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Clinical and Molecular Significance of Poly (ADP-Ribose) Polymerase-1 (PARP-1) in Breast Cancer of African Women and its Potential as a Targeted Therapy

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Abstract **Background:** The therapeutic effects of Poly (ADP-ribose) polymerase-1 (PARP -1) inhibition are currently studied in a clinical trial that is recruiting African- American (A-A) women with breast cancer (BC). Although, A-A and West African women are likely to share the same ancestry, there are overwhelming evidences, that BC is undoubtedly heterogeneous which might influence results obtained in these Nationalities. Thus, this study aims to investigate PARP-1 expression in a large and annotated series of breast cancer from Nigerian women to determine its clinical and biological significance for the indigenous African women. **Methods:** PARP-1 protein expression was assessed immunohistochemically in 204 formalin fixed paraffin samples from Nigerian breast cancer women prepared as TMA. **Results:** PARP-1 was inversely associated with steroid hormone receptors (oestrogen (ER) and progesterone (PR) receptor), the Homologous Recombination marker BRCA1 associated ring domain 1 (BARD1) and p27. Conversely, a positive association was established between PARP-1 and high histologic grade, expression of basal markers (cytokeratins (CK) 5/6 and 14) and epidermal growth factor receptor (EGFR)), DNA damage-repair markers (protein inhibitor of activator signal transducer gamma (PIAS)), the BRCA1 inhibitor (metastasis tumour antigen-1 (MTA1), p53, the proliferation markers (KI-67, Phosphoinositide-3-kinases (PI3KCA)), the triple-negative and basal-like phenotypes. Outcome analysis indicated PARP-1 as a predictor of poor survival independent of tumour size, histological grade and lymph node involvement. **Conclusion:** These results provide evidence that PARP-1 plays an important role in Nigerian women with breast cancer. It is recommended that indigenous Black women from Africa are included in the ongoing clinical trial of PARP1 inhibitors that is aimed at determining the efficiency of the drug in black BC women outside United States.

Keywords: *PARP-1, African women, therapeutics potential, breast cancer*

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1. Introduction

PARP-1 plays a prominent role in the physiological state of cells through enzymatic modification of Poly (ADP-ribose) chains of target proteins [1]. Its' enzymatic activities increase in response to cellular stressors in order to participate in the inflammatory response, apoptosis and control of gene expressions, thereby ensuring the genomic integrity is maintained [1,2].

PARP-1 is also involved in DNA repair, where it functions as an important player in single strand break (ssBR) through base excision repair (BER) mechanism [3]. In addition, PARP-1 also participates in the double strand break (DSB) repair through non homologous end joining (NHEJ) pathway [3].

Mounting evidences show involvement of PARP-1 in carcinogenesis, its expression has been reported particularly in melanomas, and head and neck, prostrate, lungs and breast cancer [4,5,6,7,8]. Based on these discoveries in cancer cell lines and preclinical studies, the therapeutic effect of PARP-1 inhibition is being exploited. The principle behind PARP-1 therapy approach in the breast cancer associated gene 1 (*BRCA*-1) and breast cancer associated gene 2 (*BRCA*-2) defective tumour is to

selectively sensitize human cancer with defective homologous recombination (HR) to synthetic lethality by blocking PARP-1 dependent BER repair pathways [9,10].

In this study, PARP-1 expression was investigated in Nigerian breast cancer patient samples, which are recognised to have a high prevalence of DNA repair defects, in order to establish its clinical and biological significance and its potential usage in the management of breast cancer in Black women.

2. Materials and Methods

The patient cohort comprised of 204 formalin-fixed paraffin embedded (FFPE) breast cases from women presented at the Olabisi Onabanjo University Teaching Hospital, Sagamu, and Histopathology Specialist laboratory, Idi-Araba Lagos, Nigerian from January 2002 to December 2008. Patients' clinical history and tumour characteristics include age, menopausal status, tumour type, histological grade, tumour size, lymph node status and vascular invasion.

