Review Article

Preimplantation Genetic Testing for Breast Cancer

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Abstract

Breast cancer (BC), a malignant tumor characterized mainly by a lump in the breast and a change in breast shape, has plagued many women of childbearing age in Nigeria today. This has thus propelled many to find both prophylactic and curative agents to combat BC in affected persons. This article extensively reviews a method of preventing BC in the offspring of affected parents, known as preimplantation genetic testing (PGT) – an assisted reproductive technique that selects genetically unaffected embryo(s) to be transferred to the uterus of a mother upon *in vitro* fertilization and standard genetic analysis. The present study also seeks to present the techniques involved in PGT that have been reported to prevent the inheritance of BC, its benefits and risks, related case studies in Africa and other continents, and ethical issues surrounding the application of assisted reproduction for BC testing. To achieve these, a thorough search was conducted in reputable scientific journals of reproduction and cancer, and expert knowledge was consulted with regard to these aspects of health and reproduction. Upon reviewing this very important subject, it was confirmed that the beneficial role of assisted reproduction in the field of science and the homes of many cannot be overestimated. This review of the role of PGT in BC prevention will enlighten the understanding of many – creating awareness that with PGT, BC-affected women can have not only children, but also healthy and genetically unaffected children.

Keywords: Breast cancer, genetic analysis, *in vitro* fertilization, preimplantation genetic testing

Introduction

It is of little or no surprise that breast cancer (BC) has led to the death of many women around the globe.[1,2] As a malignancy of the breast tissue, BC has become a plague common in females of reproductive age in Nigeria and Africa as a whole.[3,4] Another crisis of BC is its high inheriting capability of the BCRA1 and BCRA2 gene mutation that leads to cancer[5] such that in addition to facing a lifetime risk of cancer, BRCA mutation carriers also need to cope with a 50% chance of transmitting the mutation to their children.[6] The gene mutation carriers, either male[7] or female, thus have a desire that their offspring are genetically normal, not carriers of the gene mutation. In the quest to fulfill this desire, several reproductive options have developed over time, two of which have proved worthy and can lead to the testing and consequent avoidance of the mutation in offspring, namely prenatal diagnosis (PND) and preimplantation genetic testing (PGT). While PND (including chorionic villus sampling and amniocentesis) involves the testing of the fetus for the presence of the BRCA1/2 mutation during pregnancy[8,9] where unfavorable results may lead to the parents having to make the difficult decision of pregnancy termination or bearing a child who has the tendency to develop breast malignancy, PGT offers a chance to select genetically unaffected embryos even before implantation.[10,11] It is important to note that PGT is a replacement of preimplantation genetic diagnosisand preimplantation genetic screening.[12] Assisted reproductive technologies (ARTs) have indeed offered a better alternative to many BRCA1/2 mutation-affected parents who, beyond the choices of gamete donation and adoption, seek to have their own biological children. With the embryos "created" and tested for *in vitro*, the major benefit of PGT for BC is that only the unaffected embryos are implanted to try to conceive, nullifying any chances of the mutation being present in the offspring and subsequent generations even in a family with a past medical history of BC. With advances in technology, assisted reproduction has only seen tremendous progression, leaving various historical landmarks that create platforms for more hope-giving techniques; some of these events include:

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Access this article online

Quick Response Code:

Website: www.nigeriamedj.com

DOI: 10.4103/nmj.NMJ_124_18

How to cite this article: Oribamise EI, Ashiru OA, Iloabachie EC, Osumah JG, Moruf OO. Preimplantation genetic testing for breast cancer. Niger Med J 2019;60:99-105.

- 1978: Birth of the first *in vitro* fertilization (IVF) baby by Steptoe and Edwards[13,14]
- 1989: First applications for PGT (in testing monogenic disorders and sex-linked disorders using polymerase chain reaction [PCR] for the Y chromosome from a blastomere) by works done by Handyside *et al*.[15,16]
- 1990: First successful PGT baby born for X-linked disorder[17]
- 1990: Discovery of BCRA mutation in BC by Hall and team[18]
- 1999–2001: PGT for late-onset common disorders with genetic predisposition[19,20]
- 2008: The first report of a baby born after PGT for the BCRA mutation[21]
- 2015: First PGT in Nigeria.[22]

Methodology of Information Gathering

During this review, the main search engine employed for the gathering of information was Google Scholar[23,24] which is known for its vastness in abstracts, papers, patents, theses, and dissertations. Citations of related studies were further searched for, and an in-depth review was carried out correspondingly. Furthermore, experts who have contributed to both fields of interest (i.e., assisted reproduction and BC) were consulted through ResearchGate for further expertise so as to arrive at this robust and well-informative review. Magazine articles and television documentaries also served as good information sources. MEDLINE and PubMed indexes[25,26] were also used as search engines in this review.

