Cardiotoxicity and pharmacogenetics of doxorubicin in black Zimbabwean breast cancer patients

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Abstract

AIMS Doxorubicin-induced cardiotoxicity (DIC) is a significant cause of mortality in cancer care. This study was conducted to establish the frequency of DIC in Zimbabwean breast cancer patients on doxorubicin and to test the DIC predictive power of genetic biomarkers. METHODS A cohort of 50 Zimbabwean breast cancer patients treated with doxorubicin were followed up for 12 months with serial echocardiography and genotyped for UGTA1A6*4, SLC28A3 and RARG. 11% of the patients experienced DIC. RESULTS The frequency of SLC28A3 (rs7853758), UGT1A6*4 (rs17863783) and RARG (rs2229774) was 60.7%, 17.9% and 14.3% respectively. No association between DIC and the three variants was observed. CONCLUSIONS This is the first study on the prevalence of DIC and associated genetic biomarker predictive evaluation in Zimbabwean breast cancer patients. The genetic frequencies observed in our study was different to that reported in other populations. A larger sample size with a longer follow up time will be necessary in future studies.

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PI Statement: The authors confirm that the PIs for this paper were Prof. Margaret Borok and Dr. Ntokozo Ndlovu and that they had direct clinical responsibility for patients.

Key words: Anthracyclines; genetics; Zimbabwe, Africa, polymorphisms; cardiology; risk monitoring; cardiovascular health.

AIMS

Doxorubicin-induced cardiotoxicity (DIC) is a significant cause of mortality in cancer care. This study was conducted to establish the frequency of DIC in Zimbabwean breast cancer patients on doxorubicin and to

test the DIC predictive power of genetic biomarkers.

METHODS

A cohort of 50 Zimbabwean breast cancer patients treated with doxorubicin were followed up for 12 months with serial echocardiography and genotyped for UGTA1A6*4, SLC28A3 and RARG. 11% of the patients experienced DIC.

RESULTS

The frequency of SLC28A3 (rs7853758), UGT1A6*4 (rs17863783) and RARG (rs2229774) was 60.7%, 17.9% and 14.3% respectively. No association between DIC and the three variants was observed.

CONCLUSIONS

This is the first study on the prevalence of DIC and associated genetic biomarker predictive evaluation in Zimbabwean breast cancer patients. The genetic frequencies observed in our study was different to that reported in other populations. A larger sample size with a longer follow up time will be necessary in future studies.

What is already known about the subject?

The incidence of cardiotoxicity has been shown to range from 3-47%. The minor alleles RARG (rs2229774) and UGT1A6*4 (rs17863783) have been observed to be risk markers for cardiotoxicity, and the SLC28A3 rs7853758 have been proven to have a cardioprotective effect against doxorubicin induced cardiotoxicity (DIC).

What does this study add?

This study demonstrates the frequency of genetic risk markers that are associated with DIC in the black Zimbabwean breast cancer patients and the incidence of acute DIC (11%).

Introduction

Doxorubicin is one of the most commonly used anthracyclines for the treatment of different types of cancers in adults and children[1,2]. Its clinical utility is limited due to side effects including cardiotoxicity when it exceeds the recommended cumulative dosage of 400 mg/m^2 - 550 mg/m^2 in adults and $>$ 300 mg/m^2 in children [3,4]. The risk of developing cardiotoxicity increases when the cumulative dose of doxorubicin exceeds these thresholds, with 700 mg/m^{2}having a risk of 48% [3,5,6]. The gold standard for monitoring risk for doxorubicin induced cardiotoxicity (DIC) is using left ventricular ejection fraction (LVEF) measured via echocardiogram[7,8].

