Miscellaneous Projects

1. Monitoring of Organic Chemical Pollutants in Placental Tissue: A new Approach to Human Environmental Bio-monitoring

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In collaboration with	***:***	***Dr. Sudha Salhan, SJH, and Dr. SK Raza, DRDE, Gwalior***
Duration	***:***	***2002 – 2006***

Aims, Objectives & Background

During the past several decades international community has become increasingly aware of the hazardous impact of chemical pollutants in the food chain and concerns has been raised regarding the effects of these toxicants on population in general and pregnant and lactating women in particular. Previously Dr. S. Sriramachari had demonstrated that placenta could be used as a model for monitoring of inorganic elements and heavy metals in human population. As a follow up of the earlier study, this study was taken up with an aim to establish the utility of human placenta as a tool for comprehensive biomonitoring for organic pollutants and to demonstrate the feasibility of monitoring region specific organic pollutants in placenta. It was also aimed to establishing standard operating procedures (SOP) for the above two objectives.

Pregnant women attending Antenatal Out Patient Department Clinic of Safdarjung Hospital and / or admitted for delivery were screened for possible exposure to pollutants and a proforma questionnaire was filled to record the occupation, family background, obstetrics, clinical and gynaecologic history, dietary and smoking habits etc., socio-economic background and possibilities of exposure to pollution as per the self-reported history recalled by the patient. Informed consent of the patients was also recorded in the proforma along with delivery and sample details. OPD cards of the patients marked for collection of placenta at the time of delivery. Any women having pregnancy related problem such as PIH, PET, and Proteinuria etc. or suffering from diseases such as diabetes, tuberculosis etc. was excluded from the study. Women who reported exposure to

agricultural chemicals during pregnancy due to involvement in agricultural activity by themselves or their family members were selected and followed up for subsequent collection of placental sample at the time of delivery. A total of 100 samples have been collected which include 60 samples who women who no direct exposure and 40 samples from women who reported exposure to agricultural chemicals at some point of time after getting pregnant. In addition, fifty samples of blood and milk have also been collected.

Last year we had reported the detailed procedure for extracting the pollutants from placental samples. Briefly, the placenta was collected immediately after delivery wrapped in acetone washed aluminum foil and brought to the laboratory, washed with normal saline, cut pieces and stored in liquid nitrogen. The placental tissue was homogenized at high speed, extracted with n-Hexane and centrifuged at 3000 rpm. The clear layer of supernatant collected was concentrated by nitrogen purging. The extract was reconstituted in n-hexane and pollutants were analyzed using Gas chromatography. Standard Operating Procedure for collection of placental sample, extraction of organic chemical pollutants from the placental tissue, cleanup and concentration by nitrogen purging has been finalized. Extracts have been prepared for all the 100 placental samples as per the finalized SOP and 40 extracts were concentrated by gentle nitrogen purge technique.

Work done during this year

During the year under report, Placental extracts and commercially procured mixtures of pesticide standards were analyzed by Gas Chromatography and the retention times in GC for all pesticide/pollutant standard and thirty-one placental tissue extracts were recorded and compared.

On the basis of matching retention time with the known pesticide standards, the Gas Chromatography has revealed possibility of presence of pesticide residues in the placental extracts. For the purpose of comparison, the study group was divided in two categories, viz., urban not directly exposed housewives and rural women with possibility of exposure to agricultural chemicals. A comparison of GC retention times showed presence of a large number of organic and agricultural chemicals such as Primiphos methyl, op-DDE, Endrin, Malathion, Pyrene, Pyrethrin, Naphthalene, Phosalone and biphenyls in 13 (42%) sample

extracts out of 31 analyzed. Primiphos methyl, which is used for preservation of stored house hold food grains and for control malaria, was detected in 7 urban house wives constituting 22% of the samples analysed. Two samples from rural population showed presence of Biphenyls, one revealed presence of op-DDE while both Primiphos and Phosalone were detected in yet another rural sample. Surprisingly Malathion and Biphenyls were found in 2 different samples and Pyrethrin in one sample from urban women reporting use of only mosquito repellent. No consistent correlation could be observed with age, occupation, exposure history and residential location.

