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Dr. B.K. Anand Oration

Hypothalamic Regulation of Energy Homeostasis

Neurophysiological mechanisms underlying obesity and diabetes mellitus

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Preamble :

Although the story of Dr. Anand's discovery of a set of neurons in lateral hypothalamus involved in feeding behaviour is known to several investigators, it is perhaps worth recapitulating so as to enable the younger biomedical scientists to share the excitement of the events of November, 1950 when Dr. Anand joined Prof. Brobeck in Prof. Fulton's Lab at the Yale University School of Medicine. Having observed a few times the technique of stereotaxy as used by Brobeck, Anand wished to repeat Brobeck's experiments in which lesions of ventromedial hypothamalus (VMH) resulted in hyperphagia and obesity in the rat (1). He followed the same procedure with meticulous precision in 6 rats, but the animals, instead of getting hyperphagic and obese, refused to eat even if food was placed in their mouth. They lost weight and died in a few weeks time.

On autopsy, it was found that rather than well circumscribed discrete lesions in the VMH as produced by Brobeck, Anand's experimental rats had 'giant' lesions in the hypothalamus, destroying both ventromedial as well as a part of lateral hypothalamus. Looking back, Anand wondered as to what had gone wrong. He had precisely followed the same procedure and there was nothing wrong with his technique. It was then found that there was a malfunction of the instrument and instead of delivering a current of 2 milliamperes, the instrument delivered a current of 20 milliamperes, thus destroying a much larger area which included both VMH and lateral hypothalamus (LH). With Sheringtonian logic, Anand, with his brilliant intellect and incisive mind hypothesized that big lesions also destroyed the neurons in the lateral hypothalamus which were possibly responsible for initiating feeding behaviour.

Experimental evidence was soon generated to support this hypothesis by producing discrete lesions in the lateral hypothalamus. The destruction of these neurons led to a complete cessation of feeding by the animals. Anand, with Brobeck, in their paper in February, 1951 reported that a small area had been

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localized in rats in the extreme lateral part of the lateral hypothalamus, in the rostrocaudal plane (2). Bilateral destruction of this area resulted in a complete cessation of eating. A unilateral lesion had no such effect. This lateral area was designated a "feeding centre" (2). It was considered to be responsible for central hunger reaction or the urge to eat. As they say, the rest is history.

The second epoch-making publication by Anand and Brobeck appeared in November, 1951 and needs to be cited verbatim : 'It is thus clear from these operations that bilateral destruction of the lateral portion of the lateral hypothalamus, in the same rostro-caudal and horizontal planes as the ventromedial nucleus, abolishes the food intake of rats, even in animals which have previously been made hyperphagic and obese. None of the animals in which this area of the lateral hypothalamus has been bilaterally destroyed has ever eaten any food during entire period of its postoperative survival- and this in spite of our many attempts to put food near to or even inside the mouth' (3). These significant observations set the tone and pace for subsequent work in this area. The following decade generated active interest in this field and supportive evidence came through several studies, some of which were conducted in Anand's lab after he joined AIIMS in 1956 (4).

The next landmark was the work based on the recorded activity of single neurons from VMH and LHA. An increase in the firing rate of neuron units in the VMH in the fed state was demonstrated, along with a corresponding decrease in activity from LH in such a satiated state. On food deprivation, there was a reversal of single unit activity in these areas with an increase in the firing rate of neurons in the LH. Thus a reciprocal relationship between the activities of neurons in VMH and LHA was firmly established. More importantly, these studies also provided the basis for postulated 'glucoreceptor' neurons in these hypothalamic centers (5). The data published in the paper in Am. J. Physiol. is now considered a classic in the neurophysiology of feeding behaviour (6).

This was the state of art in 1966-67. There existed overwhelming evidence for hypothalamic regulation of feeding behaviour. It is at this time that I initiated collaboration with the group of Prof. G.S. Chhina and Prof. Baldev Singh in the department of physiology where several young doctoral and postdoctoral students, including Dr. V. Mohan Kumar were involved in active research.

In view of my deep interest in the study of basic mechanism in diabetes, a chronic diabetic state was produced in the rhesus monkey with I.V. injection of Streptozotocin, which results in extensive necrosis of pancreatic β-cells. Biopolar EEG recordings showed a slower activity in the VMH and a faster activity in the LHA after induction of experimental diabetes. EEG activity from other areas such as pre-optic and cerebral cortex remained unaltered in these animals (7). Following insulin administration, these EEG abnormalities were reversed. In a series of publications, it was concluded that it was not the amount

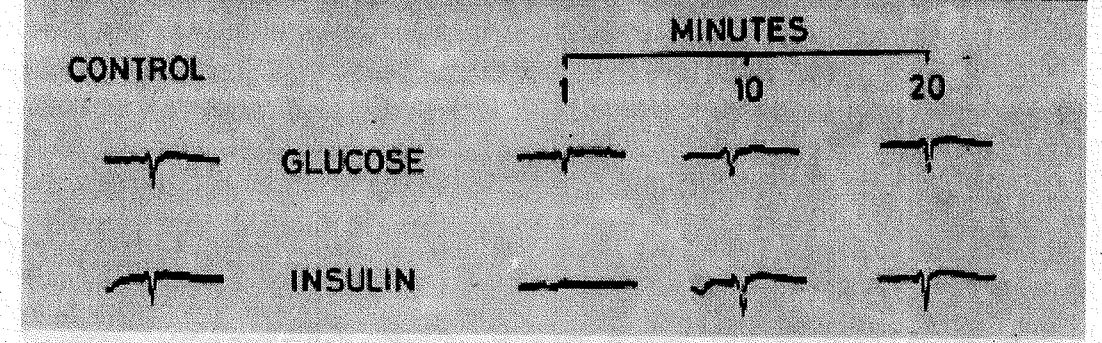
of glucose reaching hypothalamus, but its *net* utilization by these neurons that reflected their activity. For the first time, sound experimental evidence had been generated to explain the possible pathophysiological basis of hyperphagia in human diabetes.