Candidate gene and genome-wide association studies have established genetic variants associated with DIC [9–13] .These include genetic variations in genes involved in anthracycline metabolism and transportation. A meta-analysis by Aminkeng *et al* showed that the evidence was strongest and most consistent for an association of RARG (retinoic acid receptor gamma) rs2229774, SLC28A3 (solute carrier family 28 member 3) rs7853758, and UGT1A6 (UDP glucuronosyltransferase family 1 member A6), rs17863783 variants with DIC. Based on current evidence, Canadian Pharmacogenomics Network for Drug Safety (CPNDS) recommends genotyping children who are taking doxorubicin for UGT1A6*4 (rs17863783), SLC28A3 (rs7853758) and RARG (rs2229774) haplotypes[9].

As most of these pharmacogenomics studies have been performed in pediatric patients receiving doxorubicin [14–18], the generalizability of these findings to adults and other anthracyclines is unknown. In addition to the three genetic markers that have been identified in children, there are other genetic markers that have been associated with the DIC[9], however they are not well characterized and they have not been replicated in most of the studies. Further research is thus required in adults and other understudied populations to help improve the predictive and prognostic role in predicting DIC .This study was therefore conducted to

establish the frequency of DIC in adult black Zimbabwean breast cancer patients treated with doxorubicin and to test the DIC predictive power of genetic biomarkers in this cohort.

Method

Study design & participants

The prospective study was initiated at the University of Zimbabwe, Faculty of Medicine and Health Sciences at Parirenyatwa Hospital (PH) in Harare. The study recruited 50 Zimbabwean patients above the age of 18 years receiving doxorubicin chemotherapy for breast cancer over a period of 6 months from January 2019-July 2020. The patients were sampled using purposive sampling to reflect the demographics of black breast cancer patients from Zimbabwe. Participants underwent cardiovascular assessment by a cardiologist using echocardiography to measure Left Ventricular Ejection Fraction (LVEF) at baseline, 3 months, 6 months and 12 months. Patients with prior treatment with any chemotherapy, prior chest wall radiotherapy and non-black women were excluded. Demographics, body mass index (BMI) and physical performance of the patients was recorded at the time of recruitment. A blood specimen was collected at the study entry from which the genotype for the three cardiotoxicity risk variants was determined. The genotypes were checked for any deviation from the Hardy Weinberg principle (HWE) before their frequencies were determined using $\chi 2$. The association analysis of genotypes and LVEF measures was done using multivariable logistic regression. A patient with cardiotoxicity was defined as having LVEF threshold of greater than 10% reduction from the normal echocardiograms (normal [?]60%) [19,20].

Genotyping

Three SNPs (UGT1A6 rs17863783, SLC28A3 rs7853758 and RARG rs2229774) that previously showed strong evidence of association with DIC were selected [9,15]. Genomic DNA was extracted from whole blood using the Applied Biosytems MagMAX DNA Multi-Sample Ultra 2.0 Kit using an automated system (King FisherFlex Magnetic Particle Processor System). Genotyping was done using the GenoPharm® pharmacogenomics open array panel which contains 120 assays for over 48 genes. GenoPharm® uses TaqMan Assay chemistry using the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts).

Statistical analysis

Statistical analysis was done using Stata Version 16.0. Continuous variables, such as age, dose and BMI were presented as medians and interquartile ranges (IQR). Qualitative variables, such as cancer stage, performance status and co-medications were presented as numbers and percentages. Hardy–Weinberg equilibrium of variants and genotype distribution were tested by $\chi 2$ ($p > 0.05$). The association of DIC and the variants was evaluated using Pearson Chi-Square exact test. We also tested the association of UGT1A6 rs17863783, SLC28A3 rs7853758 and RARG rs2229774 with cardiotoxicity using logistic regression adjusted for age at start of treatment, cumulative anthracycline dose and BMI. Statistical significance was set at $p< 0.05$.