Pesticides extracts were also analyzed using ELISA Kits supplied by M/S Envirologix, USA. Stepwise instructions were followed. Five different kits covering main pesticide groups were used viz., DDT, Cyclodiene, Synthetic Pyrethroid, Organophosphate and Carbamate groups. A total 29 tissue extracts and 9 blood extracts were tried. The results revealed widespread positivity in all the kits tried. Presence of pyrethroides (a component of mosquito repellent) was observed in all most all the samples followed by organochlorine, Organophosphorus and Carbamate pesticides.

Although the study confirmed the hypothesis of Dr. Sriramachari that placenta could be used for monitoring of organic chemical pollutants and may be an ideal tool for HEBM, more samples need to be analysed and confirmatory techniques such as mass spectrometry need to be used to come to a definitive conclusion

Future Plan of Action

Further confirmation for the presence of these residues by GC-MS will be undertaken after the GC-MS is installed in the Institute. .

2. Study of Glomerular Structural Changes in Diabetic Nephropathy

Scientific staff	***:***	***Dr. Ila Tyagi, Dr. Sunita Saxena, Dr. AK Jain, Dr. Usha Agrawal***
In collaboration with	***:***	***Dr. Vindu Amitabh, Dr. S Lakra, SJH***
Duration	***:***	***2005-2006***

Aims, Objectives & Background

The natural progression of diabetic nephropathy over time has not been elucidated in the perspective of renal changes and morphological appearances thereof. While the full blown morphological spectrum in clinical cases of proteinuria in DM patients has been studied, the early pre-clinical changes of renal pathology are yet to be understood to enable better understanding of the events leading to proteinuria. The identification of pre-clinical and reversible changes of glomerular morphology associated with microalbuminurea with improved glycemic control can help in better patient management. Hence this study was undertaken with a view to understand these early changes in the context of clinical and biochemical parameters and find morphometric parameters which could help in diagnosis of pre-clinical DN.

Work done during this year

The study included 47 patients of diabetic nephropathy referred from Dept. of Nephrology, Safdarjung Hospital, which included 30 cases of clinical & 17 cases of pre clinical nephropathy (microalbuminurea). Seven cases of minimal change disease were used as control. Various investigations like FPG, PPG, 24 Hr urinary protein excretions and HbA1c were carried out. Renal biopsies received were studied both on Light Microscope & Hitachi H7500 Transmission Electron Microscope. Morphometry was done on digital EM images to measure Glomerular and Tubular Basement Membrane thickness in each of the three study groups, viz., clinical, pre-clinical and control.

The study revealed that there was a significant association between duration of disease and severity of disease while no significant association was observed between FPG, PPG, HbA1c and the clinical stage of disease. No significant difference could be observed in the immunofluorescence profile of clinical and pre-clinical groups.

On electron microscopic examination, capillary wall showed morphological changes in both the Glomerular Basement Membrane (GBM), which was thickened and epithelial cells, which showed variable effacement of foot processes. The study revealed increased glomerular and tubular basement membrane thickness in 82% cases, which had normal morphology under light microscope. Thus EM played an important role in discerning increase in thickness of GBM, which could be the earliest change in DN.

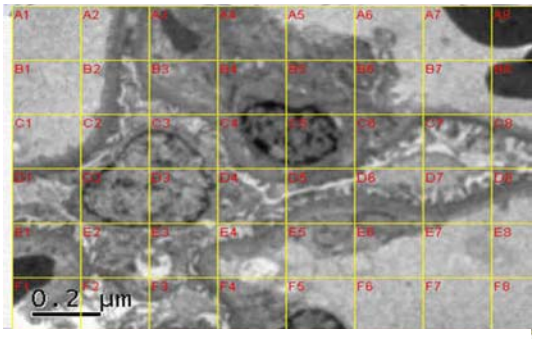

Fig. 1: Morphometric analysis of GBM thickness on electron photomicrograph of glomerulus in pre clinical diabetic nephropathy

