



ICMR

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PREIMPLANTATION GENETIC DIAGNOSIS - CURRENT AND EMERGING TRENDS

Preimplantation genetic diagnosis (PGD) is the earliest form of prenatal diagnosis through which embryos created *in vitro* are analysed for well-defined genetic defects; only those free from the defects are implanted into the womb. This technique is extremely useful not only for individuals at high risk of having a child with genetic disease but also for those being subjected to *in vitro* fertilization (IVF), enhancing their chance of an on going pregnancy as low success rates in IVF might be attributed to chromosomal aneuploidies in the embryos.

The first application of PGD was carried out in patients who were carriers of an X-linked disease and had thus one in four chance of having an affected child. The sequences on the Y chromosome were amplified by polymerase chain reaction (PCR) to discriminate male and female embryos and only the female ones were transferred. Since then, PCR has been the method of choice for several more common monogenic diseases. Later on fluorescent *in situ* hybridization (FISH) replaced PCR on sexing of the embryos and various other aneuploidy detections.

In the current age the PGD has rapidly become an essential tool for improving the success rate of assisted reproduction techniques (ART) and offering couples a

normal baby thus avoiding the need for a therapeutic abortion of abnormal foetuses.

The PGD allows to detect the karyotype of the embryos obtained through IVF as also the presence of single gene defect prior to implantation. It is gaining increasing consensus world-wide and is of particular

Table : Common genetic disorders where PGD protocols have been used

Autosomal dominant	Autosomal recessive	X- linked	Aneuploidy
Marfan syndrome	Cystic fibrosis	Duchenne muscular dystrophy	Advance maternal age
Huntington's disease	Tay sachs	Haemophilia A	Translocation carrier
Myotonic dystrophy	Lesch-nyhan syndrome	Fragile X syndrome	Gonadal mosaics
Polyposis	□-Thalassaemia	Occular albinism	
		Fever combined	
	Spinal muscular atrophy	Immuno-deficiency	

interest in ART. Majority of spontaneous abortions and implantation failures are due to the chromosomally abnormal embryos. These factors make PGD an essential part of an IVF setting.

Tremendous progress has been achieved in the field of PGD in the last one decade. Prenatal genetic diagnosis is presently being performed in more than 3000 clinical cycles world-wide resulting in nearly 700 clinical pregnancies and birth of 600 unaffected children.

The International Working Group on PGD was set up in 1990 and the first international symposium on PGD was organized in Chicago in 1991 for collecting data on world-wide basis. Recently, the Special Group in Reproductive Genetics of the European Society of Human Reproduction and Embryology (EHRE) has established a PGD Consortium for centres in Europe. The consortium is committed to collect accurate data on outcomes of PGD. The major question that needs to be addressed is accuracy and efficiency of special genetic pregnancy rate and frequency of congenital malformation. Less obvious are questions like profile of clinics offering PGD and availability of different diagnostic tests to facilitate cross-references.

The PGD represents a state of art procedure which potentially avoids the need to terminate affected pregnancies through identification and transfer of only unaffected embryos established with the help of IVF. Although a growing number of centres world-wide offer PGD, it is still not widely performed as a clinical service. Since it requires the expertise in the fields of reproductive medicine and cytomolecular genetics, additionally genetic diagnosis by a single cell PCR is technically demanding and protocols have to be stringently standardized before the clinical application.

Some of the techniques employed in PGD are outlined here:

Polar Body Biopsy

Polar bodies are biopsied to deduce the genotype of the oocyte. A slit is made in zona pellucida with sharp needles or laser to obtain polar bodies that are then drawn out of the egg.

Cleavage Stage Embryo Biopsy

It is a widely used technique on the 3rd day post insemination when the molecular embryo is usually six to eight cell stage¹. At this stage the cells are totipotent

and are usually not compacting - the quality of cleavage stage embryos in culture is variable but they can be evaluated and graded. Most centres performing PGD prefer biopsy of blastomere from cleavage stage embryo. One of the main disadvantages of blastomere biopsy is the limited amount of material available for analysis.

Blastocyst Stage Biopsy

The blastocyst is a cavitated structure that contains around 100 cells and developed about 5-6 days post insemination. Blastocyst biopsy has an advantage over blastomere biopsy in that more cells can be removed for analysis and biopsy procedure is less demanding. But the disadvantages are that only about 36% embryos mature this far and since embryos should be transferred before day 5 or 6 very little time is left for the diagnosis².