In collaboration with Dr. Mohan Kumar, it was also shown in male cats that evoked responses from VMH and LHA, following stimulation of mesenteric nerves, were modified by I.V. injection of glucose or insulin. Administration of glucose produced initial decrease in amplitude, which gradually recovered while insulin produced an initial short acting inhibition followed by an increase in amplitude (8). (Fig. 1) These studies once again reemphasized the relationship between net rate of neuronal glucose utilization as an important determinent of the amplitude of evoked responses from VMH and LH.

In the late 60s and early 70s, the standard textbooks of physiology stated that insulin does not affect glucose metabolism of the brain, and that insulin does not cross blood-brain barrier. However, the data generated at the AIIMS had convincingly shown the effect of glucose and insulin on the activity of neurons in hypothalamus, and emphasized that the glucose utilization of neurons in VMH and LHA was a determinant of the neuronal activity (9).

The next logical question was : Is there a hypothalamo-insular axis? It was hypothesized that as insulin influenced the activity of VMH and LH neurons, these

Fig. 1 : Effect of glucose and insulin on ER from LHA

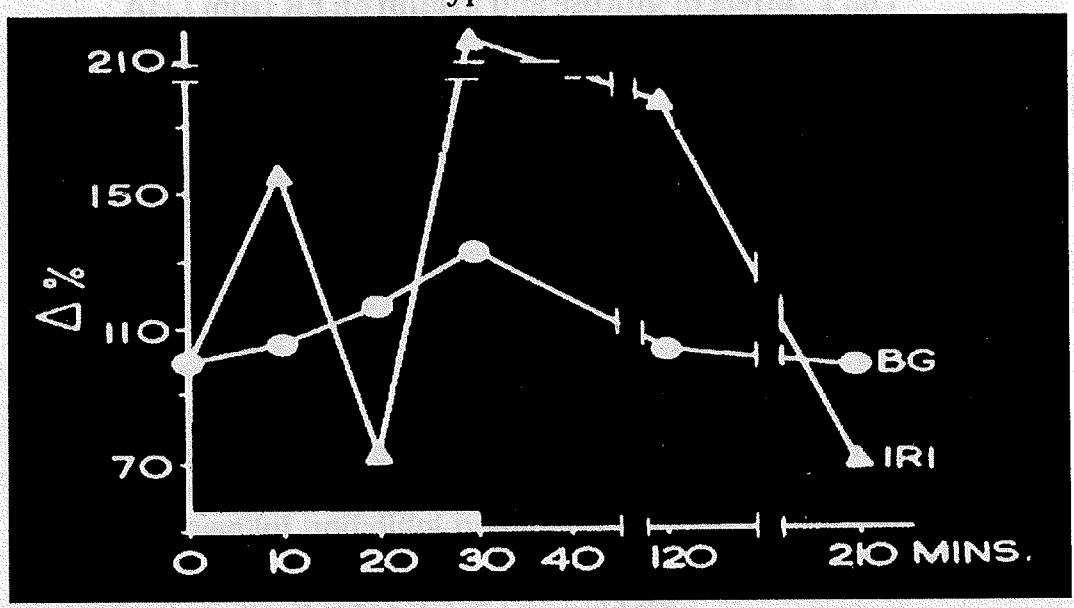


Effects of glucose and insulin on ERs from lateral hypothalamus on stimulation of mesenteric nerves. Glucose produced initial drop in amplitude which gradually recovered and then even increased. Insulin caused an initial short-lasting inhibition followed by an increase in amplitude.

(Chhina and Bajaj, 1972; Bajaj et al., 1975)

neurons may likewise affect insulin release from pancreatic β-cell. With SK Garg, a doctoral student, and under an ICMR grant to the author as Principal investigator, the effect of stimulation of different areas of brain on feeding behaviour and on circulating levels of insulin was studied (10). As observed earlier, stimulation of LHA increased food intake, while VMH decreased intake of food. More importantly, stimulation of LH in conscious restrained monkeys increased serum insulin while stimulation of VMH decreased the levels of circulating insulin. (Fig. 2) Based on the experimental data, Bajaj and Chhina proposed in 1976 the existence of Entero-hypothalamo-insular axis. It was conceptualised that in the fasted state, reduced activity in the neurons of the ventromedial hypothalamus with a reciprocally increased activity in those of the lateral hypothalamus would result in the initiation of feeding behaviour providing a neural or neurohumoral signal to the β-cell, and initiating what may be termed as the cephalic phase of insulin release. Intestinal motility, blood flow and rate of nutrient absorption would also be altered through the autonomic responses. Food intake would also result in the release of gastrointestinal hormones (*presently* called *Incretins)*, some of which will further increase insulin secretion from the β-cell. Finally, a rise in blood glucose will directly stimulate the glucose receptor in the β-cell, thus further increasing the levels of circulating insulin and enhancing glucose utilization in the body as well as in the hypothalamic neurons. Presence of food in the intestines and increased glucose

Fig. 2 : Δ Blood glucose & serum insulin : Lateral Hypothalamus stimulation



BG = Blood glucose; IRI = Immunoreactive insulin

utilization by insulin release provided afferent inputs initiating satiety behaviour and cessation of food intake (7,11).