Results

Study population

A total of fifty patients were enrolled into the study and of those 28 (56%) patients were followed up to 12 months (Figure 1). Of the patients analysed the median age was 48 years (IQR 44.5-59.0). Most of the patients (86%) had advanced stages of breast cancer (stage III/IV) with a good Karnofsky performance status between 80-90%. The median LVEF measurement at 12 months was 61.5% (IQR 58.1-77.3) and the median cumulative doxorubicin dose was 238.89 mg m^{-2} (Table 1).Two (7%) out of the 28 patients received single agent doxorubicin, 9 (32%) received doxorubicin plus cyclophosphamide doublet and 17 (61%) of patients received doxorubicin, cyclophosphamide followed by paclitaxel(Table S1) Three (11%) patients developed cardiotoxicity and cumulatively these patients received the following doses of doxorubicin 240 mg m^{-2}, 281 mg m^{-2} and 360 mg m^{-2}. The final LVEF measurements of the three patients after the 12-month follow up were 18.2%, 34.9% and 39%. One of the patients received single agent doxorubicin while the remaining

two received a combination therapy of doxorubicin plus cyclophosphamide followed by paclitaxel. The three patients were all hypertensive and additionally one had diabetes mellitus.

Genetic results

Of the 50 patients initially recruited, 47 patients were genotyped successfully. Hardy-Weinberg equilibrium (HWE) was calculated for each SNP and no genotype deviated from HWE ($p > 0.05$) (Table S2). The cardioprotective variant SLC28A3 rs7853758 was identified in 31 (67%) patients. The cardiotoxic risk variants UGT1A6 rs17863783 and RARG rs2229774 were present in 10 (21.2%) and 5 (10.6%) respectively. Among the 28 patients who were followed up to 12 months, 12 (43%) of them carried the protective variants SLC28A3 rs7853758, 1 (4%) carried UGT1A6 rs17863783 and 2 (8%) carried RARG rs2229774. Three (11%) patients carried both SLC28A3 rs7853758 and UGT1A6 rs17863783 while 1 (4%) carried both SLC28A3 rs7853758 and RARG rs2229774 variants. Eight (29%) patients did not have any genetic variation. A single patient (4%) carried all the three variants. All three patients who developed DIC carried SLC28A3 rs785375 variant and none of them carried RARG rs2229774 variants. One of the 3 patients carried UGT1A6 rs17863783.

Association between the variants and Doxorubicin Induced Cardiotoxicity

We evaluated the association between SLC28A3 rs7853758, RARG rs2229774 and UGT1A6 rs17863783 variants and DIC. No covariate had an independent significant effect on DIC from the univariable analysis (SLC28A3 rs7853758 ($p= 0.408$), RARG rs2229774 ($p= 0.471$), UGT1A6 rs17863783 ($p= 0.354$), age ($p= 0.124$), BMI ($p= 0.451$) and cumulative dose ($p= 0.357$)). Using the stepwise method approach in multivariable analysis, we further explored whether cardiotoxicity was influenced by age, BMI and genotypes. None of these covariates were significantly affecting changes in cardiotoxicity. Thus, there was no significant relationship between cardiotoxicity and genotype, age and BMI.

Determination of the predictive value of genotyping test

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated to determine the predictive value of the test. The SLC28A3 rs7853758, UGT1A6 rs17863783 and RARG rs2229774 genotypes were associated with the following parameters: sensitivity of 13% and specificity of 90% in predicting cardiotoxicity (Table 2). The PPV of the genotype test was 33.3% while the NPV of the test was 72.0%. Both PPV and NPV are influenced by the prevalence of DIC.

Discussion

The incidence of cardiotoxicity (11%) in our population was comparable to rates in the literature [21–23] which ranged from 9%-12%. However, it is important to note that these studies were performed in Western populations and are associated with late-onset DIC, with a sample size of more than 1000 and longer follow up time of 5 years contrary to our study which had a smaller sample size with follow up time of 1 year. Other studies conducted in Asia[24–26] reported higher incidence of DIC ($> 30\%$) . This suggests that our patients may be experiencing higher incidence of cardiotoxicity or an early onset cardiotoxicity.