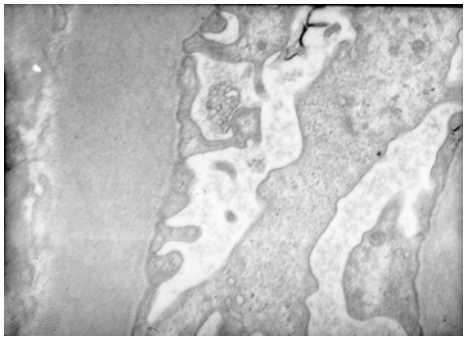

Fig. 2: Electron Micrograph from a case of diabetic nephropathy showing thickening of glomerular basement membrane

3. *Application of Human Epidermal Sheets Cultured from Autologous Epidermal Stem Cells in Burns Patients in Phased Manner*

Scientific Staff **:** ***Dr. LK Yerneni***
Duration **:** ***2005-2010***

Work has been undertaken towards development of protocols for safety, purity, identity, potency and stability of cultured epidermis. Looking at the stringent international regulatory framework on the use of autologous cells manipulated ex-vivo intended for wound healing, creation of a clean room as per the GMP norms, Schedule M, Part 1A of the Indian Drugs and Cosmetic Act, has been taken up as precedence before further clinical applications are undertaken.

4. *Cell Culture Contamination with Mycoplasma in Basic and Applied Biomedical Research*.

Scientific Staff **:** ***Dr. LK Yerneni, Mr. Ashok Kumar***
Duration **:** ***2005-2008***

Aims, Objectives & Background

It has been noticed that there is general the lack of awareness about *Mycoplasma* contamination in cell culture laboratories in our country and its significance in basic and applied biomedical research.

The project has been envisaged to study the frequency of *Mycoplasma* contamination in various reputed institutes of Delhi and to identify a protocol for detection of *Mycoplasma* in human and other mammalian cell cultures. To verify effectiveness of various antibiotics and *Mycoplasma* removal agents for elimination of *Mycoplasma* contamination from infected cell lines and to find out an ideal dose of anti- *Mycoplasma* antibiotics like Ciprofloxacin, and Gentamycin for their routine use in cell cultures in place of Penicillin and Streptomycin that have least anti- *Mycoplasma* activity.

Work done during the year

Out of a total of 82 samples analyzed by Hoechst and immunofluorescent methods, the routine screening at microscope level revealed only 5%. However, upon thorough analysis of the digital images of photomicrographs using Adobe's Photoshop by increasing the image size without loosing the resolution and adjusting the brightness and contrast, 18 cell lines out of 77 were positive for *Mycoplasma* by both immunoflorescent Assay using a mycoplasma-specific antibody and Hoechst (DNA) staining. In fact, 23 cell cultures were Hoechst positive out of which 5 turned out to be contaminated with bacteria as revealed by the total loss of cultures in antibiotic-free medium.

The cell cultures collected were further subjected to PCR analysis using *Mycoplasma* detection kit supplied by American Type Culture Collection (ATCC), USA. The kit was claimed to detect largest number of cell culture contaminating species of *Mycoplasma*. However, by the time 77 samples were analyzed by PCR, the kit was withdrawn from the market due to quality assurance issue. Our results with the PCR method revealed an exorbitant 81% positivity.