Glucose sensitive neurons in VMH : Supportive Evidence

In addition to the evidence provided through studies referred to above, supportive evidence was generated by other investigators using mice that had been injected with gold thioglucose. As is well known, gold is toxic to neurons and leads to their destruction. When gold is coupled to glucose by sulphur (aurothioglucose : ATG; auro, gold; thio, sulphur) and injected in the mice, extensive damage is done *only* to those neurons in VMH which take up and metabolise glucose. Following the injection of ATG, mice become hyperphagic within 15 hrs. followed by continuing hyperphagia that subsequently leads to obesity (12). Interestingly, such chemical lesions cannot be produced when other goldthio-compounds are injected (13). Furthermore, damage to the VMH is minimized if mice are injected with 2-deoxyglucose (2 DG) prior to the injection of ATG as the former blocks 'glucoreceptors' (14). Likewise, the damage is minimal if ATG is injected in animals previously made diabetic (15). On the contrary, the damage to VMH by ATG is greater if mice are injected with insulin prior to the administration of ATG (16). Subsequent studies provided further support as ATG-induced damage was prevented by the administration of betathioglucose, an antimetabolite of glucose (17). Similarly, administration of phlorizin, which inhibits glucose transport, prevented ATG induced hyperphagia and obesity (18).

Point : Counter Point

The chronology of this line of research dated from late 60s and extended into late 80s. It provided strong support for the hypothesis generated by us, which in turn was based on early work by Anand and his coworkers, using entirely different experimental design and research methodology. Nevertheless, as in any scientific and intellectual pursuit, counter challenges were mounted by a number of investigators, principally pioneered through a paper published in 1974 by Richard Gold entitled "Hypothalamic obesity : the myth of the ventromedial nucleus" (19). Even prior to this publication, doubts were being expressed regarding the precise location of target neurons exclusively in the VMH to warrant its recognition as 'satiety centre'. In a series of experiments, lesions limited to the VMH in guinea pigs of either sex failed to produce anticipated excessive weight gain. Indeed when Rosen, a doctoral student, failed to produce hyperphagia with VMH lesions in the male rats (20), she concluded : 'The production of obese rats by neurological damage must be an art rather than a science'.

Additional studies of lesions produced with ATG in mice hypothalamus showed initial changes localized in the area ventral to VMH, and including the arcuate nucleus (ARC), the damage later spreading to VMH (21). These observations questioned the role of VMH as the exclusive site which when damaged resulted in production of hyperphagia and obesity. The new experimental data suggested that damage to ARC may also play a significant role.

While these studies doubted the exclusive role of VMH in the neuroregulation of satiety behaviour, Gold's research in female rats convincingly demonstrated that electrolytic lesions restricted *only* to VMN produced neither hyperphagia nor obesity. Such lesions cause obesity *only* when they extend *beyond* VMN. The magnitude of obesity was related to the extent of area damaged *adjacent* to VMN (22). It was hypothesized that obesity observed in rats with lesions extending just rostral to VMH was due to damage to the ventral noradrenergic bundle (VNAB). Lesions of the VNAB at the level of midbrain cause a significant reduction in norepinephrine in hypothalamus, thus resulting in hyperphagia and obesity (23). Several investigators confirmed Gold's observations and reported that long parasagittal knife cuts extending laterally to anterior hypothalamus were more effective in producing hyperphagia and obesity (24). Finally it was shown that although electrical stimulation of VMH suppressed feeding in food deprived rats, parasagittal knife cuts between VMH and LH failed to prevent the stimulation-induced inhibition of feeding. What sounded like a requiem for the VMH was the observation by Leibowitz et al. that lesions of paraventricular nucleus (PVN), *with no damage to VMH*, resulted in hyperphagia and obesity in rats (25).

As if these counterarguments were not enough to alter noise : signal ratio in this cacophony, evidence was generated that obesity resulting from VMH lesions was metabolic in origin, and not primarily as a result of hyperphagia. Weanling rats given VMH lesions do not display hyperphagia or excess weight gain but develop marked increase in body fat content (26). The pathophysiological basis for this was considered to be hyperinsulinemia in nonhyperphagic weaning rats with VMH lesion (27). Subsequently, hypothalamic obesity was shown to be reversed by subdiaphragmatic vagotomy (28). It was considered that VMH obesity was the result of an increase in all vagally mediated (parasympathetic) reflexes and that hyperphagia was secondary to such alterations in visceral metabolism.

Resurgence of Interest in VMH :

The revival of interest in the role played by neurons in the VMH (and LH) is due to the availability of newer techniques of molecular biology which have facilitated our understanding of the role of autonomic nervous system and neuroendocrinal regulation of energy balance (29). There is now substantial evidence that the VMH plays a key role in generating and regulating autonomic responses, both parasympathetic and sympathetic, and that any alterations of these responses through VMH lesions contribute to obesity. The consensus seems to be that VMH lesions result in a metabolic disorder leading to obesity, *in addition* to causing hyperphagia which is possibly independent of metabolic events and which also contributes to the causation of obesity.

Presently, there is a general consensus that a physiological system maintains homeostasis of energy stores in response to

varying availability of food and changing demands of energy expenditure. The energy demands of the body under resting basal, active and stressful conditions are adequately and appropriately responded to in a short period of a few minutes, by glucose which constitutes a most dependable energy supply source on a short term basis, although in the long term, body adipose tissue responds to the needs for the maintenance of energy balance. The evidence for the neuroendocrinal regulation of energy balance had been previously reviewed and it was stated : 'the rate of glucose utilisation seems to be the set point in the regulation of entero-hypothalamo-insular axis. However, this may be so for the maintenance of energy balance on a short term basis. Adipose tissue functions as the major source of energy fuel; during starvation, glycogen stores in the human body may sustain life for less than 24 hours while energy stored as triglycerides can maintain supplies to vital organs for 30-60 days. It is therefore possible that control of triglyceride storage may be of considerable influence as a long range regulator of entero-hypothalamo-insular axis' (8).

Hypothalamic Regulation of Energy Homeostasis :

The subject has been recently reviewed (29) and relevant excerpts are cited here so as to maintain narrative continuity. The hypothalamus is the principal brain region that acts as a key determinant in the integrated control of feeding, energy homeostasis, and regulation of body weight. Hypothalamus senses neural, endocrine, and metabolic signals, integrates these inputs, and engages distant effector pathways, resulting in behavioural, autonomic, and endocrine responses (30). The hypothalamic control and regulatory mechanism is mediated through a complex array of neuroendocrinal signaling pathways involving synthesis and release of several neurotransmitters and neuropeptides. These include monoamine neurotransmitters, such as 5-hydroxytryptamine, norepinephrine, as well as orexigenic neuropeptides (neuropeptide Y, orexins A and B) and anorectic peptides (cocaine-and amphetamine-regulated transcript: CART; pro-opiomelanocortin : POMC and related peptides i.e. αMSH) (31). A brief perspective of the role and action of these peptides is provided in the following paragraphs.

