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APPLICATION OF BIOCHEMICAL GENETICS TO GENETICALLY CHARACTERIZE MOSQUITO VECTORS

Of all the insects that transmit diseases, mosquitoes represent, by far, the greatest menace. Mosquitoes are pestiferous insects, which are responsible for the transmission of various dreadful diseases and the WHO has declared the mosquito "Public Enemy Number One"[1]. Mosquito-borne diseases cause havoc in developing countries, both in urban and rural population and the loss in terms of human lives is irrevocable. A major portion of the National Health budget is spent on the control of these diseases. Reliable methods for identification of vectore mosquitoes are desirable to distinguish between the harmless majority and the dangerous minority.To determine the species of medical importance, morphological characteristics of mosquito eggs, larvae, pupae and adult males and females are helpful, but the existence of many sibling species, (i.e., morphologically indistinguishable species) with contrasted bio-ecological characteristics makes it necessary to employ other methods to define and recognize many of the harmful species.

The sibling species complexes are defined as pairs or groups of biologically distinct species that are morphologically identical, or nearly so. Sibling species often have contiguous or overlapping geographical distributions where they remain reproductively separate through assortative mating due to various isolating mechanisms.[2] The discovery of more and more sibling species among mosquitoes and other arthropods, by analysis of population genetics, increasingly undermines the taxonomic reliability of anatomical features for proof of specific identity.

Studies of reproductive compatibility and incompatibility can give valuable clues to ta xonomic relationships among mosquito species, but much more understanding of these processes is needed. Such work will involve ethology, ecology and endocrinology in relation to genetics and taxonomy. These approaches can provide better ways of defining species, which may then be identified by simpler criteria and characteristics. Apart from the co-adapted lock and-key arrangements of external genitalia of conspecific females and males, one must try to analyse the role and formulae of pheromones that appear to function for specific sexual purposes either by contact[3] or remotely epigamic behaviour of mosquitoes including male swarming and inter-sexual communication by sight and specific sounds[4-6] as well as the cytogenetical and physiological systems. Cross-mating and cytogenetic studies have revealed three species within the taxon Anophelese farauti (s.l.), provisionally designated as An. farauti No.1 and An. farauti No.2[7,8].

Apart from the study of polytene chromosome characteristics, the usefulness of mosquito cytogenetics is mainly to provide a basis for biochemical and molecular investigations. Thus taxonomic status and population genetics of the vectors are extremely important because of the wider involvement of this species in the transmission of human viral pathogens.

Both cytotaxonomy and zymotaxonomy, together with more basic morphological methods, have proved so successful for the identification of mosquitoes and the interpretation of vector population genetics that there is seldom any perceived need for more elaborate and expensive biochemical or molecular techniques. Electrophoresis is the main technique which is rapid and also sensitive enough to detect minor differences and hence can examine a large number of specimens. The main factors which influence electrophoretic studies of proteins are genetic variation, age, sex physiological conditions and environment. In many organisms within the same individual there are enzymes with similar catalytic properties but differ in their primary structures which are called isozymes and are the products of separate genes. Specific allozymes have been detected for many mosquitoes and in some cases the isozyme characters appear to be correlated with vector status or another biological attribute.

Historically, population genetics is based on superficial phenotypes or metrical characteristics having complex polygenic inheritance and considerable environmentally induced variation. But, in addition to these obvious characteristics there are genetically determined differences at the protein level which are much less subject to environmental influence. The genetic code of DNA is translated into proteins and there are frequently subtle, non-functional variations in the structure of homologous proteins in different individuals originating from small variations in the genotype. These phenotypes, which can be detected by biochemical means, in particular electrophoresis, are relatively easily studied both in natural and laboratory populations. This provides a rapid and efficient way of obtaining much of the basic genetic information crucial to the genetic manipulation of different population.

Protein Structure

Most proteins, in particular those with enzyme function, contain amino-acids with electrically charged side chains. Arginine, histidine and lysine are positively charged while aspartate and glutamate carry a negative charge. Thus virtually all proteins have a net charge depending on the relative proportions of amino acids, unless they are at their isoelectric point, the pH at which the net charge is zero.

The basis of electrophoretic separation is that proteins of different net charge and different molecular size will migrate at different rates within an electric field. Over the past 30 years isozymes have been used extensively as markers to analyse the genetic structure of natural population. In diploid, sexually reproducing animals each chromosome pair consists of homologous chromosomes one derived from each parent. Every gene (or locus) is composed of two parts, the alleles, one on each of the homologous chromosomes. Each allele is composed of a section of DNA with the same or similar base sequences. When the alleles are co-dominant (which is the most common condition for protein producing loci) each allele forms half of the total amount of polypeptide. However, if one of the alleles contains a slightly different sequence of bases the locus will produce two polypeptides with the same function but differing from each other by minor amino acid substitution. A locus in a species is considered polymorphic if the most common allele does not occur at least 99% of the time (some definitions say 95%).