Data relating to survival were collated in a prospective manner including breast cancer specific survival (BCSS), defined as the interval (in weeks) from the date of the primary treatment to the time of death, and disease free interval (DFI), defined as the interval (in weeks) from the date of the primary treatment to the first loco-regional recurrence or distant metastasis. Patients were followed up for at least 60 months (260 weeks).

Patient management was based on classical chemotherapy and eighty five out of the patients received radiotherapy.

The immunoreactivity scoring and categorisation of steroid hormone receptors (oestrogen (ER) and progesterone (PR) receptor), the Homologous Recombination marker Breast Cancer associated gene 1 (BRCA1), BRCA1 associated ring domain 1 (BARD1), p27, (cytokeratins (CK) 5/6 and 14), epidermal growth factor receptor (EGFR), DNA damage-repair marker (protein inhibitor of activator signal transducer gamma (PIAS), the BRCA1 inhibitor (metastasis tumour antigen-1 (MTA1), p53, the proliferation markers (KI-67), and Phosphoinositide-3-kinases (PI3KCA) were defined in this series as previously described [11,12]. The American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for HER2 Testing in Breast Cancer was used for c-erbB2 (HER2) assessment [13]. Equivocal (2+) cases were confirmed by chromogenic in-situ hybridization (CISH) as previously described [14]. For molecular classification, Nielsen's method [15] was used. This comprises of Luminal A (ER, PR positive and HER2 negative), Luminal B (ER, PR HER2 positive), Basal (ER, PR, HER2 negative and CK5/6 and or EGFR positive), HER2 (ER negative and HER2 positive) and an unclassified group (ER, PR, HER2 CK5/6 and EGFR negative).

The Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria, recommended by McShane et al [16], were followed. This study was approved by the Medical Advisory Committee, Olabisi Onabanjo University Teaching Hospital and by the Nottingham Research Ethics Committee 2 under the title of "Development of a molecular genetics classification of breast cancer".

2.1. Tissue Microarray Construction.

Two hundred and four samples from Nigerian cohort were constructed as tissue microarrays (TMA) as previously described [11,12]. Breast tumour cores were taken from each FFPE donor tissue block that has been marked for the most representative points of tumour (both peripherally and centrally). A precision instrument (ALPHELYS MiniCore®) was used to take representative cores of tissue (0.6mm diameter, 3mm height) from each sample, which was arrayed into recipient paraffin.

2.2. Immunohistochemistry Method

The standard strept Avidin –Biotin complex method as previously described in [11,12] was used for the experiment following antigen retrieval. The antigen retrieval was performed by microwaving the slides at 800W for 10 minutes followed by 560W for 10 minutes in citrate buffer (1M Sodium Citrate at pH of 6.0), thereafter, the samples were allowed to cool in running water immediately. The primary antibody for the biomarkers was incubated for 60 minutes at room temperature. Diaminobenzidine tetrahydrochloride (DAB) solution was incubated for 10 minutes after which copper-sulphate solution (0.5% Copper Sulphate in 0.8% Sodium Chloride) were applied to the slides and incubated for 10 minutes each and counter stained with haematoxylin for 2-3 minutes, followed by rinsing in tap water. Slides were de-hydrated by immersing in three alcohol baths for 10 seconds and cleared in two xylene baths followed by application of cover slip. Negative and positive controls were performed by omitting the primary antibody and including control tissues as specified by the antibody supplier respectively.

2.3. Immunohistochemical Scoring

The percentages of PARP-1 nuclear immunoreactivity staining of invasive malignant cells within the TMA cores scores of 0-100 were considered. All samples were scored by one observer (J A) and a further observer (Dr Andrew Green of the department of Histopathology, University of Nottingham Teaching Hospital, City hospital, Nottingham) countered scored proportions of these samples. The whole tissue mounts and TMA samples were scored twice without knowledge of the patient outcome. The median of the percentage of the staining of PARP-1 biomarker frequency histogram distribution was used to dichotomised into 0-79% negative/low/moderate and >80% as high expression cut- off points.