In this study we could not verify clinical risk factors to be significant risk factors for developing DIC, of which previous studies have identified hypertension, female sex, age, cumulative anthracycline dose, as risk factors for DIC [3,14,27] . It may be possible that the small sample size in our study could have reduced the statistical power to demonstrate the significance of association of the clinical risk factors with DIC [12,28–33]

If we considered doxorubicin cumulative dose, we observe that there was no clear association between cumulative doxorubicin dose and cardiotoxicity ($p = 0.357$), which is not as expected[11,12,18,34–38]. However, the high frequency (60.7%) of the cardioprotective variant could have influenced this dose relationship in our study. The cardioprotective variant (SLC28A3 rs7853758) is important because it affects the pharmacology of doxorubicin and reduce incidence of cardiotoxicity[39,40].

The UGT1A6 rs17863783 variant results in altered enzymatic activity, increasing the risk of developing DIC in those carrying at least one copy of the variant [9,18,30] . One of the patients who carried UGT1A6

rs17863783 developed DIC in our study. RARG rs2229774 variant occurred at a significantly low frequencies (14.3%) in our study, compared to Asian populations (21.6%) suggesting a possible decreased expression of the topoisomerase II gene (TOP2B). A reduction in expression of this gene has been shown to be cardioprotective[1,14,18,30]. None of the patients who developed DIC carried RARG rs2229774.

PPV and NPV are influenced by the prevalence of the disease that is being screened. In our study the prevalence of DIC was 11% and resultantly our PPV and NPV was 33.3% and 72.0% respectively. This was comparable to PPV of 34.4% and NPV 90.9% obtained in other studies[9,19,41]. With current care, the estimated average lifetime cost of DIC is $8,667 per treated patient[42]. Our test will reduce DIC related life cost and deaths in 33% of patients who carry the risk variants.

To our knowledge, this study presents the first documentation of the prevalence DIC in black Zimbabwean breast cancer patients and the first attempt to evaluate the predictive value of genetic biomarkers for cardiotoxicity. The lack of significant association between the DIC and the biomarkers is either due to the small sample size or the insufficient predictive power of these biomarkers. Future studies in adults will be needed to further evaluate the possible use of these biomarkers in guiding treatment in adults. A larger sample size with a longer follow up time will be necessary for future studies to increase the predictive power of these biomarkers because chronic cardiotoxicity occurs after a long period of time. There is also need to develop African specific DIC scoring system that incorporates both genotypes and clinical data like electrocardiogram (ECG) scores and cardiac enzymes like troponins to better predict DIC.

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Conflict of interest

No conflict of interest

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Zolk O, von dem Knesebeck A, Graf N, et al. Cardiovascular Health Status And Genetic Risk In Survivors of Childhood Neuroblastoma and Nephroblastoma Treated With Doxorubicin: Protocol of the Pharmacogenetic Part of the LESS-Anthra Cross-Sectional Cohort Study. *JMIR Res Protoc* . 2022;11(2). doi:10.2196/27898

2. Magdy T, Burridge PW. Use of hiPSC to explicate genomic predisposition to anthracycline-induced cardiotoxicity.*Pharmacogenomics* . 2021;22(1):41-54. doi:10.2217/pgs-2020-0104

3. Chang VY, Wang JJJ, Keeling NJ, et al. Pharmacogenetic testing to guide therapeutic decision-making and improve outcomes for children undergoing anthracycline-based chemotherapy. *Br J Clin Pharmacol* . 2016;11(1):1-12. doi:10.1111/bcpt.13593

4. Qiu S, Zhou T, Qiu B, et al. Risk Factors for Anthracycline-Induced Cardiotoxicity. *Front Cardiovasc Med* . 2021;8(September):1-12. doi:10.3389/fcvm.2021.736854

