Resume

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PERSONAL PROFILE

- Male, Married, energetic, industrious and dedicated researcher with proven personal, analytical and organizational skills.
- Well-rounded, creative, technical problem-solver, innovative, self-motivated and optimistic.
- Tutor, trainer, and team-builder.
- Quality-conscious contributor with solid analytical and writing skills.

Science has fascinated me since my childhood that resulted in enthusiasm, love and passion for it. In my first preliminary cell biology class at higher secondary level I became conscious of the feeling that molecular cell biology, gene regulation and signal transduction was something I really enjoyed. At graduation level it was a wonderful experience to study the mechanism of action of drugs and by studying the molecular mechanisms involved in life processes created in me the zest and zeal to go into depths. Along this journey, choosing Biochemistry as the subject at Post graduation level was a big milestone when I enjoyed studying life at thinner level. Working as a Project Assistant after M. Sc under the guidance of Prof. Raghavan Vardarajan proved a beacons light to me when I learned standardizing and applying the scientific methodologies to solve scientific problems.

The way science explains the precision and accuracy of the complexities of the processes and the phenomena operating in the universe are really dazzling and inspiring the human mind. The fact that human beings can conquer the intricacies of life processes demands their understanding at molecular grassroots level. Take the example of cell division, the machinery involved in it and the delicacy of balancing it, is in fact the important contribution of cell biologists who strived in digging the depths in this field. At the same time, how the deviations from the normal pathway of cell division occurs that result in catastrophic situation for life called cancer - are the questions addressed to molecular cell biologists who are contributing worldwide to unravel the secrets of complexity of this problem at molecular fundamental level so that we can identify and devise logistic strategies for counteracting and averting successfully this dreaded threat to life.

OBJECTIVE

To contribute dedicated research at the molecular level of scientific problems in Life Sciences.

EXPERIENCE

- **Postdoctoral Fellow** (2004 - Till date) **(National Level DBT-PDF Program),** in the Lab of Cancer Biology, Center for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad under the supervision of **Dr. Syed E. Hasnain** and **Dr. Gayatri Ramakrishna.** The project title is "Molecular Etiology of Esophageal Cancer in Kashmir- a high incidence area in the world".

- **PhD** (1999 - 2004), under the guidance of **Prof. Shamim Ahmad (Supervisor)**, Microbiology Section, Institute of Ophthalmology, and **Prof. Asif Ali (Co-supervisor)**, Department of Biochemistry, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, India. The PhD thesis title was "Biochemical Studies on Antibiotic Resistance in Staphylococci from Eyes and other Clinical Sources in Health and Disease".

- **Project Assistant** (1998 - 1999), in Molecular Biophysics Unit, Indian Institute of Science **(IISc),** Bangalore India, under the guidance of **Prof. Raghavan Varadarajan**. Worked for two projects entitled "Effect of Proline to Serine mutations on the Stability of Maltose Binding Protein" and "Designing Vaccine candidate for HIV-1 using Stabilized gp120-CD4 complex".

SUMMARY OF THE WORK DONE

1. Molecular etiology of esophageal cancer in Kashmir- a high incidence area in the world:

Esophageal cancer is sixth most common cause of cancer deaths worldwide. Esophageal cancer displays unique epidemiological features that distinguish it from other malignancies. It shows marked geographical variation, with exceptionally high rates (some of the world's highest for any cancer) ranging from 3 per 100 000 per year reported in Western countries to 140 per 100 000 reported in Central Asia. Regions with high incidence are generally located in poor parts of the world (Fleischer and Haddad, 1999). A high incidence "cancer belt" extending from northern Sinkiang, through the former Soviet Union Republics of Kazakhstan, Uzbekistan and Turkmenistan to northern Afghanistan and northern parts (Caspian Littoral) of Iran, has been identified in the world (Blot et al., 1999). Kashmir valley borders the cancer belt on the south with incidence of 27 and 42 in females and males, respectively, per 10^5 individuals, the highest incidence in India (Siddiqi et al., 1989). In Kashmir valley there has been efforts highlighting the incidence of ESCC, the possible exposure of the local population to various dietary carcinogens and their *in vitro* effect. Intake of salted tea and the habit of consuming sun-dried foods, which promote nitroso compound formation, are associated with increased risk. Mutations in *TP53* (Mir et al., 2005) and infection of high-risk human papillomaviruses (HPVs), particularly the HPV types 16 and 18 (Katiyar et al., 2005) have strongly been implicated in human esophageal carcinoma in Kashmir. These results have put new questions regarding the etiology of the disease in which diet, ethnicity and environmental factors play a vital role, substantiating further investigation on involvement of other crucial oncogenes, tumor suppressor and xenobiotic metabolizing genes in the progression of ESCC.