2.4. Statistical Analysis

Statistical analysis was performed using SPSS 16.0 statistical software. Chi-squared analyses were used for inter-relationships between the PARP-1 expression, clinicopathological parameters and other biomarkers. The Kaplan–Meier survival method and the log-rank test were used for survival curves. Multivariate analyses using Cox proportional hazard regression models were performed and from the model both the risk factor and 95%

confidence intervals were generated. A two-sided *p*-value of <0.05 was considered significant.

3. Results

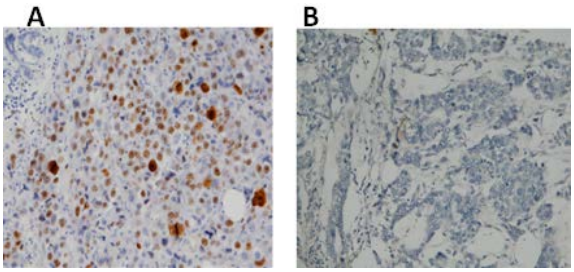

Figure 1. (A) and (B) showing positive and negative PARP-1 immunochemistry expression. Mag X 20

PARP-1 immunohistochemistry staining was observed in the nucleus (Figure 1). One hundred and eight (52.9%), 96 (47.1%) tumours were considered positive and negative for PARP-1 immunoreactivity expression by using this appropriate cut off points (Table 1).

Table 1. Frequency of PARP-1 immunohistochemistry staining in Nigerian BC women

PARP-1 expression	Frequency (%)
Positive	108 (52.9)
Negative	96 (47.1)

The statistical correlation between PARP-1 and clinicopathological characteristics showed a significant association between PARP-1 and tumour grade, where those tumours that expressed PARP-1 were poorly differentiated (p=0.02) and primarily of lesser tubule formation (p=0.006). There was no other significant association observed between PARP-1 and clinical history and other tumour parameters (Table 2).

Table 2. Relationship between PARP1 expression and clinicopathological parameters in Nigerian breast cancer

Variables	PARP1			
	Negative (%)	Positive (%)	χ2 value	p-value
Age (years)				
≤ 50	65 (67.7)	71 (65.7)	0.89	0.76
> 50	31 (32.3)	37 (34.3)		
Lymph node involvement				
Negative	7 (7.3)	11 (10.2)	0.52	0.46
Positive	89 (92.7)	97 (89.8)		
Menopausal				
Pre	65 (67.7)	74 (68.5)	0.02	0.90
Post	31 (32.3)	34 (31.5)		
Mitotic figure				
Low	64 (66.7)	60 (55.6)		
Medium	18 (18.8)	28 (25.9)	2.66	0.26
High	32 (14.6)	20 (18.5)		
Nuclear pleomorphism				
Small uniform cells	0 (0.0)	1 (0.9)	1.84	0.39
Moderate increase in size	33 (34.4)	30 (27.8)		
Marked variation	63 (65.6)	77 (71.3)		
Tumour Size (cm)				
≤2.0	7 (7.3)	11 (10.2)	0.52	0.46
>2.0	89 (92.7)	97 (89.8)		
Tubule formation				
> 75 %	0 (0.0)	2 (1.9)		
10 -75 %	0 (0.0)	9 (8.3)	10.33	0.006
< 10 %	96 (100)	97 (89.8)		
Tumour grade				
1	0(0.0)	5(4.6)		
2	64(66.7)	56(51.9)	7.70	0.02
3	32(33.3)	47(43.5)		
Tumour type				
Typical medullary	0 (0.0)	0 (0.0)	14.03	0.05
Atypical medullary	1 (1.0)	1 (0.9)		
Tubular	0 (0.0)	0 (0.0)		
Lobular	2 (2.1)	1 (0.9)		
Ductal NST	81 (84.4)	100 (92.6)		
Mucinous	1 (1.0)	1 (0.9)		
Tubulolobular	1 (1.0)	0 (0.0)		
Lobular mixed	0 (0.0)	3 (2.8)		
Tubular mixed	10 (10.4)	1 (0.9)		
Mixed NST	0 (0.0)	0 (0.0)		
Others	0 (0.0)	0 (0.0)		

