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Research Article
Genetic Factors Associated with Response to Breast Cancer Chemotherapy in Burkina Faso: Case of the *CHEK2* 1100delC Mutation

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Abstract

Background and Objective: Breast cancer is a major public health burden worldwide. During chemotherapy treatment, the development of therapeutic resistance considerably compromises patients' prognosis. The aim of this study was to investigate the genetic factors associated with response to breast cancer treatment in patients in Burkina Faso, in particular the *CHEK2* 1100delC mutation. **Materials and Methods:** A case-control study has been performed from January, 2020 to August, 2022. Cases were patients with a poor response to chemotherapy according to RECIST criteria, controls were those with a good response. Specific parallel PCR has been used to characterize the *CHEK2* 1100delC mutation. **Results:** A total of seventy-eight patients have been enrolled in the study, including 38 cases and 40 controls. The mean age of the patients was 48.73±10.69 years. The SBRm grade II (84.62%) and non-specific infiltrating carcinoma (91.03%) were the most common. The majority of patients (67.95%) were on their first line of chemotherapy. No *CHEK2* 1100delC mutations were found among study patients. **Conclusion:** The management of breast cancer with chemotherapy requires relevant data to assess response to treatment. There were no *CHEK2* 1100delC mutations found in the study. Further studies, using sequencing methods, in particular, would make a considerable contribution.

Key words: *CHEK2* 1100delC, breast cancer, chemotherapy, chemoresistance, mutation, Burkina Faso

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer is a major public health burden worldwide. It is the most common cancer of all ages and sexes[1]. A total of 2,261,419 new cases were reported worldwide in 2020, representing around a quarter of all cancers in women. Breast cancer is the leading cause of cancer death in women, with 684,996 deaths in 2020, representing 15.5% of all cancer deaths[1]. In Burkina Faso, 1,927 new cases of breast cancer (24.9% of all female cancers) were reported, with 1,142 deaths (21%) in 2020[1].

Chemotherapy is a common systemic treatment for breast cancer, which can improve cure rates and reduce the risk of recurrence and metastasis. However, one of the main causes of mortality resulting from breast cancer is the development of resistance to chemotherapy, known as chemoresistance. Resistance to anti-cancer drugs is a complex phenomenon influenced by a variety of mechanisms. It can arise from intrinsic host factors, or be acquired through genetic alterations[2,3] or epigenetic[4,5]. Chemoresistance should be investigated if there are no significant changes according to chemotherapy response evaluation criteria. No reliable parameter or biomarker can adequately and effectively predict response to chemotherapy[6].

Chemoresistance is a major obstacle to improving clinical outcomes and remains a major prognostic challenge for breast cancer patients. It is therefore vital to be able to identify patients who will respond positively to treatment, versus those who will develop a relapse and show a limited response or a negative response to treatment.

This is the framework to the present research, which focused on genetic markers of response to chemotherapy in breast cancer, in this case the *CHEK2* 1100delC mutation. This mutation has been extensively studied in breast cancer and numerous studies have highlighted the association between this mutation and the risk of developing breast cancer[7-11].

However, results regarding the clinical and therapeutic implication of the mutation on the occurrence of metastases and recurrence are contradictory[12-15]. The aim of the study was to characterize this mutation in patients undergoing chemotherapy for malignant breast tumors in Burkina Faso, with a view to contributing to the improvement of care, in particular through the appropriate choice of anticancer therapies.

MATERIALS AND METHODS

Study site: The study has been conducted in two cities, Ouagadougou and Bobo-Dioulasso, in Burkina Faso. Patients have been recruited at Bogodogo University Hospital and SANDOF polyclinic in Ouagadougou and at Lorentia Clinic and Souro Sanou University Hospital in Bobo-Dioulasso. Molecular biology analyses have been performed at Molecular Biology and Genetics Laboratory (LABIOGENE) and at Pietro Annigoni Biomolecular Research Center (CERBA). This was a case-control study, conducted from January 2020 to October, 2022.

Sampling: Sampling was exhaustive during the study period. Seventy-eight female patients, with histologically confirmed malignant breast tumors who received chemotherapy at the study sites have been included in the study. The efficacy of chemotherapy has been assessed by the response rate obtained according to RECIST criteria (Response Evaluation Criteria in Solid Tumor)[16,17]. All patients with a poor response to chemotherapy, i.e., those whose response to treatment was marked by stabilization or progression, were therefore considered as cases in the study. Controls were patients with a good response to chemotherapy, including partial or complete response.

Data collection: Epidemiological information has been obtained by interviewing patients. Clinical and histological data have been obtained from patient files. Venous blood samples (5 mL) have been collected in EDTA-type anticoagulant tubes. Whole blood has been stored at +4 to +8°C until genomic DNA extraction.

