumour suppressor genes and oncogenes.13 CaP is an important worldwide health problem. CaP is a major age-related malignancy, which is most prevalent in the 54-74 years age bracket.14 Its main cause is genetic factor. Although advanced metastatic CaP commonly metastasises to regional lymph nodes and vertebral bones and metastasis to peritoneum leading to malignant ascites is rare.8

TP53 gene was altered in CaP. The p53 pathway is important in CaP development and progression.5 Abnormal p53 protein expression is associated with a worse prognosis after radiation therapy (RT) and androgen suppression therapy (AST).15

Increased prostate specific antigen (PSA) levels are often seen in CaP but it is also reported that there is increase in PSA in benign inflammatory disorder of prostate.16

The p53 is an important biomarker as it helps monitor CaP
conversion into castration-resistant prostate cancer (CRPC). In fact, p53 controls the androgen receptor (AR) gene, which restrains transition of CaP to CRPC. The tumour suppressor p53 gene was found to be at the hub of significant biological pathways.

Serum malondialdehyde (MDA) emphasises a convenient in vivo index of lipid peroxidation and a non-invasive biomarker of oxidative stress. A study indicated that MDA may be used for prognostic assessment of localised CaP.

There are many studies on CaP patients in Pakistan. Still, there is no local study showing correlation between p53 and MDA levels. The current study was planned to fill that gap.

Material and Methods

The analytical study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, Pakistan, and at the Centre for Research in Experimental and Applied Medicine (CREAM) for a period of one year after it was approved by the institutional ethics committee. It comprised male subjects aged 55-85 years who furnished informed consent. They were divided into patient and control groups. Prostate biopsy by transurethral resection of prostate (TURP) as well as 3ml blood samples were collected from CaP and control groups. CaP group was divided into four subgroups according to Gleason scoring. History was taken and documented at the Armed Forces Institute of Urology, Combined Military Hospital (CMH), Rawalpindi.

Tissue ribonucleic acid (RNA) was extracted by using the Genetix RNA purification kit (Ambion, USA). The total RNA isolated was then subjected to first stand complementary deoxyribonucleotide (cDNA) synthesis using RevertAid Premium First Strand cDNA Synthesis Kit (Fermentas, Germany). The first stand cDNA synthesis reaction product was directly used for polymerase chain reaction (PCR).

The cDNA synthesised from RNA of patients and controls was used for PCR amplification of p53 on real-time (RT) PCR (Smartercycler). RT-PCR was performed in 25µl tubes (Axygen, USA) containing 25 µl total reaction mixture. The reaction was prepared by adding SYBR® Green qPCR SuperMix Universal 12.5 µl, Forward primer 0.3 µl 5’GCCGACAGAGGAAGAGATC3’, Reverse primer 0.3 µl 5’CAAGGCCTCATTCAGCTTC3’, cDNA 6 µl, Taq Polymerase 0.5 µl, Nuclease free water 5.4 µl. The reaction mixture was vortexed and centrifuged for a few seconds with thorough mixing.

Thermal cycling conditions was included 5 minutes at 95°C for template DNA denaturation followed by 35 cycles of amplification, each consisting of 3 steps: 30 seconds at 94°C for DNA denaturation into single strands; 40 seconds at 55.6°C for primers to hybridise or "anneal", and one minute at 72°C for extension and final 10 minutes at 72°C. PCR was carried out in Smartcycler (Cepheid, Germany). Five standards were run in triplicate tubes in Smartcycler for RT-PCR.

Levels of serum MDA were determined by commercially available competitive inhibition enzyme immunoassay kit (CUSBIO BIOTECH CO., China). Samples were run in batches and test was performed according to instructions of the manufacturer.

Mean ± standard deviation (SD) was compared between the groups by independent t test. Non-parametric data was analysed by Mann-Whitney U test. Negative correlation was determined between the expression of p53 and MDA levels by using Spearman’s correlation. RT-PCR in CaP group was analysed compared to controls by delta-delta cycle threshold (ΔΔCt) method. The efficiency of our experiments of p53 expression was tested through real-time quantitative polymerase chain reaction (qPCR). The efficiency was 1.06. SPSS 20 was used for data analysis.

Results

Of the 32 subjects, 16 (50%) were CaP patients and 16 (50.0%) were controls. Overall mean age of the subjects was 71.69±6.04 years (range: 55-85 years). Mean PSA was 106.02±74.79 ng/ml (range: 7-187 ng/ml).

In terms of Gleason scoring, there were 3 (18.8%) patients in grade 6CaP group, 5 (31.2%) in grade 7, 5 (31.2%) in grade 6 CaP group, 5 (31.2%) in grade 7, and 3 (18.8%) in grade 9 CaP group.

Meanvalue of MDA in CaP patients was

Table-1: Significance of malondialdehyde (MDA) in different prostatic carcinoma (CaP) patients.

