kumar Godatwar (National Institute of Ayurveda, Jaipur) in his plenary talk presented reappraisal of the concepts of *Kāma Vājikarana* and *Śhukra*. Health professionals need to develop comfort and competence to address the concerns of their clients at a level appropriate to themselves, their clients and the clinical situation, with the option of referral to higher levels of management. Research indicates that sensitization/desensitization in attitudes and values, basic knowledge of the genital structure, the sexual response cycle and changes in the life cycle as well as competence in communicating with a client on sexual and relational issues are considered essential (core) components of most sexuality education programmes. The average age when people first receive sex education in India is 15.6 years, while the world average is 13.2 years. B. Srinivas Prasad (Shri B.M.K. Ayurveda Mahavidyalaya and Hospital, Shahapur, Belgaum) spoke on etiopathogenesis of male infertility, discussed the principles of ayurvedic management of male infertility, *panchakarma*, management of azoospermia, varicocele, etc. Madhava Diggavi (Government Taranath Ayurvedic Medical College, Bellary) spoke on understanding of *samprapti-ghataka* in male infertility and counselling. S. I. Neeli (KLES Prabhakar Kore Hospital, Belgaum) spoke on investigations regarding male infertility and their clinical interpretation, and discussed the latest protocols followed in evaluating reversible and irreversible conditions in infertile male, significance underlying medical pathology, genetic and/or chromosomal abnormalities that may affect either the patient or his offspring. Niranjan Rao (S.D.M. College and Hospital of Ayurveda, Udupi) spoke on medical management of male infertility: *samprapti vighatana-chikithsa*, *Dhatu saamya-chikithsa*, *panchakarma*. T. Shridhara Bairy (Folklore Medicine Research Centre) in his keynote address spoke about single herbal drugs in male infertility. Understanding the dynamic properties of herbs in the light of ayurvedic principles can help us choose them. Sometimes just one herb, if it is well suited to the individual, can be effective. It is important to strengthen *agni* and cleanse *ama* before giving proper treatment, or combine the appropriate herb with light, warming and stimulating herbs like *Ela*, *Shunti* or *Pippali* to make them easier to digest.

R. S. Hiremath (Shri B.M.K. Ayurveda Mahavidyalaya and Hospital) spoke on single mineral drugs concerned with male infertility. He discussed about different drugs like *Vajeekara*, *Shukravardhaka*, *Shukrasthambhaka*, *Dhwajabhanganashaka*, *Shukramehanashaka* and *Ksheenashukra*. Anup Thakar (Gujarat Ayurved University, Jamnagar) spoke on recent researches in ayurvedic management of male infertility and clinical assessment of certain *vajikarana* drugs in the management of male infertility.

In the panel discussion and valedictory function, clinicians stressed the need for a holistic approach to the management of infertility, ranging from the basics such as sex education and medication to advanced forms of treatment, including *in vitro* fertilization, intra-cytoplasmic sperm injection and other assisted reproduction techniques.

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RESEARCH NEWS

Malaria control: New avenues

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Malaria is an acute infectious disease caused by the parasites, *Plasmodium* spp. and spread by the vector, the female anopheles mosquito. Today over 3.2 billion people in 107 countries/territories are living under the threat of malaria[1]. It is spreading worldwide due to the emergence and spread of drug-resistant strains. This poses major health and economic problems for the population living in endemic areas as well as travellers[2]. If no new control measures are developed, the number of malaria deaths is projected to double in the next 20 years[3].

Therefore, there is an urgent need for developing new strategies to control malaria. Several new drugs are under development, which are likely to be used in combinations to slow the spread of resistance, but the high cost of treatments would make these drugs difficult to sustain. Malaria vaccine, RTS, S/AS02, has shown promise in endemic areas and will shortly enter further trials. Other vaccines are being studied in clinical trials, but it will probably be at least ten years before a malaria vaccine is ready for widespread use[4]. The other approach involves control through vector, and in the last few years there have been several developments in this field.

Riehle *et al.*[5] looked into natural resistance of mosquitoes to *Plasmodium.* According to them, *Anopheles gambiae* population in a West African malaria transmission zone has naturally occurring genetic loci that control mosquito infection with the human malaria parasite, *Plasmodium falciparum*. This genetic resistance can segregate as a simple Mendelian trait. They have sampled the isofemale pedigrees from wild mosquitoes as female *A. gambiae* mate only once and each mosquito pedigree is the progeny of a single pair cross that has occurred in nature. The strongest *Plasmodium* resistance loci cluster in a small region of chromosome 2L and each locus accounts for at least 89% of parasite-free mosquitoes in independent pedigrees. All the clustered loci form a genomic *Plasmodium*-resistance island and are responsible for most of the genetic variation for malaria parasite infection of mosquitoes

in nature. Among the candidate genes in this chromosome region, RNA interference knockdown assays confirm a role in *Plasmodium* resistance for *Anopheles Plasmodium*-responsive leucine-rich repeat 1 (APL1), which is similar to molecules involved in natural pathogen resistance mechanisms in plants and mammals.