In a species there may be a large number of (30 or more) alleles at any locus. However, in the individuals there are only two possibilities; the alleles at a locus are identical (homozygous) or different (heterozygous). If for example, in a population there are two possible alleles A and B, for a locus then three genotypes are possible. Two homozygotes AA and BB and one heterozygote AB. Each homozygote will produce only one type of polypeptide but they will be different from each other, whereas the heterozygote will produce both polypeptides.

Analysis of Zymograms

When individuals in a population exhibit variation in protein structure the zymograms will conform to those expected under simple models of single locus Mendelian inheritance with co-dominant expression.

If the population is in Hardy-Weinberg Equilibrium the calculated and observed heterozygosity will not be significantly different. The mean heterozygosity per individual is the mean of the proportion of loci at which

each individual is heterozygous, summed over all individuals. Thus if, on an average, an individual is heterozygous at 12 of the 40 loci examined, its mean heterozygosity is 0.3.

All these practices require genetic characterization of the stocks under investigation and analysis of results uninfluenced, as far as is possible, by environment. Biochemical genetics is the only practical method of obtaining a sufficiently detailed characterization of a statistically acceptable number of individuals within a population.

Isozymes may be differentially expressed during the life cycle. Sex specific proteins may be of value in determining the sex of an individual before the gonads are morphologically developed. Environmental factors and disease may result in differential expression of isozymes and changes in other proteins. For the developmental programmes biochemical genetics permits the evaluation of the degree of homozygosity and the genetic similarity of populations making designed crossings more likely to be productive. These techniques also make it possible to monitor genetic changes in colonized populations thus permitting the detection and correction of unintentional inbreeding and gene loss.

The breeding of specific rare alleles into populations to serve as genetic tags is likely to be a very valuable development. It will permit the evaluation of the performance of different stocks of mosquito species in the same environment and can be used with mosquitoes where initial size or life styles precludes normal tagging methods. Evaluation of the performance of animals stocked in natural conditions is possible following allozyme tagging. With hybrid mosquitoes electrophoresis allows the detection of the relative contribution of maternal and paternal genomes. In addition, the characterization of species makes it possible to predict, to a certain extent the outcome of hybridization. Few attempts have been made to explore specific biochemical characteristics of mosquitoes other than allozymes, and yet the available results are not discouraging.

Zymotaxonomy

It seems desirable to explore further the functional value of allozyme changes in connection with vetor competence[9,10], vectorial capacity[11,12] and other aspects of mosquito biology and speciation.

Diagnostic allozymes have been found for specific identification of nearly all the mosquitoes investigated for this purpose, but there is apparently no way of predicting which enzyme systems are most likely to be involved in interspecific divergences.The study of mosquito allozymes has therefore been extended beyond the species level[13-28].Of course, it is always necessary to identify mosquito specimens by morphological or cytotaxonomic methods as far as possible before subjecting them to the destructive process of electrophoresis for zymotaxonomic purposes.

It is relevant to emphasize how much the evidence of allozyme frequencies contributes to precise characterization of local demes and populations of Aedes. aegypti[29-37] and of Culex.pipiens [38,39], so that their evolutionary divergences and ecological adaptations can be interpreted in relation to vector functions. Equivalent studies on vector population genetics of other Anophelines and Culicines, by means of allozyme frequency comparisons, should help provide the background for more appropriate antivectorial measures to be implemented against each population of medically important mosquitoes in turn.

To identify those mosquitoes that are of vectorial importance, control them and monitor disease transmission rates, it is a pre-requisite to recognize vector genotypes as well as the species themselves. Such approaches may give some false negative results, although positive vector competence can be confirmed by following the inheritance patterns of factors responsible for these traits which occur at variable frequency in populations of vector species[9, 40-43].

Another important source of intraspecific variation comes from the way that some species, or at least some populations, of mosquitoes show individual variation in response to certain aspects of the environment. It is difficult to establish whether this heterogeneity is due largely to chance or is governed by inherent polymorphism.

Even in the days when all species were thought to be identifiable from external morphological features, it required considerable skill, experience and tedious effort[44] to undertake the primary taxonomy and then to heck the identity of representative mosquito specimens being evaluated for epidemiological purposes or subjected to control measures. Fortunately, the basic expense are not disproportionately greater for cytotaxonomy,

which requires only a few chemicals and couple of good microscopes, or for zymotaxonomy, which needs less capital investment for equipment but rather more recurring expenses for chemicals. Assume that these techniques, when integrated with field ecology and laboratory genetic studies[45] can be employed to identify any mosquito of interest and clarify its taxonomic and vector status, as exemplified by the combination of procedures used for interpretation of the sibling species comprising the An. gambiae complex then it must be decided whether this level of attention is affordable and is required to provide adequate information on disease transmission or anti-vectorial operations.

These mosquito vectors have well adapted for life in intimate association with humans. It is widely accepted that only virus strains that replicate efficiently in humans and produce high levels of viraemia are transmissible by mosquito species. Genetic variation in virus strain is thought to affect virulence, accounting for the changing patterns of disease. The movement of the viraemic people by both local and international air travel seems to be the most likely mechanism for the movement of these viruses and the significant variation in the oral susceptibility to these viruses in the various strains of mosquito vectors. In order to test these changes in the vector competence of the vectors collected from an area endemic for these viruses, it is essential to know the presence of different geographical populations of these species from different areas which can be tested and the data can be interpreted in the light of known epidemiological information in the area. We can explore the population structures of the mosquitoes by biochemical markers.