5. Tonk ECM, Gurwitz D, Maitland-Van Der Zee AH, Janssens ACJW. Assessment of pharmacogenetic tests: Presenting measures of clinical validity and potential population impact in association studies.*Pharmacogenomics J* . 2017;17(4):386-392. doi:10.1038/tpj.2016.34

6. Bini I, Asaftei SD, Riggi C, et al. Anthracycline-induced cardiotoxicity in patients with paediatric bone sarcoma and soft tissue sarcoma. *Cardiol Young* . 2017;27(9):1815-1822. doi:10.1017/S1047951117001536

7. Ohtani K, Fujino T, Ide T, et al. Recovery from left ventricular dysfunction was associated with the early introduction of heart failure medical treatment in cancer patients with anthracycline-induced cardiotoxicity. *Clin Res Cardiol* . 2019;108(6):600-611. doi:10.1007/s00392-018-1386-0

8. Sallustio BC, Boddy A V. Is there scope for better individualisation of anthracycline cancer chemotherapy? *Br J Clin Pharmacol* . 2021;87(2):295-305. doi:10.1111/bcp.14628

9. Aminkeng F, Ross CJD, Rassekh SR, et al. Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity.*Br J Clin Pharmacol* . Published online 2016:683-695. doi:10.1111/bcp.13008

10. Park B, Sim SH, Lee KS, Kim HJ, Park IH. Genome-wide association study of genetic variants related to anthracycline-induced cardiotoxicity in early breast cancer. *Cancer Sci* . 2020;111(7):2579-2587. doi:10.1111/cas.14446

11. Schneider BP, Shen F, Gardner L, et al. Genome-wide association study for anthracycline-induced congestive heart failure. *Clin Cancer Res* . 2017;23(1):43-51. doi:10.1158/1078-0432.CCR-16-0908

12. Christidi E, Huang H, Shafaattalab S, et al. Variation in RARG increases susceptibility to doxorubicin-induced cardiotoxicity in patient specific induced pluripotent stem cell-derived cardiomyocytes.*Sci Rep* . 2020;10(1):1-13. doi:10.1038/s41598-020-65979-x

13. Dent SF, Botros J, Rushton M, et al. Anthracycline-induced cardiotoxicity in patients with early-stage breast cancer: the Canadian Cancer Trials Group (CCTG) MA.21 experience. *Breast Cancer Res Treat* . 2020;184(3):733-741. doi:10.1007/s10549-020-05887-w

14. Aminkeng F, Bhavsar AP, Visscher H, et al. Anthracycline-Induced Cardiotoxicity in Childhood Cancer. *Nat Genet* . 2015;47(9):1079-1084. doi:10.1038/ng.3374.A

15. Aminkeng F, Ross CJD, Rassekh SR, et al. Pharmacogenomic screening for anthracycline-induced cardiotoxicity in childhood cancer. *Br J Clin Pharmacol* . 2017;83(5):1143-1145. doi:10.1111/bcp.13218

16. Sági JC, Egyed B, Kelemen A, et al. Possible roles of genetic variations in chemotherapy related cardiotoxicity in pediatric acute lymphoblastic leukemia and osteosarcoma. *BMC Cancer* . 2018;18(1):1-14. doi:10.1186/s12885-018-4629-6

17. Garcia-Pavia P, Kim Y, Restrepo-Cordoba MA, et al. Genetic Variants Associated With Cancer Therapy-Induced Cardiomyopathy.*Circulation* . 2019;140(1):31-41. doi:10.1161/CIRCULATIONAHA.118.037934

18. Loucks CM, Yan K, Tanoshima R, Ross CJD, Rassekh SR, Carleton BC. Pharmacogenetic testing to guide therapeutic decision-making and improve outcomes for children undergoing anthracycline-based chemotherapy.*Basic Clin Pharmacol Toxicol* . 2022;130(S1):95-99. doi:10.1111/bcpt.13593

19. Visscher H, Ross CJD, Rassekh SR, et al. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. *J Clin Oncol* . 2012;30(13):1422-1428. doi:10.1200/JCO.2010.34.3467