Correlation of incidence with probable causative factors:

It was found that the incidence of *Mycoplasma* contamination was 5% in those cultures with Gentamicin or Ciprofloxacin as compared to 27% with Penicillin and Streptomycin. There was a significantly ($P<0.001$) high (66.67%) incidence of *Mycoplasma* contamination in cultures handled by technical personnel as compared to researchers (14.29%) indicating that technicians could be more accountable for *Mycoplasma* contamination and its spread in cell cultures. Additionally, it appears that non-adherent cell lines are significantly ($P<0.05$) more (42.86%) prone to get *Mycoplasma* contamination than adherent cell lines (17.65%). The correlation of incidence of *Mycoplasma* contamination with the type of culture revealed no significant incidence with continuous cell lines and primary cultures. The primary cultures may be getting *Mycoplasma* contamination from the source tissues as it is known (*McGarrity et al 1985; Barile and Rottem, 1993*). There is a high 66.67% incidence with the use of membrane filters of larger pore size of 0.45 μm, a lesser incidence of 21.62% with pore size of 0.22

μm and nil with the smaller pore size of 0.1 μm indicating a significant ($P<0.001$) increasing trend of contamination with the use of larger than 0.1μm pore size filters. It may be inferred that due to their smaller size of about 0.3 μm, flexible and plastic nature of the cell body, the mycoplasma could possibly pass through larger pore size membrane filters during filter sterilization. This consequently could spread to other cell cultures, media, sera and related solutions. The frequencies of *Mycoplasma* contamination with the use of Type I and Type II laminar flow and periodic fumigation in laboratories do not play any significant role in the spread and control of *Mycoplasma* contamination in cell cultures.

Conclusions:

Detection of *Mycoplasma* contamination in cell cultures by immunoflorescent assay and Hoechst (DNA) staining give results within 2 hours while broth-agar culture method is time consuming and needs more expertise and there is either a high false positivity with group-PCR based detection methods or requires a battery of species specific PCRs. Additionally, we recommend simultaneous employment of both of these tests and analyzing the digital images using Photoshop so that (a) even low level contamination becomes apparent and (b) the presence of bacterial contamination could be excluded by the differential staining.

Indiscriminate use of antibiotics, handling of cultures by inexperienced technicians and use of membrane filters with larger than 0.1 μm pore size may increase *Mycoplasma* contamination chances. Continuous cell lines seem to be more vulnerable to *Mycoplasma* contamination as they are repeatedly handled over a long period.

5. Evaluation of Human T-cell Lymphotropic Virus Type-1 (HTLV-1) in Blood Donors and Patients with Leukemia/Lymphoma from Delhi

Scientific staff : ***Dr. Ranvir Singh***
In collaboration with : ***Dr. Sumita Saluja, SJH***
Duration : ***2005-2006***

Aims, Objectives and Background

A number of evidences indicate the association of HTLV-1 with various diseases including adult T-cell leukemia / non-Hodgkin"s lymphoma , HTLV-1 associated myelopathy (HAM/Tropical spastic paraparesis(TSP), development of inflammatory diseases in various organs such as eyes, lungs and joints, inflammatory ocular diseases such as endogenous uveitis, episcleritis, retinitis pigmentosa and degenerative choroiditis, etc. In these conditions, the presence of HTLV-1 can be associated as an accompaniment of repeated blood transfusions. However, recently in a pilot study, *Ramalingam et al (2001)* have reported a strong association of HTLV-1 with haematological malignancies (8/86 patients) and for both horizontal and vertical transmission of the infection in South Indian population.

In view of the reported association between HTLV-1 and hematological malignancies in a comparatively smaller number of subjects (n=86), the current investigations were planned to study the association between HTLV-1 and hematological malignancies. Such a study carried out in another population namely the North-Indian subjects will confirm the above mentioned serological evidences and if confirmed may lead to the possible introduction of HTLV-1 monitoring policy in the National Blood Transfusion Services.

Work done during the year

A total of 455 blood samples, comprising 390 sera from blood donors (119 HIV seropositive & 271 HIV seronegative) and 65 sera from patients with hematological malignancies, were collected and tested for anti-HTLV-1 antibodies through Particle

Agglutination Test (PAT) during 2004-2005. No sample was found to be having anti-HTLV-1 antibodies. The sera were collected from hematology division, Safdarjang Hospital, New Delhi and Institute of Pathology (ZBTC), New Delhi.

Future Plan of action

Evaluation of HTLV-1 in patients with Thalassaemia.