CHROMOSOMAL RADIOSENSITIVITY AND INSTABILITY IN TRIPLE NEGATIVE AND/OR YOUNG BREAST CANCER AND FANCONI ANAEMIA PATIENTS IN SOUTH AFRICA

ABSTRACT

Introduction: Breast cancer is the leading cancer in women in South Africa (SA). Triple negative breast cancer (TNBC) is clinically characterised by the lack of expression of estrogen, progesterone and HER2/NEU receptors. These breast cancers occur frequently in young African women and are associated with aggressive disease progression, poor prognosis and *BRCA1* mutations. TN patients with operable tumours may undergo surgery under general anaesthetics. Treatment of TNBC poses a clinical challenge as these tumours are unresponsive to hormonal or HER2 targeted therapy. Defects in *BRCA1* and other DNA repair genes contribute to chromosomal instability and radiosensitivity and cause irregularities in the cell cycle checkpoints in the S/G2 phase.

Studies have shown the overlap of breast cancer susceptibility genes and Fanconi Anaemia (FA) genes. FA is an autosomal recessive disorder defined by cellular hypersensitivity to DNA cross-linking agents such as mitomycin C (MMC) and defects in DNA repair genes. FA patients are known to be radiosensitive and have defects with DNA repair. These patients are at high risk to develop leukaemia and solid tumours that may require radiotherapy. Diagnosis of FA patients often includes detecting chromosomal aberrations induced by a cross-linking agent. Molecular tests are also conducted to identify mutations in FA genes. It has previously been shown that FA patients undergoing radiotherapy display increased clinical radiosensitivity. Evidence suggests that FA patients are chromosomally radiosensitive to ionising radiation (IR).

Chromosomal radiosensitivity can be evaluated using the cytokinesis-block micronucleus (CBMN) assay in different phases of the cell cycle. Micronuclei (MNi) serve as biomarkers for radiation-induced DNA damage repair and defects in DNA repair mechanisms can be reflected in chromosomal radiosensitivity. A number of factors could influence the MNi yield such as storage time and temperature, and cytotoxic agents such as anaesthetics. As radiotherapy is considered a principle treatment in the management of TNBC, it is important to investigate in vitro chromosomal radiosensitivity of South African TN breast cancer patients. Chromosomal

instability and radiosensitivity of FA patients has previously not been investigated in SA. The overall aim of this study was to investigate chromosomal instability and radiosensitivity of lymphocytes in South African breast cancer patients, FA patients and parents compared to healthy individuals using the G0 and S/G2 CBMN assay. The effect of age, ethnicity and mutations in breast cancer susceptibility genes was also investigated. Furthermore, storage time and effect of anaesthetics on MNi yield was investigated.

Methods: For the G0 MN assay, heparinised blood in culture medium was irradiated at 0Gy (Baseline), 2 and 4 Gy followed by the immediate stimulation of lymphocytes using phytohaemagglutinin (PHA). Cytochalasin B was added 23 hours later to inhibit cell division. The S/G2 MN assay is a modified version of the G0 MN assay. In this assay, the cultures are first stimulated with PHA and irradiated 72 hours post stimulation. Eight hours post irradiation cells were fixed. The Mitomycin C (MMC) MN assay is similar to the G0 MN assay except the DNA damage is induced using MMC.

Results: Chromosomal instability is significantly elevated in TNBC, young and older breast cancer patients. Radiation-induced MN values in the G0 MN assay are significantly enhanced in a total unselected group of breast cancer patients compared to healthy individuals. However, when subdividing the breast cancer patients in a TNBC group, the enhanced radiation-induced MNi are not observed. We cannot demonstrate a correlation between the age of the patients and chromosomal radiosensitivity but an effect of ethnicity is noted in our breast cancer population. In the S/G2 MN assay, TNBC patients continued to exhibit a decreased chromosomal radiosensitivity. We also demonstrated that increased storage time can influence MNi yields in patients and controls; anaesthetics influenced spontaneous MNi yields.

The FA patients in our study demonstrate higher MNi when compared to parents and controls indicating chromosomal instability and chromosomal radiosensitivity in the G0 as well as in the S/G2 phase of the cell cycle. This is not seen in the FA heterozygotes. With the MMC assay, the detection of significantly higher MN is noted in as well the FA patients as well as the FA carriers.

Conclusions: Chromosomal instability and radiosensitivity of breast cancer and FA patients are notably higher when compared to healthy individuals. The association of BRCA mutations in TN and young patients highlight the importance of radiosensitivity information in the understudied SA population. FA carriers can be at risk for breast cancer with mutations associated with breast cancer susceptibility genes. As a functional assay, the MMC MN assay will be useful in the identification of FA carriers who may be at risk of breast cancer. Data on radiosensitivity of patients with defects in DNA repair genes could provide important information for radiotherapy management of cancer.