The positional cloning of ob gene in 1994 (32), and the subsequent discovery that the encoded protein, named leptin, functions as an adipocyte derived signal for the regulation of feeding behaviour, set the direction and pace of research during the last decade. Ob receptor gene was cloned a year later, in 1995 (33), and leptin receptors were demonstrated in the arcuate nucleus (ARC) of hypothalamus (34). ARC is located in the mediobasal hypothalamus adjacent to the floor of the third ventricle. It contains neurons that respond to afferent signals, predominately hormonal, which reflect and relate to the *size* and *state* of adipose tissue stores. Although leptin is secreted primarily from adipocytes and insulin is released from the endocrine panacreas, both

circulate at levels proportionate to body fat mass and exert relatively long-lived inhibitory effects on food intake via actions on their receptors in the ARC. These actions are mediated through a set of neurons in the ARC which coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP). These two peptides are potent stimuli of food intake; moreover, these peptides also reduce energy expenditure and thus promote weight gain. In contrast, the ARC also contains neurons that synthesise αMSH (Melanocyte stimulating hormone) that exert a powerful anorectic effect. αMSH is synthesized from its precursor proopiomelanocortin (POMC). Many POMC neurons also coexpress another peptide called CART (cocaine and amphetamine related transcript). Both αMSH and CART reduce food intake. α-MSH is an agonist for melanocortin 4 receptor (MC4R) which also appears to be involved in the regulation of appetite and body weight mediated through 5-hydroxytryptamine receptors, activation of which causes weight loss and deletion of which causes adult-onset obesity in mice (35).

Insulin and leptin signals, which are enhanced in a state of excess adipose tissue mass i.e. obesity, are inhibitory to NPY and AgRP neurons and facilitatory to POMC and CART neurons, the net effect of these hormones results in inhibition of feeding behaviour (36). It is through this reciprocal regulation of anabolic and catabolic neuronal circuits that insulin and leptin mediate their effects on energy balance. It was hypothesized that a decrease in plasma levels of insulin and leptin which follows a reduction in body fat mass, results in the activation of NPY/AgRP and inhibition of POMC neurons.

Insulin and Hypothalamus :

Insulin functions not only as a peripheral regulator of nutrient storage and release of circulating substrates but there is increasing evidence that in the brain, insulin is involved in a wide array of regulatory mechanisms including neuronal survival, neuronal plasticity, learning and memory, as well as energy homeostasis and reproductive function.

Essentially, glucosensing neurons are predominantly located in those areas of brain that are involved in the control of neuroendocrine function, nutrient metabolism, and energy homeostasis. A select group of such neurons use glucose as a signaling molecule to alter their firing rate both as a means of, and also as a response to, glucosensing. The VMH contains both the ventromedial hypothalamic nucleus (VMN) and the arcuate nucleus (ARC). Both contain glucosensing neurons that respond to changes in ambient glucose levels. Through effector pathways, these neurons are involved in regulation of glucose homeostasis. The population of glucosensing neurons in the VMH (VMN & ARC) is amongst the best characterized with respect of glucosensing. In the VMN, 14-19% neurons are glucose-excited (GE) and 3-14% are glucose-inhibited (GI) in type (37). Glucosensing neurons use glucose in a concentration dependent manner as a signaling molecule to regulate their membrane potential and action potential

frequency (38). It has been suggested that GE neurons are analogs of pancreatic β-cell, whereas GI neurons have some similarities to pancreatic α-cells : GE neurons and β-cells are activated and GI neurons and α-cells are inhibited by increase in ambient glucose levels (39) . The LH contains predominantly glucose-inhibited neurons (40).

Insulin Signaling in Hypothalamic Neurons

Glucokinase (hexokinase IV) is a key regulator of neuronal glucosensing, thus performing a role similar to that in the pancreatic β-cell (and α-cell) glucosensing (41). In the ARC, more than 75% of Neuropeptide Y-(NPY-) positive neurons express glucokinase (42). Many glucokinase-expressing neurons coexpress K_{ATP} channels (43). Furthermore, coexpression of GLUT-3 and GLUT-4 with insulin receptor mRNA (IR mRNA) is also reported in glucose-responsive neurons (44). Recent studies confirm that glucose-excited neurons utilize ATP-sensitive K^+ channels as their transduction mechanisms for glucosensing whereas glucose-inhibited neurons appear to utilize a nonspecific Cl^- channel. Irrespective of the type of ion channels used as a final common pathway, a large proportion of glucose-excited and inhibited (GE and GI) neurons appear to utilize glucokinase as a regulator of glucosensing. Glucokinase mRNA is selectively localized in several brain areas involved in glucosensing. It is expressed in ~ 70% of GE and ~ 40% of GI neurons (44). Final confirmation of the key role of neuronal glucokinase is based on the fact that a knockdown of glucokinase mRNA, using glucokinase siRNA in primary hypothalamic neuronal cultures, ablates the ability of these neurons to sense glucose (45).

Protein Tyrosine Phosphatase 1B (PTP1B) has been shown to be a negative regulator of insulin signaling by dephosphorylating key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor. Recent gene knockout studies in mice ($Ptpn1^{-/-}$ mice) have shown an increase in insulin sensitivity in such animals; these mice are lean and show resistance to high-fat diet-induced obesity (46,47). Studies using Ptpn1 antisense oligonucleotides, which lower PTP1B levels only in liver and adipose tissue, tended to suggest these tissues as the main sites of action for the regulation of glucose homeostasis and lipid metabolism. A recent study with tissue specific deletion of PTP1B in brain, muscle, liver or fat has shown that neuronal PTP1B also regulates insulin sensitivity as well as degree of adiposity. Indeed mice lacking PTP1B in brain show enhanced peripheral insulin sensitivity accompanied by increased insulin receptor phosphorylation in muscle and liver. Involvement of neuronal pathway controlling hepatic glucose production or affecting adipokine secretion could possibly be underlying contributory factors.