PARP-1 relationship with other biomarkers is summarised in Table 3. PARP-1 was inversely associated with steroid hormone receptors (oestrogen (ER) (p<0.001) and progesterone (PR) (p<0.001)) and HR marker (BRCA1- associated ring domain (BARD1) (p=0.002)). Conversely, a positive association was established between PARP-1 and expression of basal markers (basal cytokeratins : CK5/6 (p<0.001), CK 14 (P=0.02) and epidermal growth factor receptor (EGFR) (p=0.002)), DNA damage-repair markers (protein inhibitor of activator signal transducer (PIASγ) (p<0.001)), the BRCA1 inhibitors (metastasis tumour antigen-1 (MTA1) (p<0.001)), p53 (p<0.001), the proliferation markers (Ki-67 (p=0.02), Phosphoinositide-3-kinases (PI3KCA) (p<0.001), the triple-negative ((TNBC) (ER, PgR and HER2 negative) (p<0.001)) and basal-like phenotypes (p<0.001). There was no significant association with HER2 (Table 3). Furthermore, the co-expression of

BRCA1 and PARP-1 showed that more than 47% of the tumours that expressed PARP-1 had reduced or no expression of BRCA-1 (Table 4).

Table 3. Relationship between PARP1 expression and other biomarkers in Nigeria BC women

Variables	PARP1 Negative (%)	Positive (%)	χ2 value	p-value
Ck5/6				
Negative	56 (70.9)	40 (40.8)	15.93	<0.001
Positve	23 (29.1)	58 (59.2)		
CK14				
Negative	45 (67.2)	42 (47.7)	5.83	0.02
Positive	22 (32.8)	46 (52.3)		
MTA1				
Negative	61 (67.8)	28 (27.2)	31.85	<0.001
Positive	29 (32.2)	75 (72.8)		
P53				
Negative	29 (46.8)	16 (16.7)	16.76	<0.001
Positive	33 (53.2)	80 (83.3)		
KI_67				
Negative	20 (24.7)	11 (11.2)	5.61	0.02
Positive	61 (75.3)	12 (88.8)		
BARD1				
Negative	81 (94.2)	78 (78.0)	9.76	0.002
Positive	5 (5.8)	22 (22.0)		
EGFR				
Negative	52 (76.5)	48 (52.7)	9.38	0.002
Positive	16 (23.5)	43 (47.3)		
HER_2				
Negative	60 (76.9)	83 (84.7)	1.72	0.18
Positive	18 (23.1)	15 (15.3)		
P13KCA				
Negative	52 (56.5)	27 (25.5)	19.80	<0.001
Positive	40 (40.3)	79 (74.5)		
ER				
Negative	57 (65.5)	88 (85.4)	10.35	0.001
Positive	30 (34.5)	15 (14.6)		
PgR				
Negative	42 (60.0)	83 (89.2)	19.10	<0.001
Positive	28 (40.0)	10 (10.8)		
Triple negative				
Negative	46 (66.7)	28 (29.8)	21.83	<0.001
Positive	23 (33.3)	66 (70.2)		
PIASγ				
Negative	44 (57.9)	19 (19.6)	27.00	<0.001
Positive	32 (42.1)	78 (80.4)		
Nielsen classification				
Basal	11 (11.5)	52 (48.1)		
HER-2	11 (11.5)	10 (9.3)	37.46	<0.001
Luminal A	21 (21.9)	8 (7.4)		
Luminal B	6 (6.3)	2 (1.9)		