Studies of this nature had to be planned to understand the population structure of these mosquitoes which carries tremendous significance in estimating the differential role played to harbor dengue viruses by different adult populations and also in the transmission of dengue in a given niche as well as for designing control strategies. This inference carries a tremendous epidemiological significance, and may be utilized in planning control operations of both the disease and the vectors.

Unfortunately, however, the method is costly and time-consuming. It is not a field technique and can be undertaken only by a competent person in a laboratory with sophisticated analytical equipments.

Recently attention is given on the intrinsic factors of vector competence that control the ability of mosquito to vector arboviruses. The potential concept affecting the intrinsic factor of vector competence is the availability of interspecies and intraspecies models available in nature.

There is a need for research on the population structure to elucidate indicators of its potential epidemiological importance in arbovirus cycle. As long as a dengue vaccine is not available, control of the mosquito vector is the only way to prevent epidemics. Knowledge of the genetic structure of the vector populations is therefore required to maintain effective vector control strategies by working out the best period for initiating insecticide treatments, or studying the conditions that allow manipulated mosquitoes to spread efficiently to block dengue transmission. The knowledge of genetic characterization is useful to estimate the level of gene flow from which mosquitoes displacements can be inferred to examine the patterns of genetic diversity. This can predict patterns of spread of non-indigenous species and their potential roles in the occurrence of this emerging disease. Information about the different populations will assist in understanding arbovirus epidemiology and disease risk and in developing the control strategies for these species of mosquitoes.

The population genetic studies of Ae. aegypti have mainly explored genetic variation at a large scale among geographical regions[33] or at a regional scale[46,47]. Very few studies have examined genetic variation at a local scale (at a city or a district level), largely because of the need of highly polymorphic genetic markers.

Methods Employed

Electrophoresis

Simplified procedure of electrophoretic separation of serum protein is the best method for the separation of isoenzymes. Protein molecules migration in an electric field depends on its net electric charge and size and pH of buffers. In alkaline solution proteins are negatively changed and travel towards anode. To compare the location of enzyme bands on the gels, samples from all species included on each electrophoretic gel.

Mosquitoes can be collected by employing outdoor and indoor resting collections, human-landing collections and immature survey in the area under disease impact and also from the places without cases.

Thus mosquito samples from 4-5 different localities viz. endemic and non-endemic areas can be collected and colonized for this study. Enzyme systems utilized for this examination are acid phosphatase, alcohol dehydrogenase, aldehyde oxidase, alodolase, alkaline phosphatase, esterase, alpha glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic enzyme, octanol dehydrogenase, 6-phosphogluconate dehydrogenase, 1-pyrroline dehydrogenase, peroxidase, sorbitol dehydrogenase and tetrazolium oxidase. For each enzyme system of each strain 118 to 120 homogenates of mosquitoes will be tested[34,48]. Methodology for studying the genetic variation using different enzymes should be standardized using Polyacrlyamide gel elctrophoresis[49]. The gels can be stained by using standard enzyme-specific histochemical staining procedures[50,51].The measures of genetic variation can be based on detection of differences in migration of enzyme phenotype and also by finding out the polymorphic loci present in the different populations.

Genetic Basis of Observed Electrophoretic Variation

A locus is considered polymorphic if the frequencies of common alleles were not greater than 0.95 and reveal electrophorosis variants. Enzyme loci in which all specimens tested appear as a single monomorphic band are classified as mono morphic loci. Those with 2 or more well separated zones of activity are assumed to be the products of 2 or more loci. The enzyme loci in different tissues tested are numbered consecutively from the most cathodal to the most anodal and alleles are designated alphabatically.

Alternative enzyme forms derived from different loci and have different molecular weight but having same function are isoenzymes. Where as multiple enzymes encoded by alternative alleles at a locus are alloenzymes. In monomorphic proteins each homozygote produce one protein in gel after staining. In heterozygotes both the product of homozygotes are formed and thus 2 bands are formed in the gel. In the dimeric proteins 2 polypeptide chains are present in the protein and hence 3 bands are generally formed in heterogenous individuals. Heterozygotes produce at least two bands and as many as five depending on whether the enzyme has 1,2,3 or 4 polypeptides.

Current Scenario

Earlier population characters have been based on superficial phenotypes or metrical characteristics having complex polygenic inheritance and considerable environmentally induced variation. Genetically determined differences at the protein level are much less subject to environmental influence.