Nigeria and Preimplantation Genetic Testing for Breast Cancer

Although PGT is widely used in many other parts of the world, it is still a growing aspect of IVF in Nigeria. PGT has, in many localities, been made similar to the "designer baby" concept. Among other objectives, this article thus seeks to shed more light on the PGT technique as a beneficial one rather than a mere experimental one. In PGT, testing is carried out, and healthy embryos are chosen for the transfer and subsequent birth of a healthy child – thus, it is more diagnostic rather than therapeutic.

Nigeria is one of the developing countries, where PGT for BC may not yet be acceptable as a result of various factors which may include culture, religion, or individual beliefs. Below are some statistical statements of problem regarding BC that may influence the adoption of PGT by the medical community and the general public in Nigeria.

In 2008, it was projected by Boyle and Levin that 70% of all new cases of cancer in 2030 will be found in developing countries.[27]

Also, in 2008, Nigeria contributed 15% to the estimated 681,000 new cases of cancer that occurred in Africa.[28]

BC (50.8%) and cervical cancer (15.7%) were found to be most common in a study after an analysis of two population-based cancer registries in Nigeria: the Ibadan Population-Based Cancer Registry and the Abuja Population-Based Cancer Registry from 2009 to 2010.[29]

Furthermore, in 2010, the Estimates of Worldwide Burden of Cancer reported that some 100,000 new cases of cancer occur every year in Nigeria, with high case fatality ratio.[30]

Aside a list of such statistics, fertility clinics in Nigeria have reported their use of PGT to help diagnose BC and the consequent testimonies of healthy babies.[31]

PGT is also currently used for aneuploidy, sickle cell screening, and family balancing in Nigeria.

Procedure of Preimplantation Genetic Testing for Breast Cancer

Upon gathering relevant information from reported studies, the following are the procedures of PGT in the testing of BC.

Pre-PGT checkup: The medical and genetic history of both partners are analyzed, a pedigree analysis is carried out, and physical observations including age and body mass index (BMI) are carried out for the female as some studies have associated obesity and increased age with reduced fertility and lower IVF success rates.[32] In addition, a 2008 study found that BMI on IVF success appeared age related,[33] where a high BMI was shown to have a pronounced negative influence on fertility, but this effect diminished as the patient's age increased. Gynecological, andrological, and oncological screening procedures (which were specially recommended by Derks-Smeets *et al*. in 2014[9]) are carried out including semen analysis to observe sperm morphology, motility, and count among other factors; female hormonal assessment of estrogen, luteinizing hormone, follicle-stimulating hormone, and anti-Mullerian hormone is carried out;[34,35] and virology tests of both partners to ensure their suitability for IVF/intracytoplasmic sperm injection (ICSI) treatment are carried out.[36] ICSI is carried out to ensure that the "best" sperm is used to fertilize the oocyte and to avoid contamination of the zona pellucida with spermatozoa (i.e., DNA contamination from residual sperm adhering to the zona pellucida), which may affect the PGT analysis.[37]

Thereafter, oocyte maturation is triggered with recombinant choriogonadotropin-α, and retrieval is done about 36 h later.[38] The mature oocytes are then each fertilized by a single sperm of good morphology, and motility was identified for ICSI. The resulting embryos are then cultured for 3 (cleavage stage) or 5 days (blastocyst stage) depending on the best type of biopsy the clinic chooses.

The cleavage-stage biopsy [Figure 1] involves the removal of a single embryonic cell (blastomere), or two, at the 6–8 cells' stage, corresponding with the 3rd day of development.[39] Although removal of two blastomeres has been shown to result in a greater decline in live birth than a single-cell biopsy, there has been increased use of two-cell blastomere biopsy, it

has been used by many centers globally.[40,41] Prior to biopsy, the embryos are transferred to calcium-/magnesium-free media to facilitate blastomere removal,[39,42] after which the zona pellucida is opened with laser, mechanical dissection, or exposure to acidic Tyrode's solution.[43] The blastomere is removed after the introduction of a biopsy pipette by aspiration or by extrusion of the cell with pressure on the outside of the zona. Furthermore, this day-3 biopsy allows 2–3 days for PGT analysis to be completed if a fresh embryo transfer is desired.[44]