Activation of the Raf/MEK/ERK (MAPK) signal transduction pathway by RAS mutations has been found in a variety of human cancers. Mutations of *BRAF* provide an alternative route for activation of this signaling cascade. Stabilization of *b*-catenin followed by nuclear translocation and subsequent T-cell factor/lymphoid-enhancing factor mediated transcriptional activation has been proposed as an important step in oncogenesis. Stabilization may occur through activating mutations in exon-3 at the phosphorylation sites for ubiquitination and degradation of *β-catenin*. *β-catenin* mutations have been reported in both cell lines and human tumors.

DNA variants of genes involved in Phase I and Phase II metabolism have been described. CYP isoenzymes play a major role in oxidation of chemical compounds, often resulting the formation of highly reactive compounds that are the ultimate carcinogens. A CYP2E1 variant with higher level of expression has been reported as a risk factor for various types of cancers. Similarly the gene variants of detoxifying enzymes like GST with reduced expression or the absence of a functional enzyme increases the susceptibility to several cancers. In addition, it is hypothesizes that the reactive intermediates created during nitrosamine metabolism which are capable of DNA damage,

and the differences between individuals in their ability to repair DNA damage might be important in ESCC pathogenesis.

The development of esophageal squamous cell carcinoma (ESCC) has been linked to exposure to carcinogens such as nitrosamines that cause various alkyl DNA damages and O^6-methylguanine–DNA methyltransferase (MGMT) is a primary defense against alkylation-induced mutagenesis and carcinogenesis. Because cytochrome P-450 2E1 (CYP2E1) is involved in metabolic activation of environmental chemical carcinogens, gene polymorphisms that alter its functions may be associated with cancer susceptibility. So in addition to the other possibilities, epigenetic studies of MGMT inactivation and hyperactivation of CYP2E1 need to be addressed for understanding the etiopathology of esophageal cancer.

As a part of PDF project the following work is near completion:

- Study the role of HPV in esophageal cancer.
- Study the mutational patterns by PCR-SSCP and Sequencing in *Ras*, *Raf*, and *β-catenin*.
- Immunohistochemistry studies of the above genes mentioned.
- Study the SNP (single nucleotide polymorphism) of DNA repair enzymes (hOGG1, XRCC1, MGMT) and xenobiotic metabolizing enzymes (CYP2E1, GST).
- Epigenetic studies of candidate genes with special reference to promoter hypermethylation of O^6-methylguanine–DNA methyltransferase (MGMT), CYP2E1 and MTHFR.

2. Abstract of the PhD Thesis:

The emergence of multidrug-resistant bacteria is a phenomenon of concern to the clinician and the pharmaceutical industry, as it is the major cause of failure in the treatment of infectious diseases.

This study was undertaken with the aim of determining epidemiology of clinical and carrier staphylococci and molecular studies of their acquisition and dissemination of resistance.

The prevalence of methicillin-resistant Staphylococci was determined in 750 subjects infected/colonized with staphylococci providing 850 isolates. Of 850 strains 575 were isolated from clinical specimens, 100 from nasal cultures of hospitalized patients, 125 from nasal and 50 strains were isolated from ocular swabs of hospital workers. It was shown that 35.1% (180/513) of *Staphylococcus aureus* and 22.5% (76/337) of coagulase-negative staphylococcal isolates were resistant to methicillin. The multiple drug resistance of all MRSA (n=180) and MRCNS (n=76) isolates was detected. In case of both methicillin-resistant as well as methicillin-sensitive staphylococcal isolates zero resistance was found to fusidic acid and vancomycin while as highest resistance was found to penicillin G followed by ampicillin. It was shown that the major reservoir of methicillin resistant staphylococci in hospitals are colonized/infected inpatients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to health care workers and patients. The results were confirmed by molecular typing using PFGE by *Sma*I-digestion.