20. Pérez-Blanco JS, Santos-Buelga D, Fernández de Gatta M del M, Hernández-Rivas JM, Martín A, García MJ. Population pharmacokinetics of doxorubicin and doxorubicinol in patients diagnosed with non-Hodgkin's lymphoma. *Br J Clin Pharmacol* . 2016;82(6):1517-1527. doi:10.1111/bcp.13070

21. Cardinale D, Colombo A, Bacchiani G, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation* . 2015;131(22):1981-1988. doi:10.1161/CIRCULATIONAHA.114.013777

22. Getz KD, Sung L, Ky B, et al. Occurrence of treatment-related cardiotoxicity and its impact on outcomes among children treated in the AAML0531 clinical trial: A report from the Children's Oncology Group. *J Clin Oncol* . 2019;37(1):12-21. doi:10.1200/JCO.18.00313

23. Agunbiade TA, Zaghlol RY, Barac A. Heart Failure in Relation to Tumor-Targeted Therapies and Immunotherapies. *Methodist Debakey Cardiovasc J* . 2019;15(4):250-257. doi:10.14797/mdcj-15-4-250

24. Tan VZZ, Chan NM, Ang WL, Mya SN, Chan MY, Chen CK. Cardiotoxicity After Anthracycline Chemotherapy for Childhood Cancer in a Multiethnic Asian Population. *Front Pediatr* . 2021;9(February):1-9. doi:10.3389/fped.2021.639603

25. Hahn VS, Zhang KW, Sun L, Narayan V, Lenihan DJ, Ky B. Heart Failure with Targeted Cancer Therapies: Mechanisms and Cardioprotection. *Circ Res* . 2021;128(10):1576-1593. doi:10.1161/CIRCRESAHA.121.318223

26. Sandamali JAN, Hewawasam RP, Fernando MACSS, et al. Anthracycline-Induced Cardiotoxicity in Breast Cancer Patients from Southern Sri Lanka: An Echocardiographic Analysis. *Biomed Res Int* . 2020;2020. doi:10.1155/2020/1847159

27. Bonifaz-Peña V, Contreras A V., Struchiner CJ, et al. Exploring the distribution of genetic markers of pharmacogenomics relevance in Brazilian and Mexican populations. *PLoS One* . 2014;9(11):1-22. doi:10.1371/journal.pone.0112640

28. Lotrionte M, Biondi-Zoccai G, Abbate A, et al. Review and meta-analysis of incidence and clinical predictors of anthracycline cardiotoxicity. *Am J Cardiol* . 2013;112(12):1980-1984. doi:10.1016/j.amjcard.2013.08.026

29. Leong SL, Chaiyakunapruk N, Lee SWH. Candidate gene association studies of anthracycline-induced cardiotoxicity: A systematic review and meta-Analysis. *Sci Rep* . 2017;7(1):1-13. doi:10.1038/s41598-017-00075-1

30. Linschoten M, Teske AJ, Cramer MJ, Van Der Wall E, Asselbergs FW. Chemotherapy-Related Cardiac Dysfunction: A Systematic Review of Genetic Variants Modulating Individual Risk. *Circ Genomic Precis Med* . 2018;11(1):E001753. doi:10.1161/CIRCGEN.117.001753

31. Chang VY, Wang JJ. Pharmacogenetics of Chemotherapy-Induced Cardiotoxicity. *Curr Oncol Rep* . 2018;20(7). doi:10.1007/s11912-018-0696-8

32. Kaboré EG, Guenancia C, Vaz-Luis I, et al. Association of body mass index and cardiotoxicity related to anthracyclines and trastuzumab in early breast cancer: French CANTO cohort study. *PLoS Med* . 2019;16(12):1-12. doi:10.1371/journal.pmed.1002989

