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(RESEARCH ARTICLE)

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HLA-DR association with prostate cancer in southwestern Nigeria: A preliminary experience using tissue microarray analysis

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Abstract

Background: Prostate cancer is a disease of gargantuan proportion worldwide. Current therapeutic modalities do not guarantee long survival or cure. Immune-based therapy may be a key solution to the treatment and cure of the disease, despite the limited gain recorded presently. This study aims to determine the association of immune marker HLA-DR with prostate cancer and the usefulness of tissue microarray analysis in small biopsies.

Methods: Forty and eight formalin-fixed, paraffin-embedded prostate needle biopsies were processed by tissue microarray analysis and stained with anti HLA-DR monoclonal antibody. The age, Gleason score and Gleason grade groups were noted. Semi-quantitative IHC scoring was done and results analyzed using SPSS version 25.

Results: The age range was 54-to 89 years (mean = 68.8; median = 68.0; SD = 7.7). Majority of cases fell into the 60-79 years age group. There was moderate and strong staining with HLA-DR in 18.7% of cases. There was no significant association between HLA-DR and independent variables such as age (r=-0.015, p=0.921) and Gleason score (r=0.226, p=0.123).

Conclusions: Prostate cancer is at its peak occurrence in the 60-79 years age group and a proportion of cases express HLA-DR, an expression that is independent of age, Gleason score or Gleason grade group. Contribution of HLA-DR to prostate cancer is probably minimal. Tissue microarray technique on needle biopsies is advocated in low-income countries if tissue loss can be minimized.

Keywords: Prostate cancer; HLA-DR; Tissue microarray; Nigeria; Gleason score

1. Introduction

Prostate cancer (PCa) is the second most common cancer among men, globally. It constituted 12.3% of all cancers diagnosed worldwide in 2020 and 29.8% of cancers diagnosed in males GLOBOCAN 2020 [1]. The burden of this cancer worldwide is enormous. Studies from different parts of Nigeria show that PCa is the predominant cancer seen in males [2,3,4]. The initial diagnosis of PCa is usually based on digital rectal examination and the level of prostate-specific antigen (PSA). Presently, the only known risk factors for PCa are: age, race, including African American ancestry in the

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United States and positive family history, all non-modifiable factors [5, 6]. In a recent study by Ntekim et al PCa is the second most common cancer in males below 55 years, preceded only by hepatocellular cancer [7]. The high prevalence of PCa at autopsy, coupled with the widespread screening to determine prostate-specific antigen level might have added to the increased incidence of this cancer in some cases [8]. PCa when diagnosed early and localized is curable. However, prognosis for metastatic disease is poor. Genome-wide association studies have identified 250 genetic loci associated with PCa and further studies are on-going [9]. The increasing incidence of PCa in the young is a cause for concern. Bleyer et al in their study found an increasing incidence of PCa in all age groups between the ages 15- 40 years. [10]. No appreciable decrease in incidence of PCa has been recorded despite the use of PSA in the early diagnosis and monitoring of treatment outcome in PCa. Orchidectomy may cause temporary relief in PCa patients but the development of castration-resistant PCa and the emergence of androgen receptor V-7 in patients treated with androgen receptor axis-targeted therapies, immunotherapy and chemotherapy is a nightmare for all patients with PCa [11]. Attempts at finding biological markers of antitumor immune responses and resistance have been focused on the role of HLA molecules. Many tumor cells express class I major histocompatibility complex (MHC) but a subset also express MHC-II, an antigen-presenting complex traditionally associated with antigen-presenting cells. MHC-II has greater diversity than MHC-I and this increases the likelihood of neo-antigen recognition by T-cells [12]. Although studies on the role of HLA in PCa had been on for decades no such study has been carried out in Nigeria and many parts of Africa, despite the huge burden of PCa on the nation. Thus Blades et al. 1995 demonstrated loss of class I HLA expression in some cases of prostate cancer [13]. Conversely, Azuma and Katsuoka 1999 in their study on Japanese men showed a higher incidence of HLA-DR allele in prostate cancer patients than in normal controls [14]. This study focuses on the association of immune marker HLA-DR with PCa in Nigerians using the tissue microarray analysis (TMA) method on needle biopsy specimens. Although this technique has been perfected in developed nations it is yet to be employed in studies done in Nigeria by Nigerians.