CHEK2 1100delC mutation characterization

Genomic DNA extraction: Rapid Salting-Out technique has been used to extract genomic DNA[18]. First, a lysis buffer has been used to destroy blood cell membranes, followed by a succession of three washes to remove cell debris. Proteins have been then digested with proteinase K and precipitated with 5 M NaCl. Finally, nucleic acids in the supernatant have been precipitated with absolute ethanol and washed with 70% ethanol. DNA concentration and sample purity have been measured using a BioDrop spectrophotometer Biochrom® (Cambridgeshire, England, United Kingdom).

***CHEK2* 1100delC mutation genotyping:** Genotyping of the *CHEK2* 1100delC mutation has been performed using allele-specific PCR amplification, with the forward primer *CHEK2*e10F: 5'-GCAAAGACATGAATCTGTAAAGTC-3' and the mutation-specific reverse primer *CHEK2*delC: 5'-AAATCTTGGAGTGCCCAAAATAAT-3'. All samples showing the presence of a 183 bp band have been then amplified. For that, the same forward primer *CHEK2*e10F: 5'-GCAAAGACATGAATCTGTAAAGTC-3' has been used, combined with the wild-type-specific reverse primer. The sequence of this reverse primer was *CHEK2*e10R:

(5'-AATCTTGGAGTGCCCAAAATCAG-3'). This step allowed to check the mutant allele homozygosis. All reactions have been performed in the presence of an additional pair of specific primers, amplifying a region of the globin β-chain gene, which served as an internal PCR control. The PCR program used consisted of an initial denaturation step at 94°C for 5 min, then 40 cycles each comprising denaturation at 94°C (30 sec), hybridization at 55°C (30 sec) and extension at 72°C (30 sec) and finally a final extension step at 72°C for 7 min. The PCR product has been subjected to electrophoretic migration on 2% agarose gel for 45 min and visualized under UV light at 132 nm using the E-Box Gene Flash revelation device Vilber® (Marne-la-Vallee, France). In the presence of the *CHEK2* 1100delC mutation, a 183-bp sequence was amplified, along with a 300-bp sequence amplified by the additional primers and serving as an internal control for the reaction. In the case of the wild-type genotype, only the 300-bp control sequence was present.

Ethical and administrative issues: The study protocol has been approved by the CERBA/LABIOGENE Ethics Committee, reference N°2020/II-03-016. Free and informed written consent has been obtained from each participant, after the study objectives had been explained to them. Authorization for data collection have been also obtained from the management of each collection site.

Data processing and statistical analysis: Study data have been entered in Excel and analyzed using Stata 16 software. The Chi-square Test has been used to compare proportions between different categorical variables. The Student's t-Test has been used to compare means between different groups. All statistical tests in this study have been considered significant at $p<0.05$.

RESULTS

Socio-demographic characteristics: In all, seventy-eight patients have been enrolled in the study, including 38 cases (poor response to chemotherapy) and 40 controls (good response to chemotherapy).

The mean age of the patients was 48.73±10.69 years. Cases represented 43.75% of patients under 45 and 62.07% of those over 45. Mean BMI was 27.69 kg m^{-2} and 40.82% of over weight or obese patients were cases. A family history of breast cancer has been found in 10 patients, among which 30% were cases. There were no statistically significant differences between the socio-demographic characteristics of cases and controls (Table 1).

Clinical and histological characteristics: The distribution of patients according to clinical and histological characteristics was sown in Table 2. Among patients with a T2 tumor size, 57.89% were controls. Of patients with a T4 tumor size, 72.41% were cases. With regard to tumor size, there was a significant difference between cases and controls, with mainly T4 tumours in cases and T2/Tx in controls. As 60% of patients with two or more lymph nodes were cases. Metastases were largely absent in controls (60.98%), whereas they were present in cases (86.36%). These metastases were represented mainly by bone, lung and liver metastases. They were significantly more present in cases (M1) than in controls (M0). SBRm grade II and non-specific infiltrating carcinoma (NSIC) were the most common.

Therapeutic characteristics: Controls were mainly first-line treatment (71.70%) and multi-line cases (92%). The most commonly used protocols combined doxorubicin (adriamycin) and cyclophosphamide with 5-fluorouracil or a taxane (docetaxel or paclitaxel). The distribution of patients according to chemotherapy characteristics was shown in Table 3.