Paulson and Hawley 1991[52] showed the difference in vector competence and virus susceptibility between field collected populations and laboratory populations. Tesh et al., 1976[53] showed different geographical strains of Ae. albopictus showing varying level of oral susceptibility to Chickungunya virus. These studies suggest that selective pressures in the environment may cause change in susceptibility of the mosquito population and that vector competence may be an important risk factor for epidemic dengue transmission[54]. Susceptibility status of a mosquito species to infection with an arbovirus can change significantly in the field during different seasons with changes in temperature and rainfall. Changes in the competence of the mosquito vectors for arboviruses affects the efficiency of transmission and virus occurance in nature[55]. Susceptibility and transmissibility of different geographical strains of Ae. aegypti mosquitoes to Chickungunya virus varied distinctly[56,57]. It is very important to know about the existence of different populations in each species to estimate the level of the competence of mosquito populations to the virus present in that area. Accordingly vector control measures can be initiated in that particular area. The pattern of genetic differentiation may provide information on local dispersion pattern which is important to better understand how vector competence evolves in the field. To assess the role of the vector Ae. aegypti in the changing pattern of the dengue in Southeast Asia, a study was carried out to find out the ecology, genetic structure and vector competence in South Vietnam[58]. Similar studies were conducted in Chiang Mai (Thailand) to know the genetic structure of Ae. aegypti populations to provide estimates of their abilities to harbour and transmit viruses for better understanding of dengue transmission and to develop effective control measures[59].

Microgeographic variation in habitat types promotes significant genetic diversity within and between-population. Using isoenzyme analysis, large genetic differentiation was found among Brazilian samples of

Ae. albopictus and between them and North American samples. Infection rates with dengue and yellow fever viruses showed greater differences between two Brazilian samples than between the two North American samples or between a Brazilian sample and a North American sample for Ae. albopictus[60].Ae. aegypti populations from the Ho Chi Minh city were genetically differentiated and their infection rates differed from those of populations from the commuter belt[61]. Population genetic structure and competence as the vectors Ae. aegypti and Ae. albopictus for dengue-2 virus was studied in Madagascar[62]. Genetic differentiation in the mosquito population of Ae. aegypti was studied in French Guiana[63] and in French Polynesia[64, 65]. Effect of biocide treatment on the genetic variability of the population of Ae. aegypti was evaluated in Thailand[66]. Enzyme electrophoretic profiles of three morphologically similar Aedes species Ae. cretinus, Ae.albopictus and Ae.flaevopictus provided diagnostic characters sufficient to separate these 3 species[67]. Allozyme patterns of Ae. albopictus, was analyzed to find out genetic relationships among different populations in Thailand in an attempt to estimate rates of gene flow among populations and to identify barriers to and corridors of gene flow[68].

Genetic differentiation of Ae. aegypti mainland and island populations from southern Thailand using 24 enzymes revealed a large effective migration rate of these populations[69]. Genetic structure and oral susceptibility of Ae. aegypti to dengue virus was observed in Cambodia[70]. Analysis of genetic differentiation of Ae. aegypti from different populations was conducted to understand the genetic relationships among population in Brazil[71]. In the eastern Caribbean, the island of Martinique, the genetic polymorphism of the dengue vector Ae. aegypti was introduced and maintained by large gene flow among populations[72]. A comparison of electrophoretically detectable isozyme differences in 6 populations of An. quadrimaculatus from northern Arkansas was undertaken[73]. Minor genetic differences was detected among the geographic samples of Cx. nigripalpus from north to south Florida based on 14 isoenzyme loci[74]. Enzyme polymorphism was recorded in Cx.pipien complex[75]. Geographic genetic variation in Cx. pipiens quinquefasciatus[76--78] and Cx. tarslis from United States was studied to find out the subspecies in that complex. Evidence for microgeographic genetic subdivision[79], electrophoretic taxonomic key[80,81] and patterns of genetic variability was studied in An. quadrimaculatus[82]. Allozyme analysis was carried out in six species of the members of the An. punctulatus complex in Paupua New Guinea[83] and in the Solomon islands and Vanuatu[84]. Electrophoretic keys was used to identify members of the An. punctulatus complex of vector mosquitoes in Paupua New Guinea[85]. Population genetic structure of the major malaria vector An. arabiensis mosquitoes collected in Ethiopia and Eritrea showed geographical diversity[86]. Significant genetic differentiation was observed between rural and urban An. minimus, a major malaria vector in Vietnam[87]. Genetic differentiation of An. claviger S.S in France, neighbouring countries[88] and in Europe[89] was studied in detail. Population structure and genetic divergence of the important vector of human malaria An. nuneztovari from Brazil and Colombia was delineated[90]. Biochemical systematics and population genetic structure of important malaria vector An. pseudopunctipennis from central and South America showed high genetic distance[91].

In recent years some attempts have been made to enlarge our knowledge about the vector species involved in transmitting the mosquito-borne diseases, nevertheless, very little is actually known regarding differential roles played by different populations of these species. Proper utilisation of the results will help in planning control strategies of the vectors and disease. Estimates of gene flow have provided great insight into the epidemiology of arthropod-borne diseases like trypanosomiasis[92] or dengue[59], etc.

APPLICATION

In order to examine the genetic characterization and genetic variation of vector species different enzymatic proteins using polyacrylamide electrophoresis are employed to determine the genetic structure of vector by specific enzyme markers. This leads to better understanding of the real status of vectors populations present in different areas and involved in the actual active disease transmission. This may work as a tool for the initiation of control strategies, both for the disease and the vectors in the area.