This strategy can be used for malaria control. For example, this can be done by increasing the population frequency of pre-existing natural resistance alleles like those described above, through elevating the fitness cost of malaria parasite infection. The important feature of the observed mosquito resistance is that it segregates as a simple Mendelian trait with major effect and at reasonably high frequency in randomly sampled natural genotypes. Interestingly, many mosquito pedigrees completely eliminated the parasite, despite feeding on infected blood. It is speculated that the wild-type mosquito phenotype is resistant and that susceptibility should be attributed to specific points of failure or loss of function in the mosquito immune system[5].

Another approach is the use of natural enemies of mosquitoes that reduce the potential for malaria transmission. Entamopathogenic fungi have widely been used for the biological control of agricultural pests. This approach has also been successfully used for the control of mosquito vector populations. Blandford *et al.*[6] used the rodent malaria model (comprising *Anopheles stephensi* and *Plasmodium chabaudi*), and found that exposure to surfaces treated with hypomycetes *Beauveria bassiana* following an infectious blood meal reduced the number of mosquitoes able to transmit malaria by a factor of about 80. Fungal infection, achieved through contact with both solid surfaces and netting sprayed with hypomycetes for durations well within the typical post-feed resting periods, was sufficient to cause 90% mortality.

This strategy is useful in many ways because it is observed that daily mortality rates increased dramatically around the time of sporozoite maturation, and infected mosquitoes showed reduced propensity to blood feed. Also unlike other mosquitocidal biocontrol agents such as bacteria, microsporidia and viruses, these fungi can infect and kill mosquitoes without being ingested. Tarsal contact alone is enough to kill the insect, a characteristic shared with insecticidal chemicals. Interestingly, development of resistance against fungal pathogens has not been reported for insects, but even if resistance does occur, cross-resistance with chemical insecticides seems extremely unlikely. Unlike malaria control by genetic modification of mosquitoes, the fitness of biopesticide transgenes could be quite low and, because secondary transfer of fungi from mosquitoes is unlikely, fungal transgenes would be much easier to control than mosquito transgenes. So residual sprays of fungal biopesticides might replace or supplement chemical insecticides for malaria control, particularly in areas of high insecticide resistance[6].

A similar study was done for controlling adult African malaria mosquito (*Anopheles gambiae sensu stricto*) using fungus *Metarhizium anisopliae.* Application of this fungus has resulted in 75% reduction of transmission intensity. Simple modification of experimental parameters like increasing the size of fungus-sprayed sheets, higher conidial dose and improved conidial efficacy may lead to improved vector control[7].

A different aspect demonstrated by Marrelli *et al.*[8] is the potential of transgenic malaria-resistant mosquitoes. The strategy used for malaria control is the introduction of genes that impair *Plasmodium* development in mosquito populations[8]. The effect of the transgene on mosquito fitness is a crucial parameter influencing the success of this approach. They have previously shown that anopheline mosquitoes expressing the SM1 peptide in the midgut lumen are impaired for transmission of *Plasmodium berghei*[9]. The SM1 peptide blocks ookinete invasion of the mosquito midgut. Marrelli *et al.*[8] have shown that when mosquitoes were fed on mice blood infected with *P. berghei*, these transgenic mosquitoes showed higher fecundity and lower mortality than sibling nontransgenic mosquitoes. When mosquitoes were fed *Plasmodium*-infected blood, transgenic mosquito frequency reached ~70% by the ninth generation and remained relatively constant thereafter.

In cage experiments, transgenic mosquitoes gradually replaced nontransgenics when mosquitoes were maintained on mice infected with gametocyte-enriched parasites (strain ANKA 2.34), but not when maintained on mice infected with gametocyte-deficient parasites (strain ANKA 2.33). This result was also seen when switching of feeding on strains (strain ANKA 2.34 to strain ANKA 2.33) was done after the fourth generation of mosquitoes. No significant differences were observed when mosquitoes were fed on gametocyte-deficient parasites, indicating that progression of *Plasmodium* infection in the mosquito was responsible for the observed differences. Furthermore, no differences in egg hatching, larval development time, and adult male survivorship were observed between transgenic and nontransgenic mosquitoes when they were fed either of the two parasite strains. These findings suggest that when feeding on *Plasmodium*-infected blood, transgenic malaria-resistant mosquitoes have a selective advantage over nontransgenic mosquitoes.

These results suggest that transgenic advantage might be an important phenomenon in epidemiologically significant mosquito–parasite systems. The results have important implications for implementation of malaria control by means of genetic modification of mosquitoes. As *Plasmodium* burden of even less than five oocysts can significantly reduce the fecundity of anophelines in experimental or natural systems, it can be predicted that the transgene may confer a significant advantage and promote its spread into field populations. In the field, where infection prevalence is lower than in the laboratory systems and only a relatively small proportion of mosquitoes become infected, gene introgression is predicted to be considerably slower and possibly not of sufficient magnitude to establish the transgene in the population. However, once established, transgenic mosquitoes that interfere with parasite development should make more difficult the reintroduction of the parasite after eradication of malaria from the target area.

