Association between Cervical Cancer and HPV in Mauritius: Paradigm revisited

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Format of talk

- Introduction
- Rationale for Mauritian study
- Material and methods
- Results
- Discussion
- New study
- Molecular Biology facilities

Introduction

- Half a million new cases are diagnosed worldwide.
- Around 300,000 females succumb to cervical cancer each year
- Cancer in general accounted for 11-12% mortality in Mauritius

- Annual cancer-associated death rate of 8 in every 1000.
- The incidence of cervical cancer and ovarian cancer combined is around 27% (21% cervical and 6% ovarian)
- Cervical cancer accounts for 65% of gynaecological cancers.

Causes of cervical cancer

- Development of cervical cancer can be multi-factorial.

- Life style: number of sexual partners
- Age of first sexual contact

- Genetic predisposition
- Exposure to high grade human papillomavirus (HPV). (16, 18, 33)

HPV

- Papovaviridae with more than 100 genotypes.
- Consists of a capsid with icosahedral symmetry with 72 capsomeres
- Average diameter between 52-55nm.
- Double stranded DNA (dsDNA)
- Molecules coding for the proteins of estimated weight of $5X10^6$ Da
- Genome size of approximately 8Kb.
- ORF consist of Early and late genes

- 100 HPV types that can infect epithelial surfaces

Divided into:

- Low risk
- Medium risk
- High risk

Dependent on their association with disease.

High Risk HPV :16, 18, 30, 31, 33, and 45

Materials and Methods:

- Ethics clearance and permission obtained from MOH.

- Histology blocks from sixty five patients diagnosed with cervical cancer in the year 2000

- Controls from patients undergoing hysterectomy

- DNA was extracted from the sections by an in-house method (Rughooputh, 2003)
- Degenerate oligonucleotide primers for the detection of HPV (Ting and Manos 1990)
- Based on the ORF of genes *E1* and *L1*.
- Amplicons size vary according to HPV type.
- HPV16 - 451bp,
- HPV18 - 454bp
- HPV33- 448bp

Primers sequence

- Positive Strand Primer (MY11): 5' GCM CAG GGW CAT AAY AAT GG 3'

- Negative Strand Primer (MY09): 5' CGT CCM ARR GGA WAC TGA TC
- Where M= A + C, R = A + G, W = A + T, Y = C +T

PCR

- 40 cycles of PCR :
- Denaturation 94°C 1 minute,
- Anneal 55 ° C 1 minute
- Extension 72 ° C for 1 minute.
- Post amplification analysis
- Positive samples cleaned and sequenced.

Results

Table 1. Incidence of cervical cancer in different age groups

Age (years)	Number of positive cases
21- 30	2
31- 40	3
41- 50	13
51- 60	11
61- 70	14
71- 80	7
81- 90	3

- 19% samples PCR-positive for HPV
- Youngest patient harbouring HPV DNA was 42
- Eldest was 80 years old.
- The mean age for patients positive for HPV was 58.7 year

Bioinformatics analysis

- Sequences analysed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST)

- A comparison of different sequences showed 96-97% similarity suggesting that the HPV types were similar with minimal mutation.

Discussion

- The incidence of HPV in the cohort was 19%. Cancer was, in general, 2-6 times more common in Creoles

- Other world-wide study suggest incidence of HPV above 80% (Castellsague et al 2002)

So?

If HPV is not the main cause of cervical cancer what are the other factors?

Several questions need answering

- What is the aetiology of cervical cancer in Mauritius
- Are the patients genetically predisposed?
- Does ethnicity play a role?
- Is cervical cancer due to mutations leading to metastasis
- Acquisition of cancer due to life style, diet or environmental factors?

What needs to be done?

- Retrospective study:

Presence of HPV,

Genetic mutations,

Demographics.

- Prospective study :

Follow patients with CIN 2-3

Collect blood, urine and Pap / Histology

Questionnaire administration

What will then be achieved?

- Questionnaire:Details on life style
- Histology/ Pap: HPV status, Gene regulation
- Blood and urine: analysed using genomic and proteomics tools for biomarkers.
- Biomarkers will be useful in early detection of cancer

Cohort size

- Retrospective study 500 samples
- Prospective study 1200 samples in the next 3-4 years. These will also include controls
- Cohort will have a good representation of all ethnic groups.

What are the benefits

- Produce a robust screening test that may be non-invasive
- Determine contributing factors in cervical cancer development
- Establish who is more at risk
- Identify oncogenes or polymorphisms that contribute to cervical cancer.

Benefit to population

- Awareness campaign
- Advise people more at risk
- Early screening, decrease death rate
- Reduce bed occupancy
- Reduce financial strains both on the government and cancer sufferers.