Selective methicillin-resistant staphylococcal isolates were subjected to plasmid isolation and curing treatments to study their mode of resistance. The conjugation experiments clearly showed that resistant markers G and T got transferred from clinical *S. aureus* (JS-105) to carrier *S. aureus* (JN-49) and the ciprofloxacin (Cf) and erythromycin (E) resistance seemed to be chromosomal mediated. In one of the experiments, plasmid pJMR1O from *Staphylococcus aureus* coding for ampicillin (Ar), gentamicin (Gr) and amikacin (Akr) resistance was transformed into *Escherichia coli* (DH5α). Transformation efficiency was about 2 x 10^3 transformants/mg of plasmid DNA. The minimal inhibitory concentrations (MICs) for A and G were lower in *E. coli* than in *S. aureus*. However, the MIC for AK was higher in *E. coli* transformants than in *S. aureus*. In order to study the effect of temperature on the conjugation frequency, the temperature found in the human nose and eye environment (22 ^{o}C) was observed to be having a profound effect on conjugation frequency. The results seemed to be supporting the clinical significance of lower temperature prevailing in the nose and eye, the two sites most exposed to the external environment. Furthermore enzyme mediated resistance particularly through β–lactamase was studied in

selected strains. The substrate profile of β – lactamase so extracted was studied against different classes of pencillins and cephalosporins. The results indicated that methicillin remains the most stable penicillin to the staphylococcal β–lactamase. The anti-MRSA activities of a synthesized compound from an Inorganic Lab of A.M.U; was determined by *in vitro* testing. The results indicated that the Nickle–metalo, complex of 2-mercaptobenzimidazole has good activity against MRSA (MIC > 64 μg/ml) and demands to be analyzed for clinical applications in future.

The widespread occurrence and dissemination of *beta*–lactamase and PBP mediated resistance leading to multiple antibiotics ineffective, thus increasing the cost of health care, needs to be tackled logistically by wise and judicious use of existing antibiotics and by developing ideal and cost effective antibiotics having least chances of acquiring resistance. Furthermore the hospital acquired MRSA infections through colonization of patients and hospital workers demands an appropriate and timely measure to counteract this health problem.

3. Effect of Proline to Serine mutations on the Stability of Maltose Binding Protein:

Which involved introducing mutations at the appropriate sites, using Site directed mutagenesis, in the helices of MBP (Maltose Binding Protein). After sequence confirmation the protein was isolated from the periplasm of E-coli k-12, which harbors ppd1Plasmid. The purified protein after its molecular weight determination by mass spectrometry was subjected to various biophysical studies like the denaturant mediated unfolding using urea or guanidium hydrochloride, Differential Scanning Calorimetry (DSC), Circular Dichromism (CD), Fluorescence Spectroscopy etc. Maltose Binding Protein is a 370 amino acid, large globular two-domain protein, which exhibit, chemo-taxis and uptake of range of maltose sugar in the periplasm of *E. coli* and helps in the translocation of maltose from Periplasm to the Cytoplasm. The biophysical studies in MBP will reveal its folding dynamics and this can be further extrapolated with the same mutation studies on the other proteins.

As a part of this project I did Differential Scanning Calorimetric studies and analysis of purified MBP protein.

4. Designing Vaccine candidate for HIV-1 using Stabilized gp120-CD4 complex:

The project was **NIH** funded. The broad long term objectives of this research are to produce folded and rigidified versions of the HIV envelope glycoproteins gp120/gp140 for use as potential vaccine candidates. Earlier attempts to use gp120 as a vaccine failed to generate antibodies capable of neutralizing primary isolates of the virus. Antibodies in vaccinated individuals were typically against linear epitopes found primarily in the denatured protein. Recently the structure of core gp120 in complex with a two-domain CD4 fragment and the antigen binding fragment of a neutralizing antibody have been determined. This structure is being used as the starting point for the design of folded variants of gp120 that should be stable and adopt the gp120 conformation found in the crystal structure in the absence of CD4 and neutralizing antibody. Mutants with improved stability and rigidity are further tested for immunogenicity and corresponding antisera are also examined for neutralization activity.

As a part of this project I did the mutagenesis, expression and purification of CD4 protein besides its spectroscopic and calorimetric studies. I did five mutations in CD4 using SDM method, cloned in pET20b and pBAD vectors and optimized the protocol for their expression using different *E. coli* strains. The first purification was done by Ni-NTA column chromatography and the final purification by gel filtration and ion exchange chromatography using HPLC and FPLC. Furthermore we checked the affinity of all the CD4 mutant proteins with wgp120 and dgp120 including their mutant versions by BIACORE assay. I also did the spectroscopic and calorimetric studies of WT and mutant CD4.