Table 4. Frequency of Co-expression of BRCA-1 and PARP-1 in Nigerian BC

Variables	Frequency (%)
BRCA1 negative/PARP-1 negative	62 (37.3)
BRCA1 negative/PARP-1 positive	79 (47.6)
BRCA1 positive/PARP-1 negative	17 (10.2)
BRCA1 positive/PARP-1 positive	8 (4.8)

Univariate analysis show patients with PARP-1 positive tumour expression had poorer breast cancer specific survival (BCSS) compared to the negative expression. However, there was no significant difference in disease free interval (DFI) between those tumours that had positive and negative expression (Figure 2). Cox multivariate analysis confirmed PARP-1 as a predictor of poorer survival independent of tumour grade, size and lymph node status (Table 5).

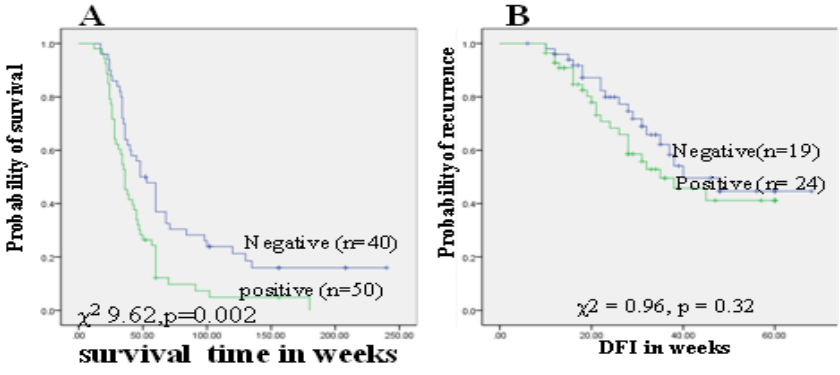

Figure 2. show the expression of PARP-1 in relation to BCSS and DFI in Nigerian BC women

Table 5. Cox multivariate analysis of probability of BCSS in Nigerian breast cancer series with PARP-1 expression

Variables	BCSS			
	p-value	Hazard atio	95 % CI	
			Lower	Upper
PARP-1	**0.004**	1.873	1.218	2.878
Tumour size	0.576	0.798	0.362	1.760
Tumour grade	<0.001	2.566	1.646	4.001
lymph_node	0.951	0.973	0.404	2.340

4. Discussion

BCs in Nigerian women are often high-grade, basal-like/triple-negative phenotype with defective DNA repair and are associated with poor outcome [17]. Majority of PARP-1 previous studies have been assessed on African descent living in western countries [18,19], despite the overwhelming evidences, that BC is undoubtedly heterogeneous in relation to its presentation at diagnosis, morphological and pathological response, molecular profile, response to treatment and clinical outcomes [20,21,22,23,24]. Although, A-A women and West African women are likely to share the same ancestry, there are many factors such as inter-racial marriage, environmental factors, socioeconomic factors and availability of treatments that could influence results obtained. Nigeria has a population of more than 150 million people; one out of every five black people in Africa is a Nigerian. Therefore, the results presented in this study probably represent a reliable account of PARP-1 behaviour in the indigenous African women with BC.

Previous studies have shown immunoreactivity of PARP-1 in carcinomas [25,26,27,28]. In agreement with previous studies, protein immunoreactivity of PARP-1 was abundantly expressed in Nigerian breast cancer.

PARP-1 as a major regulator of the base excision repair (BER) and a detector of ssBR caused by reactive oxygen species from either pathogenic or normal cellular metabolic reactions [29,30], it catalyzes ADP-ribose generated from nicotinamide adenine dinucleotide (NAD) with poly (ADP –ribosylation) to its downstream targets, which it uses to recruit other components of the BER pathway to repair ssBR [29,30]. Based on these aforementioned roles, PARP-1 has been suggested to be important in the cellular response of tumours towards chemo-and radio-therapy [31]. In the Nigerian series of tumours used for this study, chemotherapy was offered to all patients and yet the majority of them died within 5 years of diagnosis. The high level of protein expression of PARP-1 in this study indicated that breast cancer cells from Nigerian women would have repaired BER and ssBR properly, thereby becoming resistant to either chemotherapy or radiotherapy.

