Cumulative outcome of pre-implantation genetic diagnosis for sickle cell disease: a 5-year review

Saaliha Vali,1 Sunbal Mukhtar,1 Anupa Nandi,1 Kieren Wilson,1 Laura Oakley,2 Tarek El-Toukhy1 and Eugene Oteng-Ntim1

1Department of Obstetrics and Gynaecology, Assisted Conception Unit, Guy’s and St Thomas’ Hospital NHS Foundation Trust, and 2London School of Hygiene and Tropical Medicine, London, UK

Received 9 April 2020; accepted for publication 7 June 2020
Correspondence: Saaliha Vali, Department of Obstetrics and Gynaecology, Guys and St Thomas’ NHS Trust, London, Westminster Bridge Road, London SE1 7EH, UK. E-mail: s.vali@nhs.net

Summary
To review the cumulative outcome of pre-implantation genetic diagnosis (PGD) cycles performed for prevention of sickle cell disease (SCD). Couples referred for PGD for SCD between April 2012 and October 2017 were included. Ovarian stimulation was performed using a short gonadotrophin-releasing hormone (GnRH) antagonist protocol and follicle-stimulating hormone injections. The GnRH agonist was used to trigger oocyte maturation. Oocytes were fertilised using intracytoplasmic sperm injection. Trophectoderm biopsy was performed on day 5 or 6 followed by vitrification. Genetic testing was done using pre-implantation genetic haplotyping. A total of 60 couples started 70 fresh PGD cycles (mean 1.2 cycles/couple) and underwent a total of 74 frozen-embryo-transfer (FET) cycles (mean 1.3 FET/couple). The mean (SD) female age was 33 (4) years and the mean (SD) anti-mullerian hormone level was 22.9 (2.8) pmol/l. The cumulative live-birth rate was 54%/PGD cycle started and 63%/couple embarking on PGD. The rate of multiple births was 8%. The cumulative outcome of PGD treatment for prevention of SCD transmission is high and PGD treatment should be offered to all at-risk couples.

Keywords: in vitro fertilisation (IVF), pre-implantation genetic diagnosis (PGD), reproduction, sickle cell disease (SCD), single-gene disorder.

Introduction
Sickle cell disease (SCD) is an autosomal recessive haemoglobin disorder that affects 300 000 newborns globally. In England, the national newborn sickle cell screening programme revealed sickle cell disorders in England are as common as cystic fibrosis.1

SCD is characterised by lifelong morbidity and a shorter life span.2 Prenatal diagnosis in the form of chorionic villus sampling or amniocentesis offers couples who are at risk of having a baby affected with the condition to opt for a termination of pregnancy. These invasive tests are associated with a 1% risk of miscarriage.3 Non-invasive prenatal testing, which has no procedure-related risk of miscarriage, is currently unavailable on the National Health Service (NHS) because of the high false positive rate of almost 8%.4 Prenatal diagnosis may prove undesirable to couples who find the option of pregnancy termination unacceptable for religious or personal reasons. Thus, alternative options available to avoid giving birth to an affected child include a change of partner, use of donor gametes, adoption or opting to forgo having children altogether. Furthermore, several countries have introduced premarital screening for at-risk couples.5–7

For these couples, pre-implantation genetic diagnosis (PGD) offers a tangible choice. In the UK, couples who are at risk of having a child affected with SCD and have no unaffected children are entitled to a maximum of three state-funded PGD cycles. There are a limited number of centres licensed to provide PGD services in the UK of which Guy’s and St Thomas’ NHS Foundation Trust (GSTFT) is the largest. Almost half of the inner-city diverse population that the GSTFT serves are from Black and Minority Ethnic (BAME) groups, and hence the population at risk of having a baby affected with SCD is significant. A high rate of successful pregnancy would be important for haematologists to know in order to refer patients for this reproductive option, particularly if they already have a child (or know of one) who has SCD. Furthermore, none of the previous studies have assessed the cumulative success rate of this reproductive option, which could undermine effective counselling offered to at-risk couples. This cohort study provides an analysis of the cumulative outcome of PGD

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doi: 10.1111/bjh.16930
treatment in couples who have embarked on PGD for the prevention of SCD during a 5-year period in an inner London tertiary referral PGD centre.

Patients and methods

Patients

The PGD Centre at the GSTFT received tertiary referrals from regional Fertility and Haematology centres looking after couples who were identified as being at risk of conceiving a child affected with SCD. Prior to attending the PGD Centre, couples were sent a PGD information pack, including detailed information of the NHS funding eligibility criteria, PGD process and time scale, the likelihood of success, and the associated risks of the procedure. Eligible couples were seen by a senior PGD genetic counsellor and a reproductive medicine specialist, where details of the PGD treatment were explained as described previously.8

Design

A 5-year cohort study of couples undergoing PGD to prevent SCD in the offspring.

In vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) protocol

The gonadotrophin-releasing hormone (GnRH) antagonist ovarian stimulation protocol was used in all cycles and the choice of the daily dose of the follicle-stimulating hormone (FSH) injections and monitoring of ovarian response was carried out as described previously.9 Oocyte maturation was induced using 50 iu of buserelin acetate (Suprecur, Sanofi, Guildford, Surrey, UK). Transvaginal ultrasound-guided retrieval of cumulus oocyte complexes was performed 36 h after the buserelin injection and ICSI was used for oocyte fertilisation as described elsewhere.8,10,11

PGD protocol

Embryos were assessed using an embryoscope. Opening of the zona pellucida was accomplished on day 3 after fertilisation by laser penetration followed by extended IVF culture to the blastocyst stage. On day 5 or 6 after fertilisation, fresh blastocysts were assigned grades according to strict morphological criteria,12,13 which were not changed during the study period. Criteria for blastocyst suitability for biopsy have been described elsewhere.14 Biopsied blastocysts were vitrified using a Cryolock device (Biotech Inc., Alpharetta, GA, USA) and Vitrolife vitrification medium (FUJIFILM Irvine Scientific, Newtownmountkennedy, Co. Wicklow, Ireland) on the same day of biopsy. Genetic testing was carried out as described previously.15

Frozen-thawed embryo transfer

One or two genetically suitable [non-carrier (HbAA) and carrier (HbAS)] embryos were selected for transfer on day 6 of progesterone supplementation in a subsequent medicated frozen embryo transfer cycle.14

Cycle outcome

The primary outcome was the live-birth rate. Secondary outcomes were pregnancy, clinical pregnancy, implantation and miscarriage rates. Pregnancy was defined as a positive human chorionic gonadotrophin (hCG) test using a commercial urinary testing kit 11 days after embryo transfer. A clinical pregnancy was defined as the observation of fetal cardiac activity on ultrasound scan at ≥4 weeks after embryo transfer. Implantation was defined as the presence of an intrauterine gestational sac on ultrasound scan at ≥4 weeks after embryo transfer. Miscarriage was defined as pregnancy loss before 23 weeks gestation. All pregnancies were followed-up until delivery. Live birth was defined as a live born infant after 23 completed weeks of gestation.

Data collection and statistical analysis

For the purpose of this study, data were collected prospectively. Patient demographic and baseline data, PGD cycle characteristics and treatment outcomes were recorded in a relational database. For normally distributed continuous variables, data were summarised as means with standard deviations (SDs). For continuous variables, data were reported as medians and interquartile ranges (IQRs). Categorical baseline and dichotomous data were reported as absolute numbers and percentages. Univariate analysis of the study outcome measures and the associated clinical variables was performed using two-sample t-test or Mann–Whitney test as appropriate. StatView software (Statview Corp., Berkeley, CA, USA) was used for statistical analysis. A P < 0.05 was considered as statistically significant.

Ethical approval

This study was approved by the Local Research Ethics committee (Ref: 15407-1). Our study involved neither therapeutic intervention nor change of our routine IVF protocols or data collection. Each couple gave written informed consent for the use of their data anonymously for audit and research purposes upon enrolment into our IVF programme and before starting an IVF cycle in accordance with the UK Human Fertilisation and Embryology Authority (HFEA) regulations.

Results

A total of 60 couples were referred for PGD for the prevention of SCD between April 2012 and Oct 2017, and
undertook a total of 70 fresh PGD stimulation cycles. The mean (SD) interval between the initial consultation to the start of PGD treatment cycle was 5 (2) months. All cycles were funded by the UK NHS.

Table I depicts patients’ demographics. In 52 couples, both partners were sickle cell carriers with a genotype of HbAS/HbAS and in eight couples one partner was affected by the condition (HbSS) and the other partner was a carrier (HbAS). Of these eight couples, the female partner was affected in three cases (HbSS/HbAS) and the male partner was affected in five (HbAS/HbSS). In 92% of couples (n = 55), both partners were of Afro-Caribbean origin and in 8% (n = 2) one partner was of Middle-Eastern/North African ethnicity. Almost two-thirds of couples (n = 39) lived in the lowest three most deprived quintiles as measured by the English Index of Multiple Deprivation.16

Of the 60 female partners included in the study, 36 (60%) had not previously had an affected pregnancy, 11 (19%) had terminated a pregnancy affected with SCD after prenatal diagnostic testing, and 13 (21%) had given birth to either one (12) or two (one) children affected with SCD.