This fitness advantage has important implications for devising malaria control strategies by means of genetic modification of mosquitoes. Though this approach does not take into account detailed differences in lifetime fitness characteristics, according to the cumulative nature of life-table parameters, life-table trajectories are most sensitive to occurrences in early life[8]. Also SM1 inhibits development at a very early stage of *Plasmodium* development in the mosquito, preventing midgut invasion and activation of the mosquito immune system[9]. When developing future transgenes for vector-borne disease control, it is suggested that the mode of action and temporal expression

of transgene has to be taken into consideration[8].

Paratransgenic mosquitoes, where natural inhabitant bacteria of midgut are genetically altered to express antiparasitic molecules[10], are thought to be an attractive alternative to the generation of transgenic mosquitoes. This approach has the advantage that procedures for genetic manipulation of bacteria are well standardized. For such approaches to be successful, complete understanding of natural microbiota of mosquitoes is necessary. There have been few studies on the understanding of resident microbial flora in mosquitoes that utilized both culture-based[11] and culture-independent approaches[12,13]. Favia *et al.*[14] have described successful colonization by a member of the genus *Asaia* of *Anopheles stephensi* in the midgut and salivary glands. This bacterium was isolated from the laboratory-bred mosquitoes and its presence in midgut lumen was confirmed using transmission electron microscopy and *in situ* hybridization with specific probes. The authors further transformed this organism with Green Fluorescent Protein (GFP) expressing plasmid and used the transformant for the colonization of larvae and adults. Confocal microscopy revealed the presence and stable maintenance of the organism in the midgut, salivary glands and also the male reproductive system. In the male reproductive system, a large number of *Asaia* could be detected in the testes and gonoducts. This suggested possible transfer of bacteria from male to female during mating and such transfer was then experimentally confirmed by mating males carrying GFP containing bacteria and virgin females. After mating, 50% females showed presence of fluorescence-tagged bacteria in their spermatheca and gut. Interestingly, larvae raised from previously infected females also showed massive colonization by fluorescent-tagged bacteria, which is suggestive of transfer from mother to progeny. It remains to be seen whether this is due to direct transoverial transfer or indirect transfer. Irrespective of these concerns, features like the presence in larvae/adults, transfer to progeny, stable maintenance, culturability and amenability to genetic manipulation make *Asaia* a potential target to raise paratransgenic mosquitoes for malaria control.

Taken together, all these approaches provide better understanding of the genetic basis of malaria transmission by mosquitoes and open up newer avenues for malaria control. However, more information is needed before any of these strategies comes to actual field application, the most important being environmental issues[15]. Fungal biocontrol agents are already being used in agriculture, and so far no adverse effects on human health have been reported. Considering the concerns about release of transgenic organisms in the environment, increasing the population frequency of naturally occurring resistance alleles like APL1 may be more acceptable.

1. WHO and UNICEF, World malaria report, 2005.
2. Ashley, E., McGready, R., Proux, S. and Nosten, F., *Travel. Med. Infect. Dis.*, 2006, **4**, 159–173.
3. Breman, J. G., *Am. J. Trop. Med. Hyg.*, 2001, **64s**, 1–11.
4. Greenwood, B. M., Bojang, K., Whitty, C. J. and Targett, G. A., *Lancet*, 2005, **365**, 1487–1498.
5. Riehle, M. M. *et al.*, *Science*, 2006, **312**, 577–579.
6. Blanford, S. *et al.*, *Science*, 2006, **308**, 1638–1641.
7. Scholte, E.-J. *et al.*, *Science*, 2005, **308**, 1641–1642.
8. Marrelli, M. T., Li, C., Rasgon, J. L. and Jacobs-Lorena, M., *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 5580–5583.
9. Ito, J., Ghosh, A., Moreira, L. A., Wimmer, E. A. and Jacobs-Lorena, M., *Nature*, 2002, **417**, 452–455.
10. Durvasula, R. V. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 3274–3278.
11. DeMaio, J., Pumpuni, C. B., Kent, M. and Beier, J. C., *Am. J. Trop. Med. Hyg.*, 1996, **54**, 219–223.
12. Pidiyar, V. J., Jangid, K., Patole, M. S. and Shouche, Y. S., *Am. J. Trop. Med. Hyg.*, 2004, **70**, 597–603.
13. Lindh, J. M., Terenius, O. and Faye, I., *Appl. Environ. Microbiol.*, 2005, **71**, 7217–7223.
14. Favia, G. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 9047–9051.
15. Copping, L. G. (ed.), *The Biopesticide Manual. A World Compendium*, British Crop Protection Council, Surrey, 2001, 2nd edn.

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