Realising the therapeutic potential of inhibiting PTP1B in promoting weight reduction and improving insulin sensitivity and glucose homeostasis, efforts have been intensified in developing inhibitors of PTP1B which may be effective in treating insulin resistance at an early stage, thereby preventing Type 2 diabetes mellitus (T2DM) and obesity (48).

Insulin Signaling in CNS : Evolutionary Perspective

Insulin signaling in neuronal cells plays a key role not only in mammals but also in primitive organisms such as the nematode *Caenorhabitis elegans* and the fruit fly *Drosophila melanogaster* (49). Indeed, insulin signaling pathways show several similarities in *C.elegans, D.melanogaster*, rodents, and humans, thus raising the distinct possibility of an evolutionary mechanism conserved over the millennia. Neurosecretary cells in *D.melanogaster* express insulin-like peptides (dILPs). Ablation of dILP neurons results in prolonged life span, reduced fertility, increased fasting glucose levels, increased storage of lipids and carbohydrates, and reduced tolerance to heat and cold, thus highlighting the key role of these cells in the regulation of life-span and fuel metabolism (50).

Additional discoveries in this connection have clarified the role of insulin signaling in providing metabolic connectivity between nutrition, reproduction and lifespan. The initial discovery in *C.elegans* was the cloning of *DAF-2,* the gene that encodes a homologue of the mammalian insulin receptor, containing both ligand binding and tyrosine kinase domains (51). The relevance of DAF-2 to *C. elegans* physiology was initially based on its association with a stage of diapause arrest called "dauer." The *dauer* is characterized by inhibition of reproduction and reduced metabolism and growth, thereby resembling suspended animation or hibernation. Such a state is normally triggered by periods of reduced food availability. Mutations of DAF-2 were shown to produce the dauer state and also revealed DAF-2 as the first step in a signal transduction cascade homologous to the insulin pathway described in mammals. One of the proteins in signal transduction pathway is called advanced glycation end product-1 (AGE-1), a homologue of mammalian phosphatidylinositol 3-kinase (PI 3K). The knockout of PI 3K induces a phenotype dauer stage, which is characterized by increased longevity as is seen with mutation of DAF-2 (51). Another key protein is DAF-16, a member of the forkhead transcription factor family related to mammalian HNF-3 and FOXO1. The finding that DAF-16 mutation completely reversed the phenotype arising from DAF-2 or AGE-1 knockout (52) (a phenomenon referred to as genetic complementation) suggests that this forkhead protein functions downstream of the more proximal DAF-2 and AGE-1 proteins, and that its activity is normally inhibited by activation of the upstream DAF-2/AGE-1 cascade, and finally that this inhibition is a dominant component of signaling in this cascade (52).

It was suggested that increased longevity associated with the DAF-2 knockout is analogous to the effect of caloric restriction to increase mammalian longevity, since calorically restricted animals experience decrease of both circulating insulin (and hence reduced insulin receptor signal transduction) as well as of fertility. Restoration of DAF-2 in neurons was sufficient to restore lifespan and normal phase of reproduction of DAF-2 knockouts to wild-type values, and neuron-specific restoration of AGE-1 in

animals that otherwise lack this protein produced the same effect. Thus, neuronal insulin-like signaling appears to be a key regulator of various critical functions in *C. elegans*. The metabolic and reproductive defects induced by whole-body deletion of DAF-2 appear to be due in large part to abnormalities specific to the absence of neuronal insulin-like signaling.

It is of interest to note that as of March 2002 (53), *Drosophila* gene sequences have been found with highly significant ($P < 10^{-10}$) matches to 75% of the human disease loci examined. It is amazing, therefore, that fully 75% of human disease loci have counterparts in *Drosophila*. While the insulin-like receptor was first reported in this species in 1996, knockouts were found to be lethal; hence, insulin receptor-like activity is absolutely essential for life during development. However, mutations of either an Insulin Receptor Substrate (IRS) homologue termed *CHICO*, or complex heterozygotes of the insulin-like receptor, were shown to extend lifespan and reduce reproduction in a manner similar to that induced by DAF-2 mutants in *C. elegans* (54). As in *C. elegans*, lifespan extension was associated with a general growth deficiency and a decrease in cell number and size, and insulin-like signaling was shown to depend on a PI3K homologue.

Some evidence of the existence of insulin-like peptides exists in plants as well. As early as 1960, extracts of the plant *Momordica charantia linn* (bitter gourd) were shown to elicit a hypoglycemic response (55). A polypeptide was subsequently partially purified (56). After labeling with ^{125}I and further purification, this peptide was subjected to additional immunological studies in our laboratory. It was found that the material did not cross-react with anti-insulin serum. In contrast, application of wick chromatography, a technique that we had earlier found to be of value in identifying basic and acidic polypeptides (57), seemed to suggest that the hypoglycemic plant extract was an acidic polypeptide, with behaviour similar to that of the A-chain in insulin. Of particular significance is also the finding that insulins of vertebrates share a common gene lineage and have a highly conserved sequence *Gly-Phe-Phe-Tyr* (residues 23-26) in the B-chain, which constitutes a critical area of the receptor-binding region of the molecule. Our group did theoretical calculations on the minimum energy conformation (three-dimensional structure) of this conserved sequence, using a global optimization technique developed by Subba Rao et al (58). Results show that this conserved sequence has a specific conformation that gets significantly altered if either of the two *Phe* residues is substituted by *Leu* or *Ser*, resulting in a decreased activity of insulin (59). Such substitutions have been identified in a few families with hyperinsulinemia and T2DM and have been designated as insulinopathies.