**PGD cycle characteristics**

All the women underwent the short GnRH antagonist ovarian stimulation protocol and all 70 fresh PGD stimulation cycles reached oocyte retrieval. The mean (SD) number of oocytes retrieved was 16 (10), normally fertilised oocytes was 9 (6), blastocysts suitable for biopsy was 6 (4), and genetically suitable blastocysts for transfer was 4 (3).

In eight (11%) of the 70 fresh PGD cycles started, there were no blastocysts suitable for biopsy on day 5 or 6 after oocyte fertilisation. In one of the remaining 62 cycles, none of the biopsied blastocysts were deemed genetically suitable for transfer. Overall, genetic testing showed that 59% of blastocysts biopsied were unaffected by SCD and were therefore genetically suitable for transfer. Couples who had at least one genetically suitable embryo for transfer underwent a total of 76 frozen-embryo-transfer (FET) cycles Table II, of which 61 (80%) were single-embryo transfers (SET) and 15 (20%) were double-embryo transfers (DET).

**Table I. Patients’ demographics and sickle cell status.**

| Variable                        | Value       |
|---------------------------------|-------------|
| Maternal age, years, mean (SD)  | 33 (4.3)    |
| Paternal age, years, mean (SD)  | 37 (5.4)    |
| Maternal BMI, kg/m², mean (SD)  | 25 (3)      |
| AMH level, pmol/l, mean (SD)    | 22.9 (2.8)  |
| Sickle cell carrier status, n (%)| Both 52 (87) |
|                                 | Mother affected, Father carrier 3 (5) |
|                                 | Father affected, Mother carrier 5 (8) |

AMH, anti-müllerian hormone; BMI, body mass index.

**PGD cycle outcome**

There were no cases of misdiagnosis in any of the PGD cycles. Following the 76 FET cycles performed, a positive urinary hCG test was detected in 43 cycles (57%) and 42 of these went on to achieve a clinical pregnancy (55%), with an implantation rate of 50% (45/91). Of these, four miscarried before 12-weeks gestation (9% miscarriage rate) and 38 had live births resulting in a live-birth rate of 54% per fresh PGD cycle started and 63% per couple starting PGD treatment. Of the 38 live births, there were 35 (92%) singleton births, two twin births and one triplet birth after a double embryo transfer whereby one sac resulted in monozygotic twins and a singleton in the second sac.

Amongst the SET cycles, the clinical pregnancy rate per embryo transfer was 59%. Of the 15 DET cycles, the clinical pregnancy rate per embryo transfer was 40%. No cases of misdiagnosis were reported in this series.

The three cases in which the female partner was affected by SCD (HbSS/HbAS) involved women aged 31, 33 and 35 years. One woman (aged 35) underwent three cycles and the other two women went through one cycle each. All three women successfully gave birth. The live-birth rate per fresh PGD cycle started was 60% and per couple was 100%.

**Discussion**

The uptake of PGD and the number of genetic conditions for which PGD treatment is approved have been rising in the UK in recent years. Therefore, the need for an accurate assessment of the cumulative chance of success per cycle for couples embarking on PGD treatment is paramount to enable effective patient counselling. As a primary prevention measure, timely identification of carrier couples is key to the

**Table II. Pre-implantation genetic diagnosis (PGD) treatment outcome.**

| Variable                        | Value       |
|---------------------------------|-------------|
| Number of couples treated       | 60          |
| Number of fresh PGD cycles started| 70         |
| Mean number of PGD cycles/couple| 1.2         |
| Mean number of cycles that did not reach biopsy stage | 8 |
| Number of cycles not reaching embryo transfer | 1 |
| Total number of FET cycles      | 76          |
| Mean number of FET cycles/couple| 1.3         |
| Single-embryo transfers, n (%)  | 61 (80)     |
| Double-embryo transfers, n (%)  | 15 (20)     |
| Positive pregnancy test, n (%/FET cycle) | 43 (57)    |
| Clinical pregnancy, n (%/FET cycle) | 42 (55)    |
| Miscarriages, n (%/pregnancy)   | 4 (9)       |
| Live births, n (%/fresh PGD cycle started) | 38 (54)    |
| Live births, n (%/couple having PGD treatment) | 38 (63)    |

FET, frozen-embryo-transfer. Values are provided as mean values or percentages.
success of PGD, offering at-risk couples a valuable reproductive option and gradually reducing disease burden over time.