<table>
<thead>
<tr>
<th>Cancer groups</th>
<th>Mean ± SD (µg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaP</td>
<td>19.27 ± 18.75</td>
<td>0.035*</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.72 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Gleason score 6</td>
<td>10.39 ± 3.96</td>
<td>0.052</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.72 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Gleason score 7</td>
<td>27.30 ± 27.58</td>
<td>0.098</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.72 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Gleason score 8</td>
<td>13.42 ± 9.75</td>
<td>0.044*</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.72 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Gleason score 9</td>
<td>20.43 ± 21.06</td>
<td>0.246</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.72 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant difference
SD: Standard deviation.
19.27±18.75 µg/ml and in the controls it was 0.72±0.08 µg/ml (p=0.035). Mean MDA value of each Gleason scoring was established (Table-1). Only the comparison of Gleason scoring 8 of CaP was statistically significant (p=0.04). Rest were statistically non-significant (p>0.05) compared to the controls. Mean p53 expression in CaP patients was 19.17 ± 2.6 and in the control group it was 14.36 ± 2.01 (p<0.05).

Mean cycle threshold (CT) values of expression of p53 (Figure 1) and real-time qPCR CT value of data were noted (Figure-2). CaP group had 0.18-fold decreased expression of p53 compared to controls.

Expression of p53 and levels of MDA had a weak negative relation (p>0.05). Outcome illustrated that MDA and expression of p53 had an inverse relationship (Table-2).

**Discussion**

Tumour cells usually have an imbalance redox status resulting in damage to DNA, protein and lipids as well. In our findings, MDA levels were significantly high in Pakistani population of CaP compared to normal controls. In Israeli population the results were quite different, localised CaP had no statistically significant rise in MDA levels. Only the metastatic disease of CaP had statistically significant high value in MDA levels. In 2011, Brazilian population also showed the same results that MDA levels was not statistically significant with normal control. In 2006, significant increase in MDA was seen in Turkish population. A similar pattern was also found in both Turkish and Macedonian population in 2009. Another study in Turkish population also showed that the levels of MDA were significantly higher in plasma in CaP. Another study illustrated that blood MDA levels were statistically significantly high in localised prostate cancer compared to normal controls.

![](image)

**Figure-1:** Mean cycle threshold (CT) value of Control and prostate cancer (CaP) group (p-value < 0.05).

**Figure-2:** Cycle threshold (CT) values of prostate cancer (CaP) patients by real time quantitative polymerase chain reaction (qPCR). Fluorescence is taken on Y-axis and cycles at X-axis. The background is set 15 fluorescence. CT values are reported where primary curve is crossing the threshold.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>p-value</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde</td>
<td>19.27</td>
<td>4.84</td>
<td>0.621</td>
<td>-0.14</td>
</tr>
<tr>
<td>Expression of p53</td>
<td>19.17</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p value is correlation coefficient or Spearman (p).
Evaluation of correlation between expression of p53 and Malondialdehyde levels in prostate cancer patients

Our results showed that MDA level was significantly high yet it is not significant if we compare the Gleason scoring with the normal control. Another study illustrated that although blood MDA levels were significantly high yet it was not the same in tissue with Gleason sum in CaP patients. Significantly high level of MDA was observed in Indian population. According to a study in Indian population, MDA levels were significantly higher compared to the controls.

MDA may be used as a bio-marker for CaP in Pakistani population. MDA is not a useful biomarker in different Gleason scores of prostate cancer except Gleason score 8. In 2012, it was found that MDA was statistically increased with progression of CaP and Gleason score. Further studies should be planned on levels of MDA in different Gleason scores of CaP with larger population groups.

The p53 gene was involved in numerous pathways, which were interlinked for progression of CaP. Many studies have revealed that p53 is involved in different cancers. Many biological pathways were affected by alteration of p53 gene. In recent years, p53 was also found to be at the hub of significantly predicting biological pathways. A similar study was done in 2012, according to which, functional status of tumour suppressor p53 was important in progression of CaP. It was detected that p53 was genetically altered in histological localised organ-confined CaP as well as non-organ-confined CaP and distant metastasis.

Our results suggest that expression of p53 was significantly decreased in CaP. According to the outcomes of our experiments, p53 may be used as biological marker for CaP. Similar results were also observed in an earlier study which showed that a positive expression of p53 messenger ribonucleic acid (mRNA) is involved in CaP.

Our study focussed on correlation of expression of p53 and MDA in CaP group. Results obtained appeared statistically non-significant, but it has a weak inverse correlation between expression of p53 and MDA in CaP. It was our first step to find the actual role of expression of p53 in CaP. More research work should be undertaken to elucidate the actual mechanism involved in CaP.

Conclusion
MDA may be utilised as a biological marker of CaP in future. It is also appears that MDA may have some role in the progression of disease. There was a significant relationship between MDA and Gleason score 8.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: National University of Sciences and Technology, Islamabad.

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