***CHEK2* 1100delC mutation genotyping:** The A260/280 nm absorbance ratios of the extracts ranged from 1.8 to 2. The average DNA concentration was 37.46 ng UL^{-1}. These purities

Table 1: Distribution of patients by socio-demographic characteristics

Parameters	Cases (%)	Control (%)	Total	p-value
Age				
≤45 years	14 (43.75)	18 (56.25)	32	0.464
>45 years	24 (52.17)	22 (47.83)	46	
BMI				
Normal (18-24.99 kg m^{-2})	18 (62.07)	11 (37.93)	29	0.070
High (≥25 kg m^{-2})	20 (40.82)	29 (59.18)	49	
Family history of breast cancer				
Present	03 (30.00)	07 (70.00)	10	0.205
Absent	35 (51.47)	33 (48.53)	68	

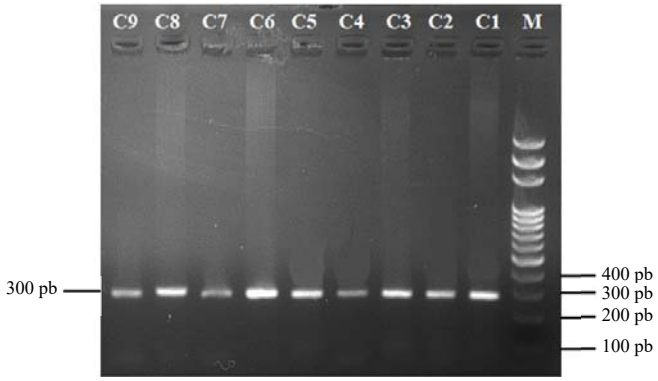

Fig. 1: Agarose gel representing *CHEK2* 1100delC mutation PCR products
M: Molecular weight marker of 100 bp and C1-C9: Samples (cases)

Table 2: Distribution of patients by clinical and histological characteristics

Parameters	Cases (%)	Control (%)	Total	p-value
Tumor size (T)				
T1	00 (00.00)	01 (100.0)	01	0.017
T2	08 (42.11)	11 (57.89)	19	
T3	02 (50.00)	02 (50.00)	04	
T4	21 (72.41)	08 (27.59)	29	
Tx	07 (28.00)	18 (72.00)	25	
Number of lymph nodes (N)				
N0	03 (42.86)	04 (57.14)	07	0.161
N1	22 (57.89)	16 (42.11)	38	
N2+	06 (60.00)	04 (40.00)	10	
Nx	07 (30.43)	16 (69.57)	23	
Metastases (M)				
M0	16 (39.02)	25 (60.98)	41	0.000
M1	19 (86.36)	03 (13.64)	22	
Mx	03 (21.43)	11 (78.57)	14	
SBRm grade				
I	02 (50.00)	02 (50.00)	04	0.995
II	32 (48.48)	34 (51.52)	66	
III	04 (50.00)	04 (50.00)	08	
Histological type				
NSIC*	36 (50.70)	35 (49.30)	71	0.264
Other histological types	02 (28.57)	05 (71.43)	07	

*NSIC: Non-specific infiltrating carcinoma

Table 3: Distribution of patients by therapeutic characteristics

Parameters	Cases %	Control (%)	Total	p-value
Line				
1st line	15 (28.30)	38 (71.70)	53	0.000
Several lines	23 (92.00)	02 (08.00)	25	
Protocol				
FAC*	11 (39.29)	17 (60.71)	28	0.018
ACT**	09 (34.62)	17 (65.38)	26	
Carboplatin/paclitaxel	09 (81.82)	02 (18.18)	11	
Others	09 (69.23)	04 (30.77)	13	

*5- fluorouracil+doxorubicin(adriamycin)+cyclophosphamide and **Doxorubicin+cyclophosphamide+taxane (docetaxel or paclitaxel)

and concentrations enabled us to consider the DNA extracts obtained for the amplification step.

After amplification by allele-specific PCR, the PCR products have been isolated by electrophoretic migration for the samples and visualized on the gel as two bands: 183 bp corresponding to the presence of the mutation and 300 bp corresponding to the control, i.e., beta-globin. None of the study samples showed the 183 bp band (Fig. 1). Thus, the *CHEK2* 1100delC mutation was not detected in any patient.

DISCUSSION

The socio-demographic characteristics with regard to 78 patients, there was no statistically significant difference between cases and controls. Thus, these two sub-populations were homogeneous and could be used for further study. As for the clinical and histological characteristics of the patients, significant differences had been found between cases and controls in terms of tumor size and the presence of metastases. In fact, cases presented mostly T4 tumors, whereas controls presented mostly small tumors (T2). Tumor size has always been used to staging breast cancer and guiding treatment recommendations. Numerous studies have highlighted the association between tumor size and response to breast cancer chemotherapy[19,20]. Thus, small tumors are more likely to respond to chemotherapy[21] and the degree of response is inversely proportional to initial tumor size in tumors over three centimeters[22].