PUBLICATIONS

1. Manuscript submitted to "Indian Journal of Medical Microbiology " for publication:

"Comparative study of pathogen frequency and antimicrobial resistance patterns among organisms from eye infections isolated in 1993 and 1999"

Shamim Ahmad, **Javid Ahmad Dar**, Shaheena Akhter

Microbiology Section, Institute of Ophthalmology, J.N. Medical College Aligarh Muslim

University, Aligarh-202002, India.

2. Manuscript submitted to "International J. Infectious Disease " for publication:

"Molecular Epidemiology of Clinical and Carrier-MRSA in a Hospital Setting in India"

Javid Ahmad Dar[1], Shamim Ahmad[1], Manzoor Ahmad Thoker[2], Jamal A Khan[3], Asif Ali[4], Mohammed Azhar Khan[1]

[1]Microbial Division, Institute of Ophthalmology, [3]Division of Bacteriology, Department of Microbiology and [4]Department of Biochemistry, J.N Medical College, Aligarh Muslim University Aligarh-202002, India.[2]S.K. Institute of Medical Sciences Kashmir-190011.

3. Manuscript under preparation:

"Tetracycline-resistance transfer from clinical to carrier Staphylococci- an in-vitro study"

Javid Ahmad Dar[1], Shamim Ahmad[1], Mohammad Owais[2]

[1]Microbiology Section Institute of Ophthalmology J.N. Medical College and [2]Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh-202002, India

4. Manuscript under preparation:

"Mutational analysis of KRAS, NRAS and BRAF genes in esophageal squamous cell carcinoma in Kashmir"

Javid Ahmad Dar[1], Mohammad Muzaffar Mir[2], Syed E. Hasnain[1], Gayatri Ramakrishna[1]

[1]Center for DNA Fingerprinting and Diagnostics, Hyderabad-50076, [2]S.K. Institute of Medical Sciences Kashmir-190011

5. Manuscript submitted to "International J. Cancer " for publication:

"No role of mutations involving exon 3 of the beta-catenin gene and Human Papillomavirus (HPV) in squamous cell carcinoma of esophagus in a high-risk population from Kashmir"

Javid Ahmad Dar[1], Pavani Sowjanya[1], Brajesh Kumar Jha[1], M Muzaffar Mir[2], Nazir A Dar[2], M Shafi Dar[2], M Maqbool Lone[3], Seyed E Hasnian[1] and Gayatri Ramakrishna[1]

[1]Centre for DNA Fingerprinting and Diagnostics Hyderabad, Departments of [2]Clinical Biochemistry and [3]Radiotherapy, Sher-e-Kashmir Institute of Medical Sciences, Soura Srinagar, J&K-190011

6. Manuscript submitted to "Carcinogenesis " for publication:

"Impact of combined genetic polymorphisms in genes of folate metabolism viz., glutamate carboxypeptidase II (GCPII), methylene tetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) on breast cancer susceptibility in Kashmiri population"

Naushad S M[1], Brajesh K Jha[1], **Javid Ahmad Dar[1]**, M M Mir, Radha Rama Devi A[1] and Ramakrishna G[1]

[1]Center for DNA Fingerprinting and Diagnostics, Hyderabad and [2]Department of Clinical Biochemistry, Sher-e-Kashmir Institute of Medical Sciences, Soura Srinagar, J&K-190011

CONFERENCES AND SYMPOSIA ATTENDED

1. "International Symposium on Cancer and Cell Death", Organized by National Centre for Biological Sciences (NCBS), TIFR, Bangalore, India. January 10th-13th, 2002.
2. "MIDCON-CM2004" organized by Department of Microbiology, Sher-e-Kashmir Institute of Medical Sciences, Srinagar Kashmir, India. Presented paper entitled "Molecular Epidemiology of Clinical and Carrier-MRSA in a Hospital Setting in India". 1st –3rd October 2004.
3. "Micro Biotech 2005" organized by the Association of Microbiologists in India (AMI) in Hyderabad. Presented Paper entitled "Alarming Methicillin Resistant Staphylococci in Ocular and Hospital Environments". December 8-10, 2005.
4. INTERNATIONAL SYMPOSIUM ON "HUMAN GENOMICS AND PUBLIC HEALTH" & XXXI ANNUAL CONFERENCE OF ISHG – 2006 organized by Jawaharlal Nehru University, New Delhi, India. Presented Poster entitled "Absence of *BRAF* and *β-catenin* mutations together with Human Papillomavirus (HPV) in squamous cell carcinoma of esophagus in a high-risk population from Kashmir". 27th February – 1st March 2006.
5. "Third Indo-Australian Conference on Biotechnology: Vaccines for cancer, infectious

diseases, life style and degenerative diseases" organized by CDFD, Hyderabad India. 6-8 March 2006.