On the basis of evidence presented thus far, it may be rationally argued that an early evolutionary role for insulin may have been to regulate metabolism through neuronal control of nutrient storage, a process tightly coupled with control of reproduction and lifespan, since both energy storage and reproduction depend upon nutrient availability. According to this hypothesis, the emergence of insulin as a key regulator

of carbohydrate metabolism in vertebrates was a more recent evolutionary development (60). Extending the concept of evolutionary perspective with competitive survival as the key, the intimate relationship between immune and metabolic responses also needs to be highlighted. It is well recognized that functional structures that control key metabolic and immune functions have evolved from common ancestors. An oft-quoted example is *Drosophila* fat body, which contains the mammalian homologues of liver, the hematopoietic system, and other related immune components. It has been recently shown that this site also corresponds to mammalian adipose tissue (61). As these specialized cells differentiate into distinct functional units or organs, they carry with them their developmental lineage. Hence, it is possible to envision a scenario where common pathways regulate both metabolic, reproductive and immune functions through the utilization of common key regulatory molecules such as glucose and fatty acids. It is of interest to note that glucosensing neurons in VMN, ARC and LHA also respond to a variety of metabolites such as lactate, ketone bodies and free fatty acids (62,63). Long chain acyl-CoA also activates K^{ATP} channel in these neurons (64) and inhibits GK activity (65). Thus glucosensing neurons indeed function as ***metabolic sensors***. Such a closely linked configuration and coordinated regulation of metabolic and immune responses is likely to be advantageous, since the organism needs to organize and redistribute its metabolic resources during stressful life situations which require urgent mounting of an immune or inflammatory response.

The Endocannabinoid System : From Anand to Anandamide

The major discovery of the last decade regarding the existence of the endocannabinoid system has a most profound physiological impact and holds a great therapeutic potential. This discovery is centered around the well known plant, *cannabis sativa or cannabis indica*. The plant grows wild all over India (and other parts of the world) and its flowering tops, resin from tops, and leaves have been extensively used for many centuries.

Marijuana cigarettes are prepared from the leaves and flowering tops of the plant while *Hashish* is prepared from concentrated plant resin. While smoking is the most common mode of use, the oral intake of *Bhang* as a concoction is practiced in several socio-religious groups, more so during festivals. As a part of folk-lore medicine, cannabis has been used over millennia for disorders as varied as joint pains and epileptic convulsions. William O' Shaughnessy brought this substance to the notice of western medicine in the middle of the eighteenth century, highlighting the 'remarkable increase of appetite' as a result of cannabis consumption (66). It was only in 1964 that its active psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated, its structure identified, and partial synthesis accomplished (67). Δ^9-THC alongwith other naturally occurring and synthetic cannabinoids, bind with two separate G protein-coupled receptors. Cannabinoid receptor 1(CB_1) (68) is localized in the CNS including neurons in

lateral hypothalamus, and in the periphery especially in the liver and adipose tissue. In contrast, cannabinoid receptor 2 (CB_2) is found primarily on cells of the immune system (69).

A complex biochemical pathway for the synthesis, release, transport, and degradation of endocannabinoids alongwith their receptors CB_1 and CB_2 constitutes a new signaling system termed the 'endocannabinoid system' (70). Since the discovery of fatty acid amide, arachidonoylethanolamide, by Devane et al in 1992 (71), who also named it as '***Anandamide***' from the Sanskrit root 'Ananda', meaning 'internal bliss', more than 3500 scientific reports have been published exploring diverse aspects of endocannabinoid system. Essentially, evidence has been generated during the last decade indicating this signaling system as a modulator of physiological functions not only in the central nervous system, but also in the autonomic nervous system, neuroendocrinal network, the immune system, the gastrointestinal tract, the reproductive system both in the male and the female, and in microcirculation.

The two most studied endocannabinoids include Anandamide and 2-arachidonoylglycerol (2-AG). Anandamide is formed by the cleavage of a phospholipid percursor, the N-arachidonoyl-phosphatidylethanolamine (NAPE). Anandamide acts as a retrograde messenger at presynaptic cannabinoid receptor CB_1 where it regulates neurotransmitter(s) release. Anandamide also acts as a neuromodulator at postsynaptic cells where it regulates excitability. Anandamide action is terminated through a two-step process that includes i) transport into cells through a specific anandamide transporter (AT), and ii) enzymic degradation by cleavage to arachidonic acid and ethanolamide by membrane bound enzyme, fatty acid amide hydrolase (FAAH) (72), which is widely distributed in the body with high concentrations in the brain and in the liver.

Cannabinoid receptors :

A reference has already been made to the two cannabinoid receptors, CB_1 and CB_2. CB_1 receptor is mainly located in the terminals of nerve cells including central neurons in the hypothalamus and other areas of brain (73), as well as in the peripheral neurons such as those in the autonomic nervous system. CB_1 receptor has also been demonstrated in the liver (hepatocytes) (74) and the adipose tissue (adipocyte) (75). CB_1 expressed in adipocytes has been implicated in the control of adiponectin secretion and lipoprotein lipase activity. In the liver, CB_1 stimulation increases hepatic lipogenesis through activation of fatty acid biosynthetic pathway. This pathway is also expressed in hypothalamus, and is considered to be involved in regulation of appetite (76). Both CB_1 and CB_2 receptors are coupled to similar transduction systems.