Analysis of Biopsied cells

PCR

Polar body or blastomere is placed in a solution to lyse the cell and release DNA. PCR reaction mix is then added. Since PCR is a very sensitive technique, the contamination with extraneous DNA can lead to misdiagnosis and transfer of affected embryos³. Introduction of automated sequencing, minisequencing and real time PCR has further refined PCR's diagnostic capabilities.

FISH

FISH is the most frequently used method for analysis of the chromosomal complement of blastomere⁴. Collected cells are spread on a slide after which DNA probes labeled with flurochromes specific for chromosomes of interest are applied. The type and number of probes used depends on the indication. Kit containing probes for chromosomes X, Y, 13,18 and 21 is available.

Comparative genomic hybridisation

Another way to identify chromosome aberrations is to use comparative genomic hybridization (CGH), a new technique introduced by two groups working on PGD⁵. Here the pre-amplified DNA from a single test cell is labeled with one flurochrome and then mixed with pre-amplified DNA from a control sample labeled with a different flurochrome with which it is compared. The mixture is applied to normal metaphase spread and the colour ratio measured.

The advantage of CGH over FISH is that the whole chromosome complement is analysed, though polyploidy and balanced translocations can not be detected. The disadvantage is the time taken for the procedure - 72 h.

Indications for PGD

Monogenic diseases

The monogenic diseases were the first genetic abnormalities to be diagnosed in embryos. The PGD in general and single cell PCR in particular are demanding and labour-intensive techniques, hence only the most common monogenic diseases have been studied extensively.

Almost 33 monogenic diseases have been listed for which PGD is available.

Sex linked diseases

Women who are carriers of a recessive X-linked disease have a 25% risk of having an affected boy. Hence, sexing of embryos with FISH has been used intensively to avoid the birth of boys affected with X-linked disease. However, half of the discarded embryos are healthy and carrier girls can not be distinguished from healthy girls by this technique.

Chromosomal abnormalities

Healthy carriers of translocations are at risk of having children with congenital anomalies and mental retardation due to chromosomal imbalances or are likely to have recurrent miscarriages or to be infertile.

Even though the first polar body from female carrier of balanced translocations can be studied, it has a major drawback of analyzing only translocations of maternal origin. Hence the most widely used method is analyzing cleavage stage embryos.

Studies carried out at several centres have shown that a large proportion of embryos are chromosomally abnormal and cannot be transferred leading to low pregnancy rate per cycle in individuals with reciprocal translocations. However, because of the occurrence of multiple miscarriage or infertility in these patients PGD is often the only way to achieve an ongoing healthy pregnancy.

The PGD aneuploidy screening (PGD-AS) has become the most widely used application of PGD, because of the relative ease of the technique compared with others and because of the large potential group of

patients. Even though the chances of misdiagnosis is there in cases where mosaicism is present, it is believed that PGD-AS increases the chances of pregnancy for patients with poor prognosis.

Future Developments

New methods for diagnosis of monogenic diseases are being developed at a rapid rate, some of which are very suitable for use with single cell PCR viz ,

Real time PCR

The accumulations of PCR products are measured during cycling, when the PCR is in exponential phase, instead of after the process is complete.

This method not only reduces the likelihood of allele drop out but also gives a more accurate idea of the homozygous or heterozygous state of the single cell.

Mini sequencing

Single nucleotide polymorphisms (SNPs) are accurately detected by this method. SNPs are widespread in the genome either as mutations or as polymorphisms, which do not affect functioning of genes. When starting from a single cell, the amplification of small stretches of DNA is much more efficient than for larger fragments, thus SNP analysis is ideally suited for single cell PCR.

Micro arrays

Micro arrays could be used to diagnose common genetic diseases caused by more than one mutation and also can be used in CGH where they could replace the metaphase spread to which a mixture of test and comparative DNA are hybridized.

The specificity of the sequences in the micro arrays would lead to shorter hybridization and computer analysis times compared to that with CGH, obviating the need for polar body analysis or cryopreservation. Other advantages of micro array CGH would be greater resolution than metaphase spreads. Standardization of the whole procedure with computers would allow more IVF Centres to use micro array CGH routinely for aneuploidy screen⁶.

Conclusions

Prenatal diagnosis of genetic disorders has been available for many decades now and has helped in reducing the burden of these severe disorders to a great extent. But this entails need for medical terminations of

pregnancy, which is not acceptable universally. Pre implantation genetic disorder has been offered with the idea of avoiding the birth of an affected child with genetic disorders without recourse to terminations. Over the last decade the PGD has received fairly universal acceptance with thousands of PGD cycles and over 1000 post PGD normal healthy children have been born already.