Trophectoderm biopsy involves the removal of 5–10 trophectoderm cells from blastocysts on day 5 or 6 after laser-assisted hatching on day 3,[45] which creates a 25–30-µm opening in the zona pellucida, allowing herniation of the trophectoderm cells through the zona as shown in Figure 2.[45,46]

Figure 3 shows the trophectoderm biopsy/blastocyst-stage biopsy. The cells are stretched out in a biopsy pipette and removed with a laser.[47-49] In comparison to the cleavage-stage biopsy, in this type of biopsy, more cells are collected for analysis, and it is believed to be less harmful to the embryos because trophectoderm cells are removed and cells from the inner cell mass are avoided.[48,50]

Although there is a third biopsy procedure, but does not apply to this study as it is only the polar body that testing is carried out on, no paternal analysis is carried out.[51]

After cells are biopsied, they are washed in preparation for the PCR procedure. Among the many other PGT techniques, PCR analysis has been reported in many of such BC-PGT cases,[52-54] of which some cases then reported the concomitant use of a comparative genomic hybridization or next-generation sequencing for further analysis.[55,56] Upon analysis completion, the embryos are classified as affected (BRCA1/2 mutation present), unaffected (BRCA1/2 mutation absent), abnormal (abnormal genotype, e.g., haploidy or triploidy), or no testing (no test result or inconclusive BRCA1/2 status). Subsequently, one or two unaffected embryos are transferred into the uterus at day 4 or 5 postfertilization. The number of transferred embryos depends on embryo quality, female age, number of previous unsuccessful attempts, and the couples' preference for transferring only one embryo. Supernumerary unaffected embryos of sufficient quality are cryopreserved and are transferred in a subsequent cycle after thawing (defined as frozen-thawed embryo transfer).[57] Pregnancy rates are reported as positive human chorionic gonadotropin tests as well as clinical pregnancy rates. The clinical pregnancy rate is diagnosed according to the standard definition, i.e., a pregnancy diagnosed by transvaginal ultrasonographic visualization of one or more gestational sacs and appreciating any pregnancy abnormalities including ectopic pregnancies. Couples are given the option of prenatal testing (PNT) to confirm the PGT outcome. Follow-up of pregnancies and children is carried out;[58] all female BRCA1/2 carriers are contacted by telephone and inquired for their health status, including testing of BC because the last PGT treatment and prophylactic surgeries are performed in the meantime. Further testing is also encouraged, even up to postnatal testing of umbilical cord blood.