Previous study suggested that DNA repair biomarkers have direct impact on the cell proliferation and cell cycle regulatory phase [32]. PARP-1 is involved in cell cycle and signalling regulators, where a reduction in PARP1 gene expression level is linked with increased genomic abnormalities [33,34]. Cheng et al., observed that PARP-1 positive relationship with p53 might play a role in immune adaptive response induced by low dose of ionizing radiation in vitro [35]. Inbar-Rozensal and colleagues reported that PARP inhibitors may be involved in signal transduction pathways and cell cycle proteins [36]. The positive relationship between the PARP-1, abnormal expression of the p53 and cell proliferation in Nigerian breast tumours would have also been responsible for poor response to the chemotherapy.

Furthermore, the majority of tumours that expressed PARP-1 did not express BRCA-1 and BARD-1; however, these tumours did express BRCA-1 regulatory proteins (PIASγ, and MTA-1). The SUMOylation pathway plays a significant role in DNA damage response [37]. PIASγ is crucial for the accumulation of ring finger ubiquitin ligase (RNF)168 on DSB sites and is also important for the efficient association of tumour protein p53-binding protein 1 (53BP1), BRCA-1 and RNF168 at DNA lesion regions [37]. 53BP1 negative expression has been linked with basal like phenotype, BRCA-1 and TNBC [38] Similarly, PIASγ depletion caused direct reduction of 53BP1 in the histone (H2Ax) positive cells [39]. H2Ax and 53BP1 are downstream mediators of ATM in the DNA damage response repair pathways [37,38]. Also, in a cell-based plasmid-integration assay, depletion of PIASγ impairs DSB repair by NHEJ [40]. In a related developments, PIASγ mediates small ubiquitin related modifier (SUMO) 2/3 conjugation of PARP-1 in BRCA-1 deficient breast tumours, with a speculation that PIASγ might reduce resistance of BRCA-1 deficient cells to PARP inhibitor [41]. Furthermore, the E3 ligase of BRCA-1/BARD1 interaction is disrupted by these SUMO proteins [39]. In BRCA-1/ BARD1 transfected cells, ectopic expression of BRCA-1/BARD1 also increases ubiquitin conjugate activity which corresponds to their functional ring domain, but this activity was lost in PIASγ depleted cells [39]. The functional link between the PARP-1 and BRCA1 regulatory proteins in disrupting the BRCA-1 binding activities at the DNA damage site might have caused a reduction in the transcription activities of BRCA-1in Nigerian tumours.

In this study, the high PARP-1 was linked with basal-like, triple negative, aggressive clinicopathological features, such as higher tumour grade that are primarily of poor tubule formation of the breast tumours and absence of expression of steroid hormones. This is in line with previous findings, where PARP-1 expression was associated with poorly differentiated tumour, ER negativity and triple negative [26,42]. Certain polymorphisms in PARP-1 are suggested to have an influence on the effectiveness of hormone therapies [43]. This association might probably have implication on the hormonal treatment as well in Nigerian women with BC.

PARP1 expression was observed to be predictors of poor clinical outcome in Nigerian breast cancer patients, independent of tumour grade, size and lymph node involvement. This study is the first study to report PARP-1 association with survival from breast cancer in Nigerian women.

In conclusion, this study provides evidence that protein expression of PARP-1 is high in tumours arising from Nigerian women. In this context, using PARP-1 as potential therapeutic drug targets in triple negative, including basal like and BRCA-1 deficient breast cancer may benefit strategic management of breast cancer particularly among the African women. It can be recommended that indigenous Black women from Africa should be included in the ongoing PARP-1 inhibitor trial

in order to determine the efficiency of the drug in black BC women outside the western countries [10].

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