2. Methods

2.1. Study population

Forty and eight consecutive cases of prostate cancer needle biopsy specimens from three tertiary health institutions in southwester Nigeria were found suitable for this study and formed the basis of this report. The cases used in this study represent those with adequate tissue left in the paraffin-embedded blocks following serial sectioning and processing for routine diagnosis as well as after microarray construction and staining with HLA-DR.

2.2. Procedure

Tisue microarray was constructed using the TMA arrayer from Pathology Devises, Inc, San Diego, CA. All cases were adenocarcinoma. The age and the Gleason scores were noted. The Gleason scores were further stratified into five different Gleason grade groups. Two samples from each core were taken for analysis using a large bore needle. Immunohistochemistry (IHC) was performed using the indirect immunoperoxidase method. Samples were stained with HLA-DR mouse monoclonal antibody (MAS 5-11966 from Thermo Fisher Scientific) and antibody diluent TA-125-ADQ also from Thermo Fisher. Heat-induced antigen retrieval was by pressure cooking. Appropriate positive and negative controls were used.

2.3. Statistical analysis

Semi-quantitative IHC score was obtained using the multiplication of intensity of nuclear or cytoplasmic staining (0-3 where 0 is no staining, 1 – weak, 2 - moderate and 3 - strong staining) with the score of the percentage of positively stained cells (0-3 where 0 is ≤ 5%, 1 is 6-25% positive cells, 2 is 26-50% and 3 is ≥51% of positive cells). The total IHC score obtainable is 9. Expression of HLA-DR was considered low if the product of the staining intensity and percentage of stained tumor cells score was ≤ 3 and high if the product was ≥ 4.25. No expression was score 0. The expression pattern of HLA-DR was correlated with age, Gleason score and grade group using SPSS Version 25. The level of significance was set at $p < 0.05$.

3. Results

The age range was 54 to 89 years (mean = 68.8; median = 68.0; SD = 7.7).

Gleason scores 8 and 9 were recorded in 60-4% of cases while grade groups 4 and 5 were seen in 66.7% of cases. There is no significant association between HLA-DR and independent variables such as age (r=-0.015, p=0.921) and Gleason score (r=0.226, p=0.123). Analysis of variance shows no significant association between HLA-DR expression and

Gleason grade group and age. The percentage distribution of respondents based on the intensity of HLA-DR staining is shown in Figure 1. High and moderate IHC staining with HLA-DR is seen in 18.7% of cases.

Table 1 Age distribution of prostate cancer (N=48)

Variable	Frequency	Percentage
Age groups (in years)		
50 – 59	6	12.5
60 – 69	20	41.7
70 – 7980 – 89	19	39.6
	3	6.3

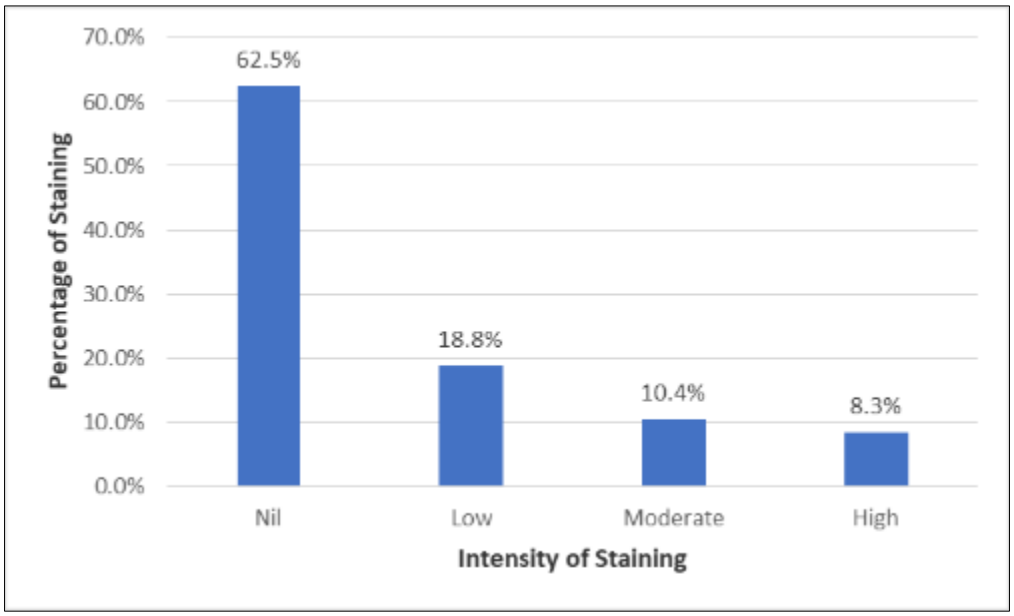

Figure 1 Percentage distribution of HLA-DR according to degree of Staining (N = 48)