Furthermore, in the study, none of the patients carried the *CHEK2* 1100delC mutation, based on the method used. Indeed, the *CHEK2* (Checkpoint kinase 2) gene is a tumour suppressor gene mapped to chromosome 22 (22q12.1), where it spans 54 kb[23]. Its canonical transcript has 15 exons and codes for a 543 amino acid, 65 kDa serine-threonine protein kinase[24]. This protein, known as *CHEK2* or *CHK2*, is involved in cell cycle control, in particular DNA damage repair. The *CHEK2* may enhance phosphorylation of the p53 gene at serine position 20, this gene constituting a tumor suppressor. In addition, *CHEK2* can prevent the binding to p53 of the murine double protein micro-2, also known as MDM2, thus improving p53 stability[25]. The p53 can induce G1 arrest by activating transcription of the p21CIF1/WAP1 gene, which inhibits the activity of the *CHEK2*/cyclin E cyclin-dependent complex. In addition to G1 arrest induced by p53 activation, activated *CHEK2* can phosphorylate and then degrade CDC25A, acting as a G1/S sensing point, thus blocking DNA synthesis.

Mutations in the *CHEK2* gene, such as 1100delC, have been implicated in genetic syndromes predisposing to cancer, particularly Li and Fraumeni syndrome. The *CHEK2* 1100delC mutation is due to a deletion of a nucleotide, cytosine, at the 1100th nucleotide of the *CHEK2* gene. This deletion results in premature termination in the kinase domain of the *CHEK2* protein. Several authors have highlighted the involvement of *CHEK2* mutations in the development of cancer. Its involvement in resistance to chemotherapy molecules has also been suggested[26-28]. Wang *et al.*[29] demonstrated that the *CHEK2* Y390C mutation could inhibit *CHEK2* efficacy in response to DNA-damaging agents, indicating that the Y390C mutation significantly altered *CHEK2* function in response to DNA damage. Another study by Luo *et al.*[30] explored the mechanism of *CHEK2* gene dysfunction in drug resistance of triple-negative breast cancer cells. Thus, the study concluded that the *CHEK2* Y390C mutation induced cell resistance to cisplatin. Furthermore, the *CHEK2* Y390c mutation could impair cisplatin-induced inhibition of cell apoptosis and cell cycle arrest[30].

No cases of the *CHEK2* 1100delC mutation were found among the patients in the study. Several studies found low or no frequencies of the mutation in various cancers, including breast cancer[10,31,32]. An international study, including patients of diverse origins, concluded that the *CHEK2* 1100delC mutation seemed to be reserved for women of European origin, including Ashkenazi Jews and French-Canadians. Few data are available on Burkina Faso. Because *CHEK2* is a tumor suppressor gene, it is involved in DNA repair and chromosome stability. In this context, it could be mutated and become carcinogenic[32,33]. The *CHEK2* mutations have been widely shown to be involved not only in the development of cancer, but also in resistance to anti-cancer therapies[26-28,34].

At the end of the study, several observations can be made. Firstly, the main limitation was the size of the sample as only 78 patients, given the study's inclusion criteria. Further studies with more representative sample sizes would be of considerable value. In addition, a specific PCR-allele technique was used. It would be essential to be able to apply highly sensitive sequencing techniques, which would enable more robust conclusions to be drawn. Beyond these limitations, recommendations can be made regarding the management of breast cancer patients. Before starting chemotherapy, it would be useful to study the mutational profile of patients, in order to effectively adapt the choice of molecules to be used.

CONCLUSION

The current results found no cases of *CHEK2* 1100delC mutation in the population. The study involved 78 patients with breast cancer, some of them with a good response to chemotherapy, others with failure. An allele-specific PCR was applied to the samples and there were no cases of *CHEK2* 1100delC mutation. Further research is needed to broaden the study population and to use more sensitive methods such as sequencing techniques, which would also enable other mutations to be targeted.

SIGNIFICANCE STATEMENT

In Burkina Faso, many studies have focused on the genetic aspects of breast cancer, but very few in their association with therapeutic failures. The aim of this study was

to identify genetic factors implicated in chemo-resistance in breast cancer, in order to improve patient management. Patients with histologically confirmed breast cancer were recruited. In these patients, a mutation likely to lead to chemo-resistance was sought using a molecular biology technique. No cases of this mutation were detected in the study population, therefore, treatment should not be readjusted. Thus, the cases of therapeutic failure in the study patients were not due to this mutation, but to other genetic mechanisms. Future research should therefore target other genes potentially involved in chemo-resistance.

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