To achieve better targeting of dengue vector control and elucidation of infestation origins, isoenzyme electrophoresis can be used to define patterns of genetic differentiation of gene flow between these populations for understanding factors that contribute to the spread of dengue fever. Therefore it is highly essential to explore the population structure of the mosquito vectors.

It is possible to detect allelic and non-allelic forms of the species tested which will enable to find out differences between homozygotes and heterozygotes. The genotype taxonomic survey of natural habitats can identify genetically distinct population and endangered species and a map indicating the availability of different population can be drawn.

Studies on sibling species involving LDH electrophoretic variants to distinguish species A and B make it possible to correlate malaria endemicity with species A and B distribution. Low vectorial potential of species B explains the lack of malaria transmission observed in eastern UP northern Bihar and Southern Karnataka. But in upper Krishna dam area in Gulburga district species A constituted 90% and malaria incidence is very high.

Insecticide responces at the sibling species level can also be monitored.Species A is more susceptible to DDT than B.

Interspecies and Intraspecies genetic variation can also be monitored. Rare alleles present in these mosquito vectors are used in genetic tagging or marking of sibling species. By specific patterns of enzymes a key can be produced in solving identification problems.

Conclusions

These studies will bring out genetic structure / characterization of different mosquito populations using electrophoretic polymorphism. The enzyme system will provide diagnostic characters sufficient to separate the different populations actually exist within the species of mosquito vectors. This will be an attempt to better understand the geographical differentiation of populations of these vectors. This will pave the way for biochemical taxonomy (molecular taxonomy) for the identification of wild caught mosquitoes of the morphologically similar populations of the same species and clarify taxonomic status of these mosquitoes. These genetic markers will be used to estimate the amount of genetic divergence among geographic populations as well as to analyze the genetic structure of these populations. Population genetics approaches provide an important framework for understanding the actual populations that contribute to the spread of mosquito-borne diseases and will clearly provide additional information on the genetic divergence of the vectors which will help to explain the differences in the transmission pattern throughout its geographical range. This will further help to initiate studies to find out whether there is variation in the oral susceptibility due to the genetic variability of mosquito populations. The observed genetic variability values from the different localities of these mosquitoes when compared with the genetic variability level of the colony samples, will demonstrate the effect of inbreeding of the colonized mosquitoes. Microgeographic genetic subdivisions of these species can also be brought out. Genetic diversity usually caused by environmental factors, genetic drift, inbreeding, population breeding structure can be determined. If there is a strong genetic differentiation observed among populations of these species; it may be associated with different abilities to harbor and transmit the different pathogen. Proper utilization of these results will help to initiate vector competence studies and subsequently planning for control strategies of different vectors and diseases. Estimation of gene flow will provide a great insight into the epidemiology of these diseases.

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This write-up has been contributed by Dr.P. Philip Samuel, Research Scientist, Centre for Research in Medical Entomology, Madurai.

ABSTRACTS

Some Research Projects Completed Recently

Effects of non-ionic polymers of surfactant additives on stress induced apoptosis in type II pneumocytes

The present study was initiated to evaluate the effect of nonionic polymers or surfactant additives on stress induced apoptosis in tumorigenic human lung epithelial cell line A549. The cell line procured from National Centre for Cell Sciences, Pune was maintained in the prescribed culture medium supplemented with 5% FCS/antibodies and under specific culture conditions (37^{0}C, 5% O_2/95% air incubator). Cell populations with 70-80% confluency, exhibiting 100% viability and excellent morphology were used for the study. Cells incubated in the absence of apoptotic inducers e.g etoposide, U.V. light or hyperoxic exposure served as control group and those incubated in the presence of apoptotic inducers served as test group.

The exposure of the cells to hyperoxia/etoposide/ UV could result in increased apoptosis. Presence of surfactant resulted in a decrease in the percentage of apoptotic cell death in all the cases. Further, the extent of apoptosis was reduced in the cells exposed to hyperoxia/UV in presence of PEG/dextran alone or along with surfactant. However, an increase in the percentage of apoptotic cell death was noticed in case of cells exposed to etoposide in presence of PEG/dextran alone. These results were further substantiated by cell death detection ELISA. Formation of the nucleosomal DNA ladder is the hallmark of apoptosis so analysis of the pattern of DNA isolated from the cells exposed to different stress inducers was done. The ladder like appearance of the fragmented DNA was quite prominent in cells exposed to O_2 for 8h. No such ladder was observed in the control cells. Very faint ladder was detected in the cells exposed to hyperoxia in presence of surfactant/nonionic polymers like PEG/dextran. Exposure of cells to etoposide/UV also resulted in formation of ladder which was not very prominent in these cells in presence of surfactant/ nonionic polymer. Further, the oxygen exposed cells and the cells exposed to etoposide/UV showed maximum FITC-dUTP end labeling which pointed to the higher percentage of apoptosis in these cells as compared to the control cells and also the cells cotreated with the modulators.

Western blotting was performed to assess the expression level of Bcl-XI (antiapoptotic protein) and Bax (proapoptotic protein), which revealed a decreased

expression of Bcl-XI in the stress (hyperoxic, etoposide/ UV) exposed cells. Bcl-XI expression was comparably same in the control cells and also the cells exposed to stress in the presence of modulators like surfactant or nonionic polymer. Expression of proapoptotic protein Bax was found to increase in the cells exposed to hyperoxia or other stress inducers as compared to the control cells.