Table 2 shows the distribution of the independent variables (age, HLA-DR, Gleason score) as continuous variables

Table 2 Distribution of independent variables

Variables	Minimum value	Maximum value	Range	Mean	Standard deviation	Median	Interquartile range	Mode
HLA-DR Score	0	6	6	1.0	1.8	0.0	1.0	0.0
Age (years)	54.0	89.0	35.0	68.8	7.7	68.0	11.8	70.0
GLEAN-SON Score	6.0	10.0	4.0	8.0	1.2	8.0	2.0	9.0

Figure 2 shows the age distribution of Gleason score. Higher scores 8 and 9 predominated in most age groups. The same pattern is seen with the Gleason grade groups (Fig 3). The different degrees of expression of HLA-DR are shown in Fi. 4

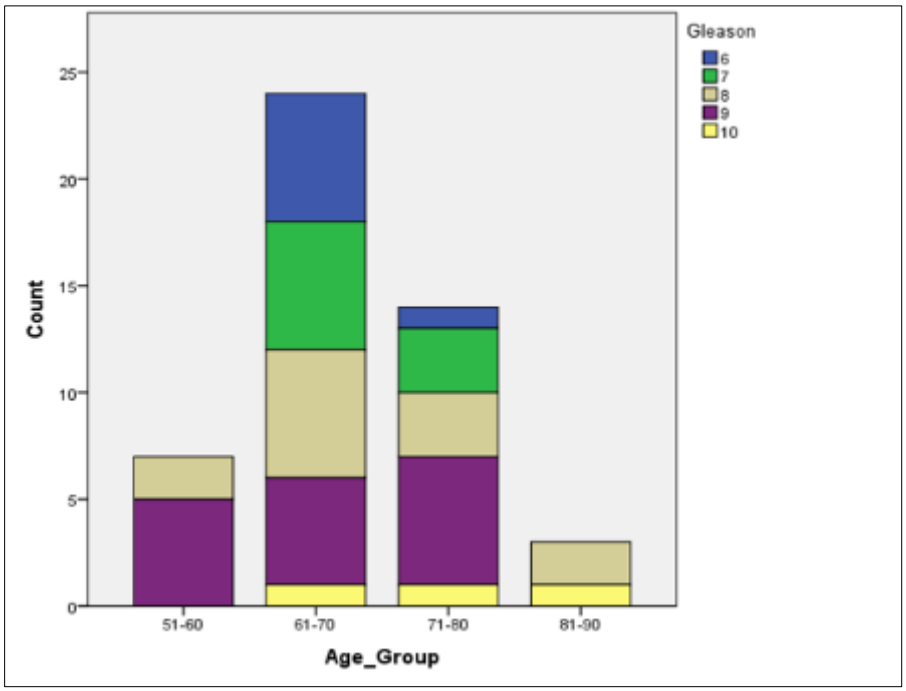

Figure 2 Age distribution of Gleason score

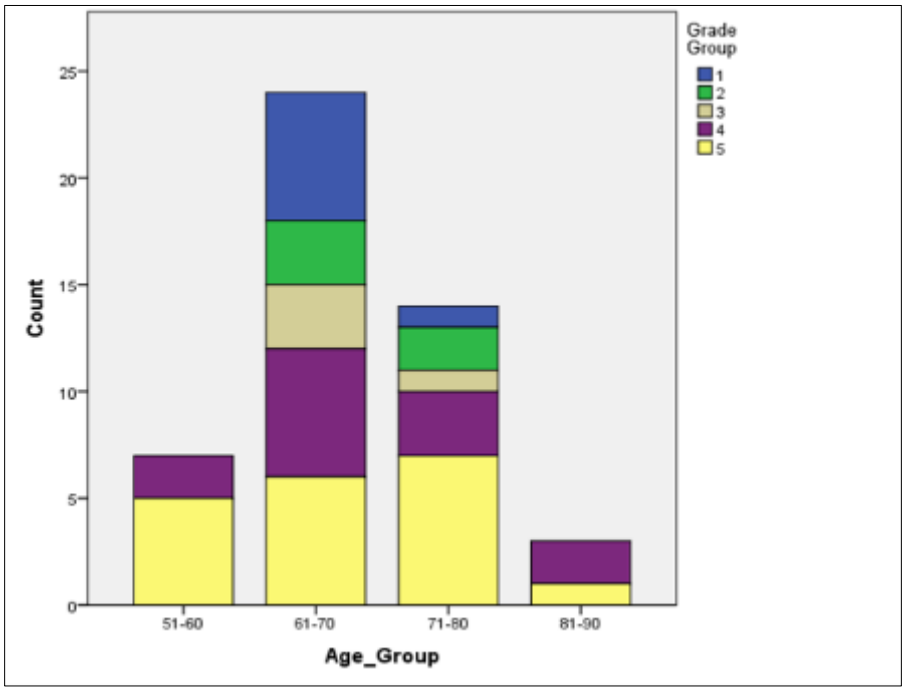

Figure 3 Age Distribution of Gleason Grade Group

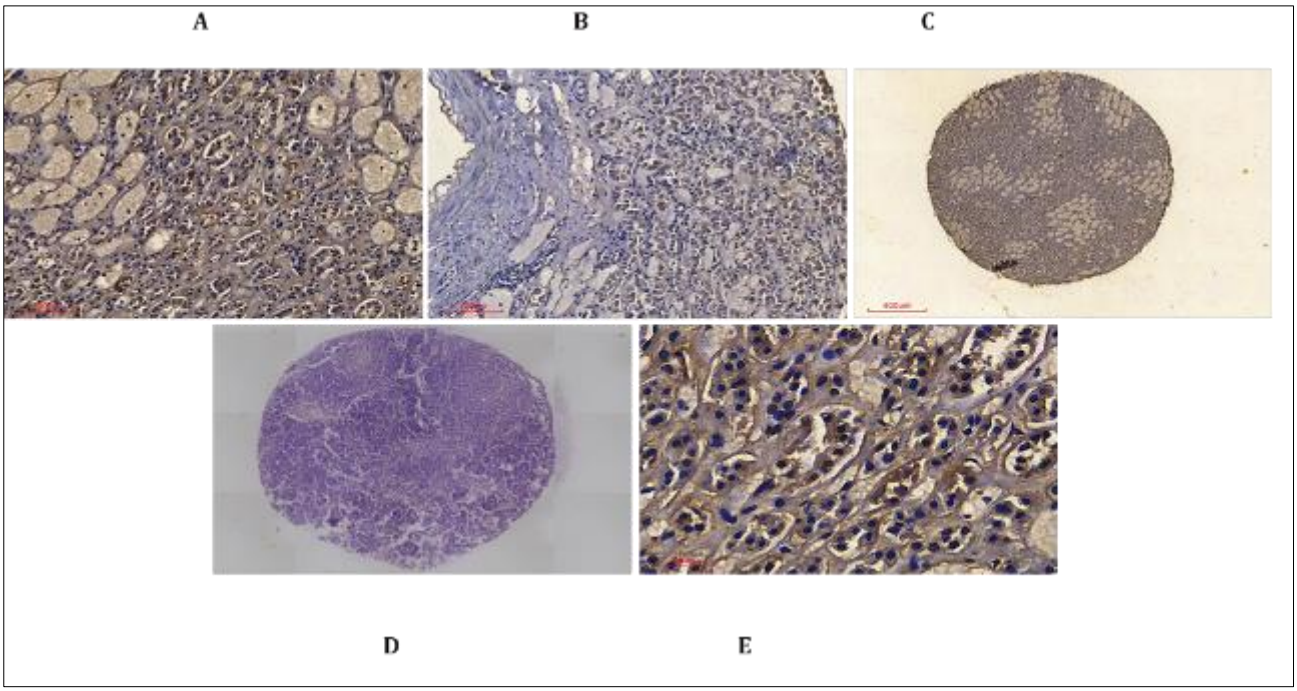

Figure 4 Photomicrographs showing different levels of expression of HLA-DR

Cytoplasmic staining with HLA-DR B. Moderate staining intensity with HLA-DR C. Low power view D. H&E stain E, Strong staining