EDUCATIONAL QUALIFICATION

EXAM/DEGREE	BOARD/ UNIVERSITY	Division	Year of Passing	SUBJECTS
DBT-PDF	CDFD, Hyderabad, India		July 2004 -Till date	Cancer Biology
Ph. D.	Aligarh Muslim University, India		2004	Biochemistry
NET (LS), July 1999	CSIR-UGC		1999	Life Sciences
M.Sc.	Kashmir University, India	Ist	1998	Biochemistry
B.Sc.	Kashmir University, India	Ist	1994	Chemistry, Botany, Zoology, English.

RESEARCH TECHNIQUES AND SKILLS

- DNA/RNA Isolation and Purification from bacteria and mammalian systems.
- PCR bases Line-blot for screening HPV.
- Isolation of DNA from formalin-fixed, paraffin embedded tissue.
- Tissue culture and anti-cancerous activities of herbal extracts.
- Single-Strand Conformational Polymorphism (SSCP) and Hetroduplex analysis (HDA) for detection of mutations.
- Immunohistochemistry.
- Site directed mutagenesis-using PCR (Polymerase Chain Reaction).
- Gradient PCR for confirming site directed mutations.
- Western blotting.
- ELISA
- Protein expression, isolation and purification techniques.
- Eletrophoretic and chromatographic techniques in frequent use.
- Basic Biochemical tests and Microbiological techniques.
- Antibiotic resistance studies of bacteria using Disk diffusion method.
- MIC determination using Agar and Micro dilution techniques.
- Plasmid isolation and curing.
- Conjugation and Transformation.
- Restriction mapping of *Staphylococcus aureus.*
- B-lactamase and Chloramphenicol acetyl transferase tests.
- Denaturant mediated denaturation of proteins.
- Differential scanning calorimetry (DSC).
- Circular Dichromism (CD).
- Fluorescence spectroscopy, Mass spectrometry, Spectrophotometry.
- High performance and Fast performance liquid chromatography (HPLC and FPLC respectively).

COMPUTER KNOWLEDGE

MS-office (Power point, MS-excel, MS-word) Corel draw, Page maker, Adobe Photoshop, Adobe Illustrator, Statistical Packages (SPSS for Windows, Origin50, Statistica).

AWARDS AND FELLOWSHIPS

1. Qualified National Eligibility Test (NET) for Lectureship/JRF-1999 conducted by University Grants Commission (UGC) and Council for Scientific and Industrial Research (CSIR), New Delhi.
2. Selected in the National Level test for M.Tech. Biotechnology program-1999, conducted by Jawaharlal Nehru University, New Delhi.
3. Qualified National Level Preliminary Common Entrance Test-2000, for PhD from JNCASR, Banglore India.
4. Selected for National Level DBT-PDF Program-2004 of Department of Biotechnology (DBT), Government of India.

REFERENCES

Prof. Shamim Ahmad, PhD., "DAAD" Fellow (W. Germany), "JSPS" Fellow (Japan) Microbiology Section, Institute of Ophthalmology, J.N. Medical College, Aligarh Muslim University, Aligarh-202002, U.P. India. Ph: +91-571-2720148 Mobile: +91-9897452023 E-mail: shamimshamim@rediffmail.com	**Dr. Syed E. Hasnain**, Ph.D., FASc., FNASc., FNA, FTWAS Vice-Chancellor Hyderabad Central University, A.P. India. Tel: +91 - 40 - 23132000, Mobile: +91- Fax: +91- 40 - 27155479, 610 E-mail: ehtesham@cdfd.org.in
Prof. Mir Muzaffar, PhD. Associate professor, Clinical Biochemistry Sher-e-Kashmir Institute of Medical Sciences, Soura Srinagar-190011 J&K India. Ph: 91-09419484920 E-mail: mirmuzaffar@rediffmail.com	**Prof. Raghavan Varadarajan**, PhD. Associate professor, Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, Karnataka, India. Ph: 91-80-23092612 E-mail: varadar@mbu.iisc.ernet.in

I hereby declare that the above details are true to the best of my knowledge.

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