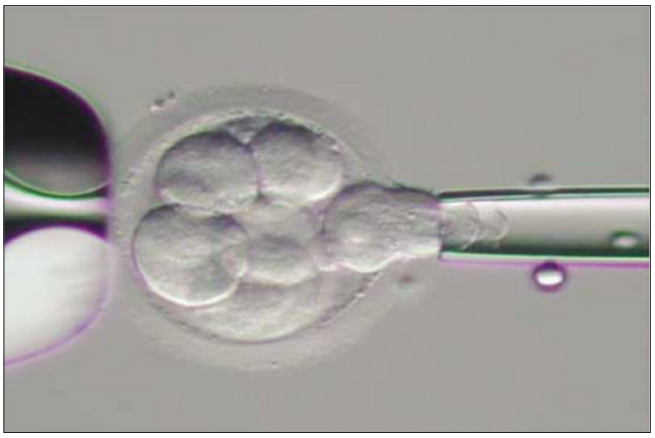

Figure 1: Cleavage-stage biopsy

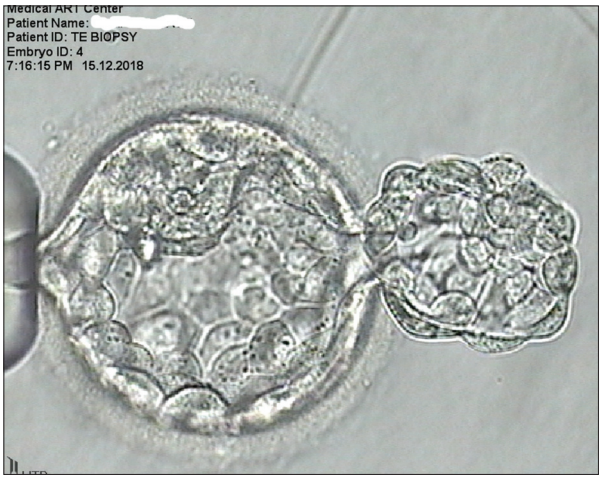

Figure 2: Herniated trophectoderm cells ready to be biopsied

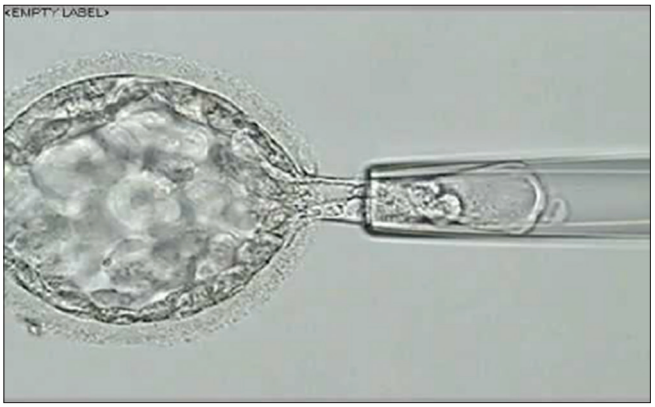

Figure 3: Blastocyst-stage biopsy

Psychological Impact of Preimplantation Genetic Testing toward Breast Cancer

Receiving a testing of BC or having a BRCA mutation is an anxiety-provoking event that can impact patients' psychosocial

well-being. Because fertility preservation must be discussed as early as possible after a BC testing, women with a BRCA mutation face the additional burden of making future-oriented reproductive decisions in a short time frame while under significant stress.[59] Some centers and studies have even reported the measurement of psychological impact of cancer and/or carrying a BRCA1 or BRCA2 mutation and the implications for future childbearing using the Impact of Event Scale.[60] The thought that there is a whooping 50% chance of one's offspring may also be diagnosed with the mutation and pass through the same traumatizing experience is another associated psychological burden; this thus leads them to make the decision of applying assisted reproduction (also considered costly) which they otherwise will not have employed, especially for those without fertility problems. A study by Woodson *et al*. in 2014 with the aim of evaluating how BRCA genetic test result disclosure and patient characteristics influence attitudes toward PGT and future childbearing in counseling women with possible or identified BRCA mutations showed that 83% of the women said that PGT should be available to families with inherited cancer syndromes.[61] Similarly, another 2014 study, but with respect to PGT–PND comparison, was carried out to determine how couples with a BRCA1/2 mutation decide on PGT and PNT for hereditary BC and ovarian cancer (HBOC) syndrome, where a group of well-informed, reproductive-aged couples carrying a BRCA1/2 mutation were interviewed and their reproductive decisional motives and considerations were studied; it was then observed that none of the couples who opted for PGT or conception without testing found the use of PND with possible pregnancy termination acceptable.[62]

Religious Bias

The attitude of the population of religious groups in several countries has a strong influence on the practice of assisted reproduction, PNT and PGT,[63] with abortion being of major worry in PND unlike the case of PGT where there is a preselection of embryos, and therefore abortion is avoided.[64] While the Roman Catholic Christian holds that the fetus has attained its status as a human being at conception, thus disapproving PGT,[65] the Jewish religion's view is that an unborn fetus is not considered a person until birth, but a part of the mother's body and not a separate being until it is delivered; in fact, they hold that prior to 40 days after conception, the fertilized egg – still an early zygote – has no status at all, it is not a person or "nefesh" (soul) and thus can be disposed of. The Islamic law may accept research on excess embryos resulting from IVF in order to increase their "ilm" (knowledge), and this may be possible in cases where it will be for the sake of the individual embryo. Researches conducted on preembryos should be limited to therapeutic research, and this includes genetic testing of a portion of the embryo, one blastomere, or its nucleus for a specific genetic defect. They view PGT as God-given knowledge in medical science to further help humans understand medical genetics. Research aimed at changing the inherited characteristics of preembryos is forbidden.[66] A study in Malaysia also reported that Christian scholars are very skeptical of the long-term use of PGT because of its possible effect on the value of humanity and the parent–child relationship.[67] Like Malaysia, Nigeria is a multireligious country, where the society places a high value on marital relationships and on the traditional concepts of family; more research should thus be carried out to see if the patronage of PGT for BC and other testing is based largely on religious views.