33. Roncato R, Cecchini E, Dalle Fratte C, et al. Cancer Pharmacogenetics: perspective on newly discovered and implemented predictive biomarkers. *Pharmadvances* . 2021;3(2):357. doi:10.36118/pharmadvances.2021.03

34. Kim YA, Cho H, Lee N, et al. Doxorubicin-induced heart failure in cancer patients: A cohort study based on the Korean National Health Insurance Database. *Cancer Med* . 2018;7(12):6084-6092. doi:10.1002/cam4.1886

35. Mbavha BT, Kanji CR, Stadler N, et al. Population genetic polymorphisms of pharmacogenes in Zimbabwe, a potential guide for the safe and efficacious use of medicines in people of African ancestry. *Pharmacogenet Genomics* . 2022;32(5):173-182. doi:10.1097/FPC.0000000000000467

36. Yoon GJ, Telli ML, Kao DP, Matsuda KY, Carlson RW, Witteles RM. Left Ventricular Dysfunction in Patients Receiving Cardiotoxic Cancer Therapies. *J Am Coll Cardiol* . 2010;56(20):1644-1650. doi:10.1016/j.jacc.2010.07.023

37. Nagy M, Attya M, Patrinos GP. Unraveling heterogeneity of the clinical pharmacogenomic guidelines in oncology practice among major regulatory bodies. *Pharmacogenomics* . 2020;21(17):1247-1264. doi:10.2217/pgs-2020-0056

38. Vargas-Neri JL, Carleton B, Ross CJ, Medeiros M, Castañeda-Hernández G, Clark P. Pharmacogenomic study of anthracycline-induced cardiotoxicity in Mexican pediatric patients. *Pharmacogenomics* . 2022;23(5):291-301. doi:10.2217/pgs-2021-0144

39. Scott E, Hasbullah JS, Ross CJD, Carleton BC. Reducing anthracycline-induced cardiotoxicity through pharmacogenetics.*Pharmacogenomics* . 2018;19(15):1147-1150. doi:10.2217/pgs-2018-0124

40. Magdy T, Jouni M, Kuo HH, et al. Identification of Drug Transporter Genomic Variants and Inhibitors That Protect Against Doxorubicin-Induced Cardiotoxicity. *Circulation* . 2022;145(4):279-294. doi:10.1161/CIRCULATIONAHA.121.055801

41. Rosenberg H. Cardiac function in 5-year survivors of childhood cancer. *Arch Intern Med* . 2011;171(3):264. doi:10.1001/archinternmed.2010.533

42. Dionne F, Aminkeng F, Bhavsar AP, et al. An initial health economic evaluation of pharmacogenomic testing in patients treated for childhood cancer with anthracyclines. *Pediatr Blood Cancer* . 2018;65(3):1-8. doi:10.1002/pbc.26887

Tables

Table 1: Patient demographics and clinical characteristics of the study participants

Characteristic	Frequency(%), Median(IQR) N=28
Age	48.0 (44.5-59.0)
BMI	30.1(28.7- 34.2)
Cumulative dose	238.8(233.6-240.6)
Systolic blood pressure	134.5(127.5- 150.0)
Diastolic blood pressure	90(84- 100)
Left Ventricular Ejection Fraction Baseline 3 months 6 months 12 months	63.9(59.3-71.3) 69.6(62.9-74.2) 69.6(62.9-73.4) 6
Performance status 100% 80-90% 60-70% 10-50%	1 (4.0) 24 (86.0) 3 (10.0) 0 (0.0)
Family history of breast cancer Yes No Unknown	3(10.7) 22(78.6) 3(10.7)
Hypertension Yes No	15(53.6) 13(46.4)
Diabetic Yes No	4(14.3) 24(85.7)
Radiotherapy Yes No Unknown	12(44.4) 14(51.9) 1(3.7)