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Revalidation of syndromic approach for the management of genital ulcers disease in a tertiary care centre in Northern India

The present study was carried out on a total of 217 patients (185 males and 32 females with mean age of 29□8.7 years to find out the etiology of genital ulcers in patients attending the STD OPD of All India Institute of Medical Sciences, New Delhi and evaluate the efficacy of the algorithm for syndromic management of genital ulcer diseases (GUDs) in sexually active male and female patients.

The majority of male patients (66.5%) had unprotected sexual exposure to commercial sex workers; all female patients denied history of any premarital or extra marital sexual experience. The patients with genital ulcers were treated with a combination of single dose of Inj. Benzathine pencillin 2.4 MU I/M as a single dose along with erythromycine 500mg four times daily for 7 days if the history of vesiculation or recurrences is not present. If the patients presented with vesicles or if the history of recurrences was present, patients were treated with acyclovir 200 mg.

The study showed that herpetic genital ulcer (HG) was the commonest (38.7%) cause of GUDs. Chancre was present in 24.4% of the patients followed by chancroid in 8.2% of patients. A substantial number of patients (12%) had 'mixed infection' having these 3 organisms in different combinations. Though the isolation of H. ducreyi was very low (3.2%) it showed significant resistance to ciprofloxacin (66.6%). With 'syndrome-based' management complete cure was seen in 99.1% of patients with non-herpetic GUDs and 82.6% of patients with herpetic GUDs at 7 days.

A sensitivity of 73.5% of the 3 NACO flow charts for the management of GUDs is seen for the management of syphilis and chancroid while the same in 73.4% for the HG. From a public health perspective, it is more important that the diagnostic approach to GUD has a higher sensitivity than a higher specificity. The treatment for bacterial causes will not result in bacterial syndromic treatment of GUD since many patients with HSV infection were not diagnosed on the basis of NACO flow chart for GUD. It may be epidemiologically prudent to treat all patients with GUD with a combination of treatments for syphilis, chancroid and HSV. Therefore efforts should be made to consider treatment of HSV-2 infection, which can be done by including the treatment of HG in the syndromic management. In addition, GUD should be managed by education, encouragement of condom use, and use of algorithms based on the local prevalence and observed etiology of GUD. Use of the modified syndromic management approach (by including treatment for HG) can be implemented easily by primary health care resources without highly trained staff or laboratories.

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Oxidative injury in cerebral diseases

In vivo study was carried out to ascertain whether treatment with quercetin and CDP-choline intercalated in mannosylated liposomes exert any neuroprotective effect against cerebral ischemia-reperfusion evoked oxidative damage in young or aged rat brain.

Elevation of conjugated diene levels, the index of lipid peroxidation and cell damage was reported in the cerebral cortex region after ischemia and reperfusion.

Cerebral ischemia and subsequent reperfusion caused a substantial increase in cerebral tissue conjugated diene. Quercetin and CDP-Choline treatment prevented the increase both in young and aged animals.

Edema development after cerebral ischemia resulted in the decrease in neuronal osmomolarity and a loss of BBB integrity. Deterioration of molecular and enzymatic

antioxidant defence activity in young as well as aged rat brain homogenate was observed after short-time cerebral ischemia and reperfusion. QC as well as CDP-Choline in mannosylated liposome treatment prior to ischemic insult resulted in an absolute protection to molecular and enzymatic antioxidant systems of brain of young and aged rats. Decreased protection against toxic radicals that are generated in cerebral ischemia and reperfusion may have serious consequences for the aging brain.

The present data suggested that lipid vesicle-trapped herbal antioxidant quercetin and CDP-Choline may not only be effective in combating the cerebral ischemic reperfusion oxidative damage but also to prevent cerebral edema development both in young and old animals. The mechanism how quercetin and CDP-Choline in mannosylated liposome exerts a complete protection against ischemia-reperfusion mediated cerebral oxidative damage in young and aged rats is not fully clear. Although expression of the mannose mannosylated liposomes were successfully incorporated into mouse brain.

It may be concluded that mannosylated liposome encapsulated QC not only prevented cerebral ischemia and reperfusion induced lipid peroxidation and cerebral edema development but also protected cerebral endogeneous antioxidant defence in young and old rat brain. This approach of delivering a non toxic herbal origin antioxidant (quercetin) to the brain offers the potential clinical application in human degenerative diseases in future.

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ICMR NEWS

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

Meetings of Project Review Committees (PRCs)/ Expert Groups (EGs)/Task Forces (TFs)/Other meetings held at New Delhi

PRC on Oncology	September 9, 2007
EG on Mental Health Service Needs and Service Delivery Models in Disaster (Earthquake) affected Population in Gujarat	September 17, 2007
PRC on Cardiovascular Diseases	September 28, 2007
TF on Registry of People with Diabetes in India with Young Age at Onset :	October 8, 2007
PRC on Ophthalmology	October 16, 2007
PRC on Environmental Hygiene and Occupational Health	October 17, 2007
Brain Storming Session on Dento-facial Anomalies	October 30, 2007

Participation of ICMR Scientists in Scientific Events

Dr. Sulagna Basu, Senior Research Officer, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, participated in the CLXI Conference of the Society of General Microbiology at Edinburgh (September 3-6, 2007).