Like any powerful, modern technology, the PGD is also a double-edged weapon and should be used with careful ethical, legal guidelines. It is very much labour intensive and fairly expensive technology requiring very high level of expertise and can function only in close cooperation with a high output successful IVF programme and cutting edge genetic laboratory with experience in single cell diagnosis. However, it has assumed a prominent place in preventive genetics programme because of tremendous possibilities in screening, diagnosis and management of all types of genetic disorders.

The ICMR Genetic Research Centre at the National Institute for Research in Reproductive Health, Mumbai is embarking into the field of PGD of aneuploidies; later single cell PCR for single gene disorders will be established to ensure birth of normal babies.

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This write-up has been contributed by Dr Z.M. Patel Dy. Director and Dr S.R. Menon, Technician, ICMR Genetic Research Centre, Mumbai.

ABSTRACTS

Research Project Completed Recently

Studies on oxidative DNA damage induced by organophosphate pesticides, chlorpyrifos and parathion in rats

The study was carried out in male Wistar strain albino rats to assess the toxicity of chlorpyrifos (CPF) and parathion, organophosphate pesticides, with the aim to investigate the involvement of oxidative stress and subsequent oxidative DNA damage in the overall toxicity (both acute and chronic) of these pesticides and efficacy of antioxidant vitamins in reducing the toxicity.

Treatment of CPF and parathion either alone or in combination caused accumulation of malondialdehyde (MDA) and 4-hydroxyl-2nonanal(4-HNE), the two major end products of peroxidation of polyunsaturated fatty acids (PUFA) and related esters, in all regions of rat brain and liver. This increase was more pronounced in rats receiving chronic exposure of these pesticides compared to acute exposure may be due to increased production of reactive

oxygen species (ROS) and decrease in activity of various antioxidant enzymes (ie catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase). The pretreatment of antioxidant vitamins (vit A + vit E + vit C) led to decreased levels of MDA and 4-HNE in all the three parts of brain (i.e. fore-, mid- and hind- brain regions) and liver of rats, but did not restore totally as in case of normal rats, indicating that antioxidant vitamins pretreatment was not able to offer complete protection against these pesticide induced injuries.

The level of hydrogen peroxide, the most stable non reactive oxygen species, was increased in all the tissues tested on both chronic as well as acute treatment of these pesticides. Chronic exposure of these pesticides both separately and in combination, led to more increase in the level of hydrogen peroxide in different regions of rat brain and liver. Pretreatment of antioxidant vitamins for one month caused significant reduction in the elevated

levels of hydrogen peroxide thereby indicating the protective effects of antioxidant vitamins against these OP pesticides induced oxidative stress.

The GSH/GSSG ratio was decreased due to decrease in GSH and increase in GSSG levels in all the three regions of rat brain and liver. Regional variation in neurotoxicity and liver toxicity induced by these pesticides was also seen. CPF and parathion treatment caused almost equal decrease in fore-brain GSH while parathion was more effective in mid-brain when given orally for one month. Pretreatment of antioxidant vitamins led to the restoration of GSH and GSSG levels in all the three regions of rats brain and liver.

Study revealed that acute exposure of these OP pesticides lead to decreased level of NADH as well as NADPH, which in turn decreased the ratio of NADH/NAD (total) and NADPH/NADP (total) in different regions of rats brain and liver. Decrease observed in parathion treated group was more pronounced compared to the CPF treated group. When these two pesticides were given in combination, the ratio of NADH/NAD (total) and NADPH/NADP (total) was maximally decreased. Both chronic as well as acute treatment of these OP pesticides, caused marked increase in DNA damage in liver and rat brain. Parathion treatment showed higher level of damage as length of the tail of the comets were also increased in

liver and rat brain nuclei in comparison to CPF treated animals. There was dose-dependent damage of the DNA in both liver and brain of CPF and parathion treated groups.

It was thus concluded that these pesticides generate oxidative stress in brain as well as in liver of exposed rats. The chronic treatment is found to be more damaging than acute exposure. The redox status of brain and liver was dramatically disturbed on treatment with these pesticides and the most important nonenzymatic antioxidant, glutathione (GSH), was depleted on CPF as well as parathion treatment. These pesticides showed genotoxic potential too as DNA damage was observed in both the tissues of rats given chronic or acute treatment of these pesticides. Antioxidant vitamins showed their protective effects on the pesticides induced oxidative damage. The study also proved that consumption of antioxidants may be a prophylactic measure for the people who remain regularly in touch with these pesticides including farmers and workers who are engaged in production and handling.