Several lines of evidence have firmly established the role of endocannabinoid signaling in feeding behaviour, obesity and lipogenesis (77). Experimental studies have provided support for the role of endocannabinoids in obesity. In genetic rodent models of obesity such as *ob/ob* and *db/db* mice and Zucker rats, elevated levels of endocannabinoids were demonstrated in

the hypothalamus. The levels were normal in other regions of brain, and in nonobese control mice (78). Additional evidence indicates that endocannabinoids are involved in the neural circuitry of arcuate nucleus through which leptin regulates feeding behaviour. Leptin administration in *ob/ob* mice leads to a decrease in feeding alongwith a concomitant reduction of anandamide expression in the hypothalamus. In summary, in response to an increase in body weight, when leptin levels increase, anorexigenic neuropeptides such as NPY are upregulated, and orexigenic endocannabinoids levels are decreased, resulting in a reduction in CB_1 receptor activation, and a subsequent decrease in food intake.

Daniela Cota et al. (75) in a series of experiments used male mice deficient for CB_1 ($CB_1^{-/-}$) and male wild type (WT) littermates ($CB_1^{+/+}$). *In situ* hybridization in WT $CB_1^{+/+}$ mice confirmed that CB_1 transcripts are co-localised with mRNAs of several hypothalamic neuropeptides involved in control of food intake. In particular, co-localisation of CB_1 and prepro-orexin mRNA, and MCH mRNA was demonstrated in lateral hypothalamic area. Interestingly, CB_1 is neither expressed in NPY neurons in the arcuate nucleus, nor do $CB_1^{-/-}$ mice show altered levels of NPY mRNA expression.

The lack of CB_1 in mice with a disrupted CB_1 gene causes hypophagia and leanness. No significant difference between WT and $CB_1^{-/-}$ mice was detected regarding locomotor activity, body temperature or energy expenditure. CB_1 mRNA was found in epidydeinal fat pads from $CB_1^{+/+}$, but not from $CB_1^{-/-}$ mice. Altered expression of hypothalamic neuropeptides in $CB_1^{-/-}$ mice along with altered peripheral lipogenesis supports a role of the endogenous cannabinoid system in the central regulation of food intake and peripheral lipogenesis.

Horvath (79) has provided a schematic illustration showing the relationship between hypothalamic peptidergic circuits that express CB_1 receptors. It is of interest to observe the localization of these receptors in the MCH and orexin containing neurons in the lateral hypothalamus. CB_1 receptors are produced in the cytosol of these cells and are transported to axon terminals. Here, the receptors, upon activation by endocannabinoids or other agonists, are thought to affect the release of neuromodulators (MCH, ORX, CART, corticotrophin-releasing factor) to the synaptic cleft, thus modulating putative inhibitory (-) and stimulatory (+) influences on food intake through the various elements of the peptidergic system.

Neuropharmacological approaches to management of obesity and diabetes mellitus :

Before concluding the present narrative, the arclight must shift and be finally focussed on my own abiding commitment i.e. diabetes. In 1976, I had hypothesized that : *'a clear delineation of possible alterations in the normal physiological control mechanisms involved in the entero-hypothalamo-insular axis is likely to provide better insights in the diagnosis as well as management of diabetes mellitus.* ***Future development of specific neuropharmacological agents, modifying entero-hypothalamo-insular-axis, remains***

a distinct therapeutic possibility in the management of diabetes mellitus (8) '. Three decades later, with the discovery of CB_1 antagonists and incretins the time has come for the fulfillment of this intuitive prophecy. The subject has been extensively reviewed recently (80) and the following narrative is cited from this publication.

Clinical Trials with CB_1 receptor antagonists :

Following the mandatory animal studies, investigations were initiated in 2002 in the human subjects administered Rimonabant (SR 141716), the first specific CB_1 receptor antagonist. At present, multinational, multicentred, well designed clinical trials have provided sufficient data based on either a completed 1-year or 2-year study, or in an ongoing 2-yr. study. The following clinical trials are specifically designed to study the metabolic end-points (Table 1) :

Table 1. Rimonabant in Obesity (RIO) : Clinical Trials

Clinical Trial	Duration and Design	No. of Subjects	Dose of RIO	Efficacy
RIO – North America	2-yr. study: double blind, placebo controlled	3040 subjects : overweight or obese (Diabetics excluded)	5 mg. or 20 mg., oral, daily	62.5% treated with 20 mg. over 2 yrs. lost >5% body wt. HDL ↑ 24.5%, TG ↓ 9.9%, HOMA-IR ↓
RIO – Europe	2-yr. study: double blind, placebo controlled	1057 subjects : overweight or obese (Diabetics excluded)	5 mg. or 20 mg., oral, daily	* At 1-yr., 39% lost >10% body wt. with 20 mg. dose. Also waist circumference reduced by 3.4 inches. HDL ↑ 27%, TG ↓ 10.6%
RIO – Diabetes	1-yr. study: double blind, placebo controlled	1042 subjects with Type 2 diabetes; mean BMI ~34. 2/3 of subjects on metformin and 1/3 on sulphonylureas.	5 mg. or 20 mg., oral, daily	** On 20 mg. dose, HbA1c ↓ 0.6% in all patients and to < 6.5% in 43%. HDL ↑ 15.4%, TG ↓ 9.1%, average wt. loss of 11.7 lbs.
RIO – Lipids	2-yr. study: double blind, placebo controlled	1036 subjects : Obese,(BMI 27-40) with dyslipidemia.	5 mg. or 20 mg., oral, daily	***44.3% lost >10% body wt. with 20 mg. Waist circumference reduced by average 3.5 inches.

(Data tabulated by J.S. Bajaj)

HDL, high-density lipoprotein; TG, triglyceride; HbA_1c, haemoglobin A_1c; HOMA-IR, homeostasis model assessment-insulin resistance.