Dr.V.P. Singh, Research Officer, Institute of Pathology (IOP), New Delhi, participated in the XI Workshop on Progress in Analytical Methodology for Trace Metal Speciation at Munster (September 4-7, 2007).

Dr. T. Ramanathan and Dr. Dipika Sur, Deputy Directors, NICED, Kolkata, participated in the Investigators Meeting for the Global Enterics Multi Centre Study at Mali (September 5-7, 2007).

Dr. U.D. Gupta, Deputy Director, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL&OMD), Agra, participated in the Symposium on Remaining Challenges in Leprosy at Kathmandu (September 5-7, 2007).

Dr. K. Polasa, Deputy Director, Dr. A. Lakshmaiah, and Dr. K. Madhavan, Nair, Assistant Directors, National Institute of Nutrition (NIN), Hyderabad, participated in the (i) Pre Congress Workshop, and X Asian Congress of Nutrition at Taipei (September 6-8 and 9-13, 2007 respectively).

Dr. B. Sesikeran, Director, Dr. G.N.V. Brahamam, Deputy Director (Sr. Grade); and Dr. N. Harishankar, Reseaerch Officer, NIN, Hyderabad, also participated in the X Asian Congress of Nutrition at Taipei (September 9-13, 2007).

Dr.V.M. Katoch, Director, NJIL&OMD, Agra, participated in the Evaluation of the Mycobacterial Research Laboratory at Kathmandu (September 9-14, 2007).

Dr. Nasreen Z. Ehtesham, Deputy Director, NIN, Hyderabad, participated in the discussion regarding the Possibility of Future Collaboration in the Field of Genetics and Nutrition with National University of Singapore (September 10-13, 2007).

Dr. Jayanti Mania Pramanik, Assistant Director, National Institute for Research in Reproductive Health (NIRRH), Mumbai, participated in the II International Congress on Immune Mediated Diseases at Moscow (September 10-15, 2007).

Dr. R.C. Dhiman, Deputy Director (Sr. Grade), National Institute of Malaria Research, Delhi, participated in the conference on One Hundred Years of Tropical Medicine: Meeting the Millennium Development Goals at London (September 13-15, 2007).

Shri Dinesh Kumar, Senior Research Officer, Regional Medical Research Centre (RMRC) for Tribals, Jabalpur, participated in the International Conference of Royal Society of Tropical Medicine and Hygiene at London (September 13-15, 2007).

Dr. P. Paul Kumaran, Assistant Director, Tuberculosis Research Centre (TRC), Chennai, participated in the XXIII Western Institutional Review Board Annual Training Seminar and Annual Board Meeting at Seattle (September 14-15, 2007).

Dr. A.C. Mishra, Director, National Institute of Virology (NIV), Pune, participated in (i) I Meeting of Regional Technical Advisory Group on Dengue; and (ii) Bi-Regional Programme Managers Meeting on Dengue at Phuket (September 17-18 and 19-21, 2007 respectively). Dr. Mishra also participated in the V International Bird Flu Summit at Las Vegas (September 27 – 28, 2007).

Dr. D.S. Dinesh, Research Officer, Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, participated in I Thematic Kolnet meeting at London (September 17-20, 2007).

Dr. R.K. Phukan, Sr. Research Officer, RMRC, Dibrugarh, participated in XXIX Annual Meeting of the International Association of Cancer Registration (IACRO7) at Ljubljona (September 18-20, 2007).

Dr. S.M. Mehendale, Deputy Director, National AIDS Research Institute (NARI), Pune, participated in the HIV Prevention Trials Network Executive Committee Meeting at Washington, D.C. (September 24-25, 2007).

Dr.Poonam Salotra, Deputy Director, IOP, New Delhi, participated in the Conference on Integrated Functional Genomics on the Road to Leishmaniasis Control at Worcestershire (September 24-27, 2007).

Dr. N.S. Wairagkar, Deputy Director, NIV, Pune, participated in the V Global Measles and Rubella Laboratory Network Meeting at Geneva (September 26-28, 2007).

Dr. Vrinda V. Khole, Deputy Director (Sr. Grade), NIRRH, Mumbai, participated in the meeting on Future of Male Contraception at Seattle (September 27-28, 2007).

Dr. Sunita Saxena, Director, IOP, New Delhi, participated in the 2007 NCRI Cancer Conference at Birmingham (September 30 – October 3, 2007).

Dr. S. Subramanian, Assistant Director,Vector Control Research Centre (VCRC), Pondicherry, participated in the Revision and Further Development of a Study Protocol on Monitoring and Evaluation of Programmes to Elaminate Lymphatic Filariasis at Geneva (September 30 –October 5, 2007).

Dr. R. Rajendran, Assistant Director and Mr. R. Paramasivan, Senior Research officer, Centre for Research in Medical Entomology (CRME), Madurai, participated in the Intensive Workshop on Wild Type and Genetically Sterile Aedes at Kuala Lumpur (October 1-2, 2007).