Downsides to Preimplantation Genetic Testing

Patients and clinicians must recognize some barriers to PGT, particularly for women diagnosed with cancer. The high cost of IVF and PGT is often a burden, particularly when combined with the cost of cancer treatment.[68]

Other downsides may include procedural risks: Like all types of medical treatments and procedures, PGT treatment has its own risks and uncertainties which are discussed with the patients before any treatment is carried out. They are otherwise known as "procedural uncertainties." Most of the risks involved in the PGT treatment are similar to those for the conventional IVF procedure, some of which include multiple pregnancies;[69] pelvic infections;[70] greater risk of premature delivery and delivery by cesarean section;[71] mechanical injury; and puncture of bowel, bladder, ureters, or blood vessels by the needle during egg retrieval. Some risks associated with the PGT-born babies may include premature birth, less weight than normally conceived babies at the same age,[71] risk of damage to embryos during biopsy,[72] hence the vast usage of blastocyst-stage biopsy for analysis, as this biopsy stage tends to leave the main embryonic cells unharmed; it is also important to note that those performing both the biopsy and the analysis must be highly technically-skilled.

Ethics of the Preimplantation Genetic Testing Procedure for Breast Cancer

For the sake of proper understanding, moral and ethical considerations have been defined and differentiated in previous studies – moral considerations such as individual principles regarding a person's conduct and ideals, usually internal, and ethical considerations such as social and external rules of conduct regarding human actions. In 2003, the Ethics Task Force of the European Society of Human Reproduction and Embryology (ESHRE) approved the PGT use for late-onset and multifactorial diseases, including HBOC. The ESHRE Task Force for law and ethics stated that "PGT for late-onset diseases is acceptable, in spite of the still-existing uncertainties concerning therapy in the time gap between the birth of the child and the onset of the disease." The task force also declared that "PGT can also be accepted in multi-factorial diseases like BRCA1/2 notwithstanding the uncertainties about the genetic predisposition and the epigenetic influence."[73] This study and other opinion surveys have shown that most BRCA carriers consider PGT for HBOC as an acceptable reproductive option, although interestingly, only a minority of them would

consider using PGT personally.[74,75] In May 2006, the UK Human Fertilisation and Embryology Authority (HFEA) approved the use of PGT for lower penetrance and late-onset cancer susceptibility syndromes such as HBOC.[74] Among other considerations, it is important to note that ethical decisions on scientific procedures are usually made based on the opinions of the recipients of such procedure. For example, studies examining the opinions toward PGT among men and women with a BRCA mutation have shown that 13%–33% would want to pursue PGT if they were trying to conceive.[75] Even after the ESHRE decision on PGT for late-onset decision, studies are still carried out by Menon *et al*., Quinn *et al*., and Julian-Reynier *et al*.,[74,76,77] reporting the opinions of PGT for BRCA mutation.

The PGT procedure has created controversy since its inception, with the ethics centering on arguments over the moral status of the embryo. Because PGT may result in the destruction of embryos and fetuses, controversy has risen between those that contend that all human embryos and fetuses have the same moral status as live-born persons (hence, they are also entitled to rights including that of not to be killed)[78] and those that hold that embryos and fetuses lack any properties such as sentience or cognitive traits that determine moral standing and so can be destroyed at will and that they lack interests and other moral claims.[79] PGT for late-onset genetic cancers such as BRCA1 or BRCA2 holds specific questions concerning the time lapse of testing and severity of the disease, consequently improving the ability of genetic counselors and health-care providers and providing health-care professionals with the appropriate information and support during BRCA pre- and postcounseling.[61,80]

Some of the controversial "ethical" issues that still remain in Nigeria are the transfer of multiple embryos in the quest to secure at least one fetus, thus leading to multiple births and, in some cases, risk to the life of the mother during childbirth. This, and other issues, thus led to the need of a regulating body in Nigeria. Even though a separate ethical body, such as the HFEA, is yet to be established in Nigeria, the practice of ART is currently being regulated by the guidelines of the Association for Fertility and Reproductive Health, a member society of the International Federation of Fertility Societies. Lagos State has also produced and launched the regulations and guidelines for ART practices including PGT.[81,82] Many fertility clinics, though, have in-house ethical teams to self-regulate their practice, and some also use higher governing bodies, for example, medical ART center is guided by the regulations and guidelines of American Society for Reproductive Medicine and American Board of Bioanalysis. Even though the AFRH currently stands in the gap to reduce ART abuse, there is still a call – an advocacy to establish an ethical body to strictly regulate this practice. This will also, in turn, make patients more receptive to the concept of IVF, PGT, and ART as a whole.

Conclusion

The medical community and the general public of both in the Nigeria and the world at large should have a deep understanding of the entire concept of PGT for early Breast Cancer diagnosis, as this will enable all to know the associated benefits, risks, etc., as reviewed in the present study.

Acknowledgment

The authors sincerely thank the Oribamise family, the staff of the Medical ART Center for technical help, and the members of staff of the Department of Anatomy at Babcock University for their never-ending support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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