Cost implication and measurable outcomes

- Project estimated cost Rs 5.5 M

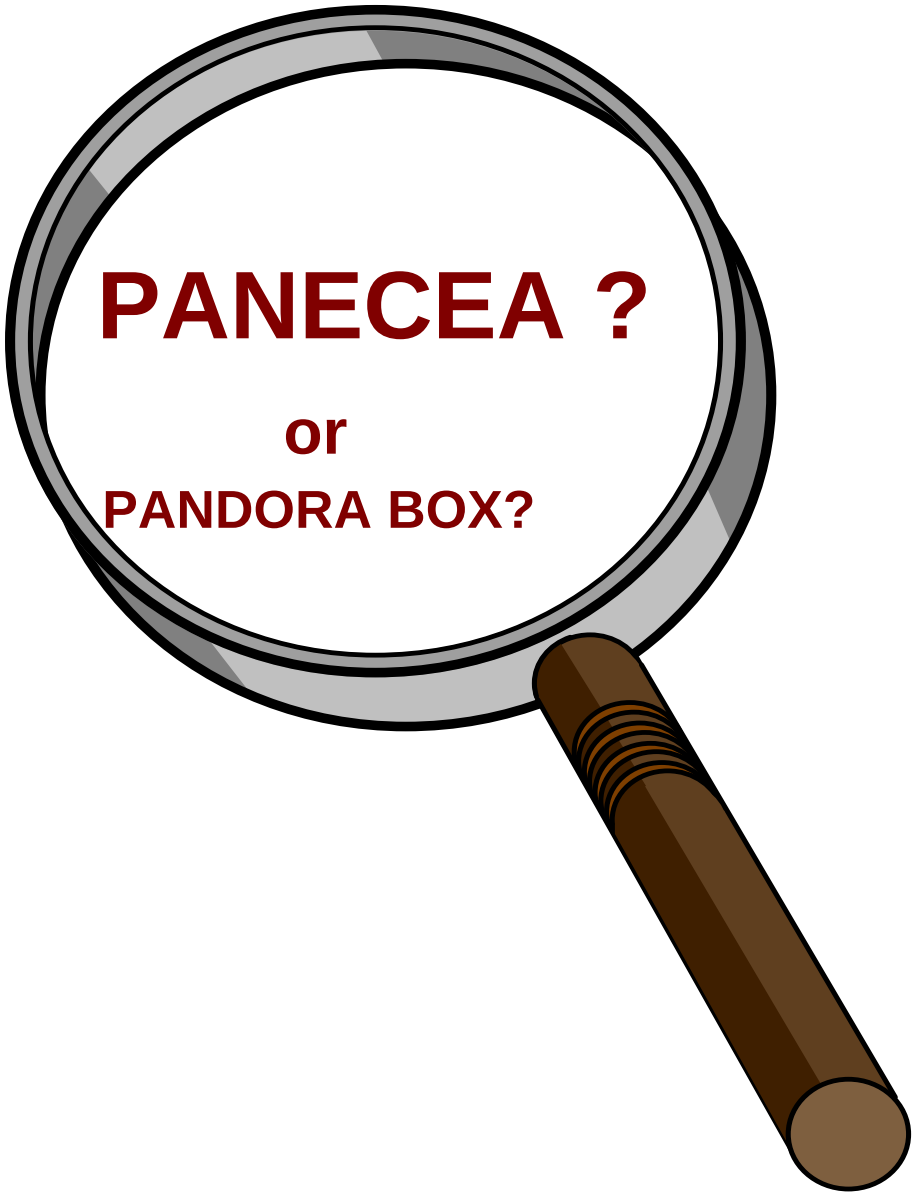
However this sum will also help in

- Technology transfer
- Setting up a one stop shop Molecular Biology Service

Research Team

- Dr Sanjiv Rughooputh (PI)
- Prof Pamela Greenwell
- Dr Shyam Manraj
- Mr Rechad Eddoo
- Mr Harris Ramuth
- Dr Nilima Jeebun
- Team members welcome!

Molecular Biology service



- Opportunities for expanding the repertoire of tests being offered
- Health tourism (e.g Apollo Hospital)
- Turn around time for some diagnostic tests
- Providing a centre of excellence in the Indian ocean for molecular biology
- Reference centre for some tests

- With globalisation, threats of emerging infections such as:

- Influenza Virus (H5N1 or other variants)
- Chikungunya
- Malaria
- Haemorrhagic viruses (West Nile, Ebola)

- Threats of Bioterrorism (e.g Antrax)