Dr. Shahnaz Vazir, Deputy Director, NIN, Hyderabad, participated in the Internadtional meeting on Child Development from a Global Perspective: Lost Potential, Modifiable Risk Factors, Successful Programmes and Future Goals at Bellagio(October 1-6, 2007).

Dr. Soumya Swaminathan, Deputy Director (Sr. Grade), Tuberculosis Research Centre (TRC), Chennai, participated in the Regional Consultation on Nutrition

and HIB/AIDS in South-East Asia: Evidence Lessons and Recommendations for Action at Bangkok (October 8-11, 2007).

Dr. G.N.V. Brahamam, Deputy Director (Sr. Grade), NIN, Hyderabad, participated in the Regional Consultation Meeting on HIV/AIDS at Bangkok (October 9-11, 2007).

Dr. Sarbani Mukherjee, Sr. Research Officer, NIRRH, Mumbai, participated in the V Annual World Congress on the Insulin Resistance Syndrome at Boston (October 10-13, 2007).

Dr. S.P. Tripathy, Deputy Director (Sr. Grade) and Dr. R.R. Gangakhedkar, Deputy Director, Dr. S.S. Kulkarni, Assistant Director and Dr. S.V. Godbole, Sr. Research Officer, NARI, Pune, participated in the AIDS Clinical Trial Group Meeting at Washington, D.C. (October 12-17, 2007).

Dr. A.C. Mishra, Director, NIV, Pune, visited World Influenza Centre in the Virology Division at UK (October 14-16, 2007). He also participated in the WHO Global Action Plan Advisory Group Meeting at Geneva (October 19, 2007).

Dr. V.M. Katoch, Director, NJIL&OMD, Agra, participated in the meeting of Joint Working Group of Indo-US Vaccine Action Programme at Washington D.C. (October 16-17, 2007).

Dr. G.B. Nair, Director, NICED, Kolkata, participated in the X Anniversary Foundation Day Ceremony of the International Vaccine Institute at Seoul (October 17, 2007). Dr. Nair also participated in II Nagasaki Symposium on Tropical and Emerging Infections at Nagasaki (November 26-27, 2007).

Dr. T. Longvah, Deputy Director, NIN, Hyderabad, participated in the VII International Food Data Conference in Brazil (October 21-24, 2007).

Dr. Anurupa Maitra, Deputy Director, NIRRH, Mumbai, participated in the LVII Annual Meeting of the American Society of Human Genetics at California (October 23-27, 2007).

Dr. S.K. Hoti, Deputy Director, VCRC, Pondicherry, participated in the meeting on Monitoring of Drug Efficacy in Large Scale Treatment Programmes in Human Helminthiasis at Washington D.C. (October 31 – November 2, 2007).

Dr. T. Ramamurthy, Deputy Director, NICED, Kolkata, participated in the Advanced Workshop on Food Safety and Food Microbiology at Cairo (November 4-8, 2007). He also participated in the Vibrio 2007 Conference at Paris (November 28 – December 1, 2007).

Dr. Neeru Singh, Director, RMRC for Tribals, Jabalpur; and Dr. P.K. Sinha, Deputy Director, and Dr. C.S. Lal, Sr. Research Officer, RMRIMS, Patna, participated in the LVI Conference of American Society of Tropical Medicine and Hygiene at Philadelphia (November 4-8, 2007).

Dr. Pradeep Das, Director, RMRIMS, Patna, participated in the Leishmania Risk Workshop at Antwerp (November 12-14, 2007).

Appointment

Dr. Neeru Singh took over as the Director of the Council's Regional Medical Research Centre for Tribals, Jabalpur w.e.f. October 1, 2007.

Training Programmes/Fellowship

Dr. P.B. Doctor, Sr. Research officer, National Institute of Occupational Health, Ahmedabad, availed Training of Technicians on Tobacco Laboratory Network at the Netherlands (September 17-22, 2007).

Dr. Hemanta Koley, Research officer, NICED, Kolkata, proceeded to avail training under the Counterpart Training Programme for the Ongoing JICA – NICED Project for Prevention of Emerging Diarrhoeal Diseases for 6 months at Japan (w.e.f. September 19, 2007).

Dr. M.V. Murhekar, Deputy Director, National Institute of Epidemiology, Chennai, participated in Management for International Public Health Course for 6 week at CDC Atlanta (w.e.f. September 10, 2007).

Dr. A.N. Ghosh, Deputy Director (Sr. Grade), NICED, Kolkata, availed training on Scanning Electron Microscopy entitled Quanta Basic and EDS Microanalysis Course at Eindhoven (October 1-5, 2007).

Dr. Jayanti Mania Pramanik, Assistant Director, NIRRH, Mumbai, availed DBT Short-Term Biotechnology Overseas Associateship for six months at Atlanta (w.e.f. October 2007).

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Dr. N.K. Ganguly, a World Renowned Scientist and Director-General of Indian Council of Medical Research, New Delhi relinquished charge after nearly Ten Years of distinguished service at the Council w.e.f. November 10, 2007.

SUBJECT INDEX

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