4. Discussion

This is the first study done in Nigeria using TMA with needle biopsy specimens. Formalin-fixed needle biopsies are the only biopsies available for the diagnosis of prostate cancer in most centers as prostatectomy is usually not done in cases of PCa. Construction of TMA from needle biopsy is challenging. Punching donor blocks and transferring into recipient block requires adequate tissue in the donor blocks. Continuous trimming and sectioning of such blocks for diagnostic purposes pre-TMA is associated with a high rate of attrition of the sample in many cases. These factors are responsible for the low number of cases in this study. Komiya et al [15] described the application and construction of a type of tissue array for small tissues which they called spiral array that could be used in prostate cancer research. Earlier on, McCarty et al [16] based on the limitation posed by the small width of a prostatic needle biopsy had proposed a method of constructing TMA from prostate needle biopsy using vertical orientation of the punched-out biopsies. This obviously requires some experience and expertise.

The peak age incidence of 60-79 years with a mean of 68.8 years in this study corresponds roughly with findings in most other Nigerian studies [17, 18]. It has been suggested that preventive strategy for prostate cancer should start at the age of 50 years but for people of African descent or with family history, age 45 years is advisable [19]. Could the reduced incidence of prostate cancer after the age of 80 years be due to hypogonadism, decreasing levels of testosterone or reduced life expectancy far below the age of 80 years? The answer may at present be in the realm of speculation.

Current treatment modalities of Pca have not proven efficient enough to stop the development of late-stage metastatic cancer, Immunotherapy is currently one of the treatments available for PCa patients. Unfortunately, current immunotherapy strategies do not prolong survival beyond a few months, largely due to inability to stimulate CD4+ helper T-cells via class II HLA pathway. Both class I and class II MHC molecules are expressed by prostate cancer cells at reduced and various levels. In this study, 18.7% of the cancer cells stained showed moderate and high expression of HLA-DR, an expression independent of Gleason score, Gleason grade group or age. In the study by Tuerff et al {20} 14 (35%) out of the 40 randomly-selected patients showed HLA-DR expression. This association was, in consonance this study, also not found to be associated with clinical characteristics such as Gleason score and PSA at diagnosis. Downregulation of HLA molecules is a known tumor immune escape mechanism. Nakamura et al {21} reported a significantly high prevalence of HLA-class I downregulation in prostate cancer and this corresponds to higher clinical grades. Low expression of class II MHC, as in this study, has been associated with favorable outcome including those treated with immunotherapy by Axelrod et al [12]. However, only a small proportion of our patients fall into this

category. MHC-II is known to have greater diversity than MHC-I. This increases the possibility of neoantigen recognition by T-cells. This immune clearance of tumor cells through activation of cytotoxic T-cells is now known to be short-lived. Doonan et al [22] have suggested the need for activation of the HLA-II pathway to induce sustained CD8+ T-cell response. They suggested the possibility of manipulating PCa cells to express class II molecules that can be manipulated for antigen processing and presentation. In another study, Doonan et al also showed that the insertion of a lysosomal thiol reductase into prostate cancer cells directly enhances HLA class II antigen processing leading to increased CD4+ T-cell activation by prostate cancer cells [23]. The implications of all this for prostate cancer patients currently is not fully known, in view of the fact that many of these research findings have not been fully and widely translated into clinical practice. With the multiplicity of research on immunotherapy, help may not be far from PCa patients who need it. But will these therapeutic manipulations be available and affordable when they eventually find their way into the clinics? For now, the best option for developing countries is to pursue the early diagnostic strategies for PCa .

List of abbreviations

- PCa - Prostate cancer;
- PSA – Prostate-specific antigen;
- HLA – Human leucocyte antigen;
- TMA – Tissue microarray analysis.

5. Conclusion

Prostate cancer is at its peak occurrence in the 60-79 years age group and a proportion of cases express HLA-DR, an expression that is independent of age, Gleason score or Gleason grade group. Contribution of HLA-DR to prostate cancer is probably minimal. Tissue microarray technique on needle biopsies is advocated in low-income countries if tissue loss can be minimized.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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