The present prospective cohort study from an inner-city tertiary PGD referral centre in London showed that for couples at risk of having an offspring affected with SCD, the live-birth rate for PGD per couple embarking on PGD treatment was 63% after a mean of 1-2 fresh PGD cycles started. These figures are significantly higher than previous reports over the last two decades, in which the live-birth rate following PGD ranged between 13% and 53%. Furthermore, recently published data from the European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium reported a clinical pregnancy rate of 31% per embryo transfer, compared to 55% clinical pregnancy rate per embryo transfer in our present study. The embryo-implantation rate was also higher in our present study compared to the study of De Rycke et al. (50% vs. 23%).

Given that currently eligible couples for NHS-funded PGD treatment are allowed up to three completed PGD cycles, the cumulative live-birth rate per couple could be even higher should unsuccessful couples opt to utilise all available funded cycles, suggesting that PGD could be a highly successful and cost-effective reproductive option for at-risk couples.

The majority of transfers in our present study were SET, resulting in a low multiple-birth rate (8%), adding to the safety and acceptability of PGD as a realistic reproductive option for couples at risk of conceiving a child affected with SCD. Our low multiple-birth rate is consistent with the upper limit of 10% set by the UK HFEA to curb the multiple-birth rate in the UK, and is considerably lower than that reported recently by the ESHRE PGD Consortium.

The favourable success rates achieved in the present study are likely to be related to a number of factors; young age of the couples included in the study (mean maternal age 33 years), the consistency of our experienced PGD team, use of trophectoderm biopsy and embryo vitrification techniques that are known to yield favourable post-thaw embryo survival and implantation rates. In addition, only high quality blastocysts were selected for the trophectoderm biopsy and vitrification, and most of the couples included in the study had no history of conception delay.

Conversely, PGD does result in the exclusion of genetically unsuitable embryos, thus the pool of embryos available for transfer is much smaller than that in the general IVF population. Furthermore, women with SCD carry unique risk factors that may negatively influence their ability to conceive including oxidative stress and ovarian sickling. Therefore, the commendable rates achieved in the present study are of the utmost significance for the SCD population.

The economic burden to the NHS across an affected individual’s lifetime needs to be carefully considered. It has been reported that the cost of an acute painful sickle episode is estimated to be between £400–600/day. Additionally, it is important to consider the sequelae of anaemia, including the requirement for repeated blood transfusions and management of organ failure. Currently, the overall cost of a PGD cycle is approximately £8000, and with further refinements, including in the technique of genetic testing and utilisation of all frozen embryos, the cost of PGD could be reduced. It is therefore reasonable to suggest that for at-risk couples, PGD needs to be part of the reproductive decision making and should be considered earlier in the process to maximise their chance of success. Point of contact clinicians such as general practitioners, haematologists and obstetricians involved in the care of patients who are carriers or affected with SCD should remain informed about the option of PGD. If utilised well, PGD can serve as an effective primary prevention measure in reducing the global burden of disease.

Despite many couples being aware of their genetic risks either due to family history, prior screening or having an affected child, a large proportion are unaware of the existence of PGD as a reproductive choice. A recent survey revealed that only 44% of couples were aware of PGD and all parents within the survey who were educated on the option reported they would consider PGD for subsequent children.

Although such a high success rate of PGD for SCD will help improve the uptake of PGD, other factors associated with the acceptance of PGD need to be considered, including moral, political and religious values. In a large cross-sectional study involving 1006 respondents, the overall support for PGD was only 66% in favour of applying PGD for diseases causing lifelong disability. In earlier studies of carrier parents, PGD is seen primarily as an opportunity to avoid termination of pregnancy, and was seen more favourably than adoption, donor insemination and egg donation. In the same study, 85% of carriers of recessive genetic disorders acknowledged that having a genetic link to the child is an important factor in choosing PGD. Therefore, carefully designed public health campaigns are required to highlight this important reproductive option and its high cumulative success rate, particularly when patients are able to utilise all the embryos created in the fresh cycle through multiple FET attempts. This enhanced awareness may also play a part in influencing a change in the governing laws of some countries to allow at-risk couples to reproduce as they now have a viable, safe, successful and cost-effective treatment option to have an unaffected child.

Author contribution

Tarek El-Toukhy and Anupa Nandi designed the research study. Saaliha Vali, Anupa Nandi, Sunbal Mukhtar and Laura Oakley analysed the data. Saaliha Vali, Kieren Wilson and Eugene Oteng-Ntim wrote the manuscript. Tarek El-Toukhy critically revised the manuscript and all authors approved its final version.
Conflicts of interest

None of the co-authors have any competing interests, nor have they received or are due to receive any payment for writing this article.

Acknowledgements

We thank all the clinical and laboratory staff at our PGD centre who provided patient care during the study period, and above all, the patients whom their treatment cycles were analysed.

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