Note: BMI – Body mass Index,

Table 2: Sensitivity and specificity of the genotype screening

			Expected test results	Expected test results	Expected
			ACT (n)	No ACT (n)	Total (n)
Observed test results	Observed test results	Positive Negative	TP (1) FN (7)	FP (2) TP (18)	TTP (3)
	Total	Total	8	20	28

TP (True Positive), FP (False positive), TTP (Total True Positive), FN (False Negative), TN (True Nega-

tive),

TTN (Total True Negative)

Figure legends

Figure 1: Study flow diagram

Appendices

Patients and Methods

This study was conducted at PH and African Institute of Biomedical Science and Technology (AiBST) laboratory. We included all the patients who met the inclusion criteria into the study. Inclusion criteria were; women aged 18 years and above with histologically proven breast cancer diagnosis, willingness to participate in the study and attend study follow-up visits as well as have been prescribed doxorubicin based chemotherapy but not received the first. The exclusion criteria were; non-black women, prior treatment with any chemotherapy and patients with prior chest wall radiotherapy. Five milliliter of whole blood was collected from each patient who met the inclusion criteria by venipuncture at entry and stored at the Zimbabwe repository. Two hundred microliters of the whole blood was used to extract DNA using Kingfisher flex Magnetic Particle Processor. Genomic DNA was extracted using the Applied Biosytems MagMAX DNA Multi-Sample Ultra 2.0 Kit using an automated system (King FisherFlex Magnetic Particle Processor System). The extracted DNA was quantified using Qubit, dsDNA BR (broad range) Assay Kit and run on the Invitrogen Qubit 4 Fluorometer according to the manufacturer's protocols. Fifty ng/µl was the recommended amount of DNA for genotyping by manufacturer hence nuclease free water was used to normalize the DNA to this recommended concentration. The extracted DNA was stored at either -20°C short term or -80°C long term before analysis. Written informed consent was obtained from all patients or legal guardians. The study was approved by the ethics committees of PH.

Genotyping

Genotyping was done using the GenoPharm® pharmacogenomics open array panel which contains 120 assays for over 48 genes. GenoPharm® uses TaqMan Assay chemistry using the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts). This open array system is customized for AiBST. In a 96 well plate, 6µl of each 50 ng/µl of DNA sample was mixed with 6µl of the Open Array Genotyping Master mix. The mixture was then shortly vortexed, centrifuged and then transferred into a 384 well plate. A no template control (NTC) was included on the plate. Using the robotic arm of the Applied Biosystems Accufill System the contents of the 384 well plate was transferred to an open array plate (33nl per well).The run was done on the QuantStudio 12K Flex Real-Time PCR System using the default settings pre-set by the manufacturer for an open array genotyping run. All calls were made at cycle 40 using the default quality value [?] 0.95 to assign a genotype call. Genotyping was done for UGT1A6 rs17863783, SLC28A3 rs7853758 and RARG rs2229774 variants using a predesigned primers and probes (Applied Biosystems). The thermal cycling conditions was as follows; Initial denaturation 95^{o}C for 10 minutes, followed by 40 cycles of denaturation at 95^{o}C for 15 seconds and annealing and extension at 60^{o}C for 60 seconds.

Cardiac examination

To determine DIC, cardiologists performed echocardiographic examinations and electrocardiographic recordings. The patients were evaluated for cardiovascular disease at entry (baseline), 3, 6 and 12 months during the study. The echocardiogram was done in the University of Zimbabwe College of Health Sciences (UZCHS) Department of Medicine and the results were made available to the treating physician. Additional information, such as BMI, cumulative dose of doxorubicin, co-medications and physical performance of the patients were recorded using a questionnaire. The endpoint were; cardiac dysfunction, as diagnosed by the cardiologist or Left ventricular ejection fraction reduction of more than 10% from baseline 60%.

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Figure.docx available at https://authorea.com/users/510964/articles/587904-cardiotoxicity-and-pharmacogenetics-of-doxorubicin-in-black-zimbabwean-breast-cancer-patients