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ICMR NEWS

The following meetings of various technical groups/committees of the Council were held:

Meetings of Task Forces (TFs)/Expert Groups (EGs)/Core Groups/Steering Committee/ and Other Meetings

EG on Mid Term Evaluation of Home-based Management of Young Infants December 7, 2006

EG on Low Dose Magnesium Sulphate Regimen For Management of Eclampsia - A Randomized Controlled Trial December 13-14, 2006

EG of the Task Force on Mental Health Service Needs and Service Delivery Module in Disaster (Earthquake) affected Population in Gujarat December 15, 2006

TF on Jai Vigyan Mission Mode Project on Community Control of RF/RHD December 19, 2006

EG of the TF on Multicentric Study on Epidemiology of Asthma and Atopy December 20, 2006

TF on Review of Cancer Management Guidelines December 30, 2006 & January 9, 2007

TF on Relation of Candidate Gene Variants Regulating Triglyceride Metabolism to Serial Changes in Childhood Body Mass Index and Coronary Artery Disease Risk Factors in Young Adulthood January 17, 2007

EG to Discuss India Country Programme for Preparedness, January 18, 2007

Control and Containment of Avian Influenza		Special PRC on North-East Projects	February 13, 2007
TF on Review of Cancer Management Guidelines	January 19, 2007	PRC on Genomics	February 19, 2007
TF on Human Genetics	January 23, 2007	PRC on Pharmacology	February 23, 2007
Core Group on Global Environmental Changes & Health	January 25, 2007	PRC on Environmental Hygiene & Occupational Health	February 27, 2007
TF on Epidemiology of Asthma and Atopy	February 2, 2007	Workshops	
TF on Screening for Cancer in Himachal Pradesh	February 5, 2007	An ICMR-INSERM Workshop on Development of Biomarkers for Cardiovascular Diseases and Diabetes was organized at Gurgaon during January 22-24, 2007.	
TF on Immunology	February 15, 2007	Pride of India Science Expo - 2007	
Steering Committee of Centre for Advanced Research on Genomics of Type 2 Diabetes Mellitus	February 23, 2007	The ICMR put up a state of art mega Science and Technology Exhibition in the Pride of India Science Expo-2007 organized as part of 94th Session of the Indian Science Congress at Anna Malai University, Chidambaram, Tamil Nadu during January 3-7, 2007. Major contributions/achievements and activities of the Council in various fields of biomedical research were displayed in the exhibition and scientists of various ICMR institutions/centres answered the queries of the visitors. The exhibition was inaugurated by Shri Kapil Sibal, Hon'ble Minister of State for Science & Technology and Ocean Development, Government of India. The ICMR Pavillion was awarded Special Appreciation Prize in the exhibition.	
TF on Multi-centric Study on Nutrition Profile of Assam, Manipur and Meghalaya	February 25, 2007	Participation of ICMR Scientists in Scientific Events	
EG on Urban Mental Health	February 27, 2007	Dr. S.P. Tripathy, Deputy Director and Dr. S.V. Godbole, Senior Research Officer, National AIDS Research Institute (NARI), Pune, participated in the Adult AIDS Clinical Trials Group 2006 Winter Meeting at Baltimore (December 4-7, 2006).	
Project Review Committees (PRCs)/Project Advisory Committees (PACs)/Project Review Groups (PRGs)		Dr. N. Selvakumar, Deputy Director (Senior Grade), Tuberculosis Research Centre (TRC), Chennai, participated in the TB Laboratory Workshop for Supranational Laboratories at Bucharest (December 5-10, 2006).	
PRC on Oncology	December 4, 2006	Dr. R.S. Yadav, Deputy Director (Senior Grade), National Institute of Malaria Research (NIMR) Field Station, Kheda, Gujarat, participated in the meeting of WHO Global Malaria Programme Working Group on Indoor Residual Spraying at Cairo (December 9-10, 2006). He also participated in a Programme for Development of a Framework for Implementing IVM for Malaria and	
PRC on Neurology	December 11, 2006		
PRC on Experimental Medicine & Surgery	December 12, 2006		
Special PRC on North-East Projects	December 13, 2006		
PRC on Gastroenterology	December 18, 2006		
PAC on Home-based Management of Young Infants	December 22, 2006		
PRG on Nutrition and Tribal Health	December 22, 2006		
PRC on Biomedical Engineering	January 10, 2007		
PRC on Orthopaedics	January 16, 2007		
PRG on Basic Reproductive Biology	February 6, 2007		
PRC on Mental Health	February 7, 2007		
PRC on Diarrhoeal Diseases	February 9, 2007		

Vector-borne Disease Control at District Level and a Case Control Study of IPVM Pilot Project in Sri Lanka (December 11-15, 2006).