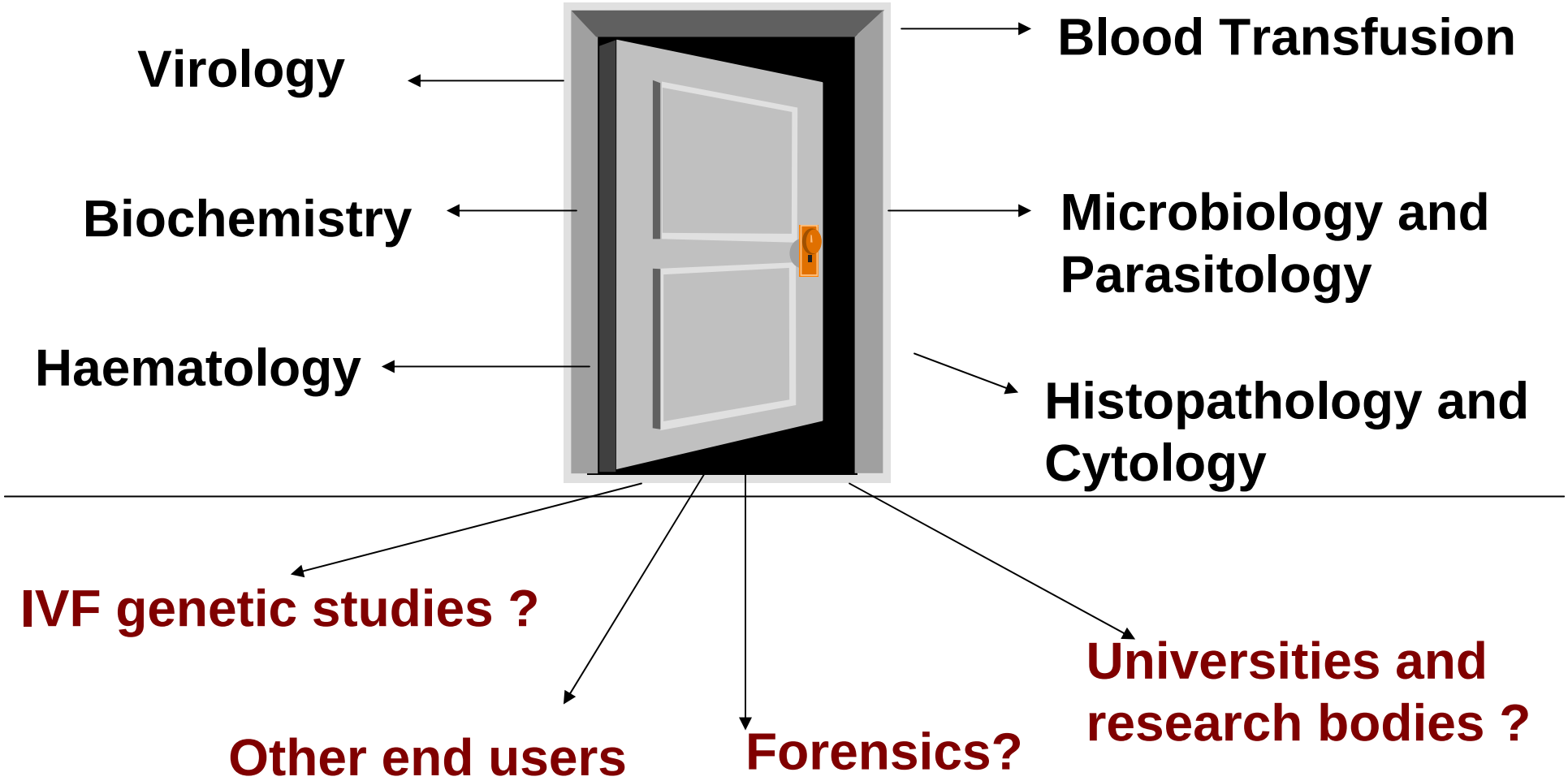
Added value to existing service

Testing for :

- CHIK
- HPV
- Meningitis
- TB (Multi drug resistance)
- STD (GC, Chlamydia, TV)
- HIV Detection and Viral load

- Thallasaemia, sickle cell
- Cancer and genetic studies (BRCA)
- HLA typing
- Malaria

Pan pathology Molecular biology suite



Future expansions

- Prenatal diagnosis of inherited diseases (DMD)
- IVF Pre implantation diagnosis of genetic defects
- Chromosome painting for defects
- FISH

Cost of implementation

- Some facilities are available, but would need pump priming funding initially from Government or benefactors (Hotels, MRC, International funding bodies)

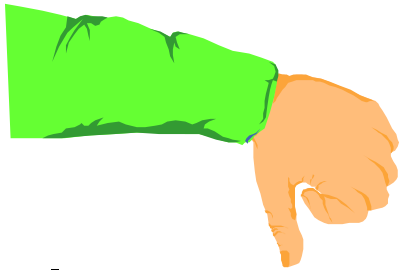
- Pan-Pathology service provided on a cost recovery and profit basis

- Initially some selected tests to be offered.

Who can buy in the project?

- Project proposal limited to laboratory medicine
- **BUT**
- Service may be offered to Forensic for DNA fingerprinting and Forensic molecular biology.
- Genetics studies by other experts.

Challenges



- Reluctance from management to buy in project
- Lack of enthusiasm from staff
- Lack of flexibility
- ICT back up
- Operational cost

- **<u>Acknowledgement</u>**
- Molecular and Medical Microbiology Research Group
- Research collaborators UK and Mauritius
- Central Health Lab (Candos)
- Ministry of Health
- Minister of Health
- MRC

Thank you for your attention

Any questions?