Dr. Neena Valecha, Deputy Director (Senior Grade), NIMR, Delhi, participated in the I Meeting of the Global Malaria Programme (GMP) Technical and Research Advisory Committee at Cairo (December 10-12, 2006). She along with Dr. V.A. Arankalle, Deputy Director, National Institute of Virology (NIV), Pune also participated in the DNDI Round table meeting on Fixed Dose Artesunate-Mefloquine and Prince Mahidol Award Conference on Essential Health Technologies on Neglected Diseases - Reaching Neglected Populations at Bangkok (January 31 - February 2, 2007).

Dr. K.D. Ramaiah, Assistant Director, Vector Control Research Centre (VCRC), Pondicherry, participated in a Workshop to Review the Impact of Mass Drug Administration on Lymphatic Filariasis Disease, Infection and Transmission at Geneva (December 11-13, 2006).

Dr. P. Jambulingam, Deputy Director, VCRC, Pondicherry, participated in the X WHO PES Working Group Meeting at Geneva (December 11 -14, 2006).

Dr. T. Ramamurthy, Deputy Director, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, participated in the IV Pulse Net Asia Pacific Planning Meeting at Nanjing (December 19-21, 2006).

Dr. Dipika Sur, Deputy Director and Dr. Byomkesh Manna, Assistant Director, NICED, Kolkata, participated in the Clinical/Epidemiological Training Session at Baltimore (January 22-25, 2007).

Dr. S.K. Niyogi, Deputy Director (Senior Grade) and Dr. T. Ramamurthy, Deputy Director, NICED, Kolkata, participated in the Discussion Meeting with Dr. Shinji Yamasaki, Professor of Laboratory of Prevention of International Epidemics at Osaka (January 25-30, 2007).

Dr. Poonam Salotra, Deputy Director, Institute of Pathology (IOP), New Delhi, participated in the Keystone Symposium on Drugs Against Protozoan Parasite at California (January 28 - February 1, 2007).

Dr. N.S. Wairagkar, Deputy Director, NIV, Pune and Dr. B. L. Sarkar, Assistant Director, NICED, Kolkata participated in the International Conference on Emerging Diseases and Surveillance at Vienna (February 23-25, 2007).

Dr. C. Padmapriyadarsini, Senior Research Officer and Dr. Geetha Ramachandran, Research Officer, TRC, Chennai, participated in the XIV Conference on Rotaviruses and Opportunistic Infections at Los Angeles (February 25-28, 2007).

Dr. Shahnaz Vazir, Deputy Director, National Institute of Nutrition (NIN), Hyderabad, participated in the Consensus Conference of Experts to Define and Discuss Responsive Feeding and Related Behaviours at Dhaka (February 25 - March 1, 2007).

Trainings/Fellowships/Associateships

Dr. S.D. Mahale, Assistant Director, National Institute of Research in Reproductive Health (NIRRH), Mumbai, proceeded to avail DBT Short-term Overseas Associateship at New York for six months w.e.f. January 1, 2007.

Dr. Lalita Savardekar, Assistant Director, NIRRH, Mumbai, proceeded to avail Western Institutional Review Board Inc. International Fellowship at Olympia for six months w.e.f. January 8, 2007.

Dr. M.V. Murhekar, Deputy Director, National Institute of Epidemiology, Chennai, participated in the WHO Global Salm - Surveillance Training Workshop at Bangkok (January 15-20, 2007).

Dr. Mihir Kumar Bhattacharya, Assistant Director, NICED, Kolkata, proceeded to avail a Counterpart Training in Japan under NICA-NICED Project on Prevention of Emerging Diarrhoeal Diseases - Phase-II for 20 days w.e.f. January 27, 2007.

Dr. Dhruva Ghosh, Senior Research officer, NIV, Pune, proceeded to avail training on Micro Array Technology for Differential Diagnosis and Surveillance of Infectious Diseases at New York for a period of 4 weeks w.e.f. February 1, 2007.

Dr. Virendra Kumar, Senior Research Officer, NJIL & OMD, Agra, availed training in the use of Scanning Electron Microscope at Japan, Singapore and Thailand during February 5-9, 12-16 and 19-23, 2007 respectively.

Dr. Anup Palit, Deputy Director, NICED, Kolkata, proceeded to avail Counterpart Training in Japan under JICA-NICED Project on Prevention of Emerging Diarrhoeal Diseases - Phase II for 2 months w.e.f. February 17, 2007.

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