** Data published after completing 1-yr (study ongoing).*

*** Data presented at American Diabetes Association meeting, June, 2005.*

**** Data presented at American College of Cardiology meeting, March, 2005.*

Table 1 summarizes the available information based on the clinical trials with Rimonabant where the focus is on metabolic end points such as body weight, blood lipids, and glycemia. In general, 20 mg. dose is more effective with regard to all metabolic parameters. As the only study so far published in a peer reviewed medical journal (Lancet) is based on 1-yr. data in the RIO-Europe trial, the following critical review with regard to efficacy and adverse effects is based on this study (81). Basal metabolic rate was estimated with the Harris Benedict formula, and 600 Kcal were subtracted by a dietician to calculate a recommended daily energy intake for each subject. Total weight loss in 1-yr. ranged from 5 Kg. in the placebo group to more than 10 Kg. in subjects on daily dose of 20 mg. rimonabant. Waist circumference reduced by 3.4 cm. in 20 mg. dose group. HDL-cholesterol increased by 22.3% (20 mg. dose group), 16.2% (5 mg. dose group) and 13.4% (placebo group). Triglyceride decrease was observed only in 20 mg. dose group; in contrast, in the placebo and 5 mg. dose groups, an increase was observed. Finally, with 20 mg. dose, there was a significant reduction in fasting plasma glucose. A similar pattern was observed for insulin concentration as well as for HOMA-IR. No significant change in fasting glucose, insulin or HOMA-IR was observed in either the placebo, or the 5 mg. dose groups.

An analysis of all the adverse events occurring in at least 5% of subjects in any group showed that the most frequently reported adverse events with rimonabant were : nausea, dizziness, arthralgia and diarrhea. These events were generally mild to moderate and presented mostly during the first month of treatment. The only adverse event which occurred more frequently in the 20 mg. groups (compared to placebo and 5 mg. group) was pertaining to mood disorders.

The authors concluded that treatment with rimonabant was associated with clinically significant weight loss and reduction in waist circumference, with additional improvements in HDL-cholesterol, and a reduction in insulin resistance. The drug showed a favourable safety profile.

In addition to the four clinical trials with metabolic endpoints (Table1), there are additional studies with rimonabant and tobacco use (STRATUS) where the role of the drug in facilitating smoking cessation, long-term abstinence, and prevention of weight gain upon smoking cessation, is being investigated. More than 6500 subjects have been enrolled in the STRATUS trials.

Epilogue :

After more than 5 decades, the journey initiated by the original observations of Dr. Anand, seems to be heading for its final destination which now appears to be well within sight. The final conclusion that can be unequivocally pronounced is that although there is no 'conventional' feeding *centre* in the LH, there are certainly well characterized neurons located in this area which coexpress orexigenic neuropeptides along with CB_1 receptors and are involved in the regulation of feeding behaviour within the metabolic framework of

maintaining energy balance. There is also substantial evidence for the existence of entero-hypothalamo-insular axis. The newer therapeutic interventions in the management of diabetes mellitus such as Incretins and CB_1 antagonists directly arise from the basic research in this area.

To many, interdisciplinary research is a most recent concept. It is not. Aristotle (384-322 BC), himself a doctor's son, went to Athens to study with Plato, and was among the early pioneers to use animal dissection for learning the interrelationship between the structure and function of various organs. With a prophetic vision, Aristotle stated :

'The natural scientist has to investigate also the basic causes of health and disease, which cannot occur in non-vital things. That is the reason why most of the natural scientists finally turn towards medical research, while the more advanced and far sighted physicians will utilize the principles of natural sciences.'

The present scientific odyssey has been most exciting and amply rewarding.

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It was my good fortune to be associated with a group of scientists of international repute constituted by Dr. B.K. Anand, who was trained as a physician but turned into a physiologist by serendipity, late Dr. Baldev Singh, the doyen of neurologists but deeply involved in basic research in neurophysiology, and Prof. G.S. Chhina, an outstanding physiologist with major interest in applied clinical research including yoga. I pay my homage to late Dr. Baldev Singh and thank Prof. Chhina, Dr. Mohan Kumar and a large number of students and colleagues with whom I worked over the years. Indeed, this was the strength of the AIIMS in its formative years that facilitated close interaction and collaborative research between clinical and basic scientists. The present faculty should be well rewarded if they continue to follow those traditions. I also thank my wife, Avninder who unhesitatingly and most enthusiastically has always supported me to continue my academic pursuits for more than 40 years.

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Current Practice in Imaging in Obstructive Biliopathy

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INTRODUCTION

The radiological evaluation of obstructed biliary tract has evolved dramatically since the early 70s. In the past, intravenous cholangiography, nuclear scintigraphy and barium meal studies were the only investigative techniques available with limited information retrieval. Now with availability of US, CT, MRI, including magnetic resonance cholangiopancreatography(1) (MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC), diagnostic approach in a patient with biliary tract pathology has been completely revolutionised with accuracy of radiological diagnosis approaching 98 percent when combined with clinical data. Sonography is usually the initial imaging modality. The first step is to determine the presence or absence of obstruction and if obstruction is present then next step is to delineate the level and if possible the cause of obstruction. Both benign and malignant lesions can cause biliary obstruction.

Ultrasonography

US is the usual screening modality in patients with jaundice. Sensitivity rate of US in evaluation of jaundice varies between 27 to 95 per cent. Extrahepatic biliary ductal dilatation precedes intrahepatic duct dilatation, therefore, meticulous attention has to be paid in scanning the common duct. The common bile duct (CBD) diameter up to 6 to 7 mm is considered normal in adults(2). On high-resolution ultrasound equipment, normal intrahepatic biliary ducts may be seen, but these should not measure more than 2 mm and should not be greater than 40 per cent of the diameter of the accompanying portal veins. Dilated intrahepatic biliary ducts appear as "too many tubes" or give "Swiss cheese" appearance.

Endoscopic Ultrasonography (EUS)

Endoscopic ultrasonography first introduced as a research tool has emerged as a significant advance in gastrointestinal endoscopy, and allows high resolution images of pancreatobiliary system(3).

CT

In addition to US, CT is often performed in the diagnosis of biliary obstruction. Both these modalities can accurately define the level and cause of

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obstruction in more than 90 per cent patients. On CT, dilated intrahepatic bile ducts are depicted as linear, branching, or circular structures of near water density which enlarge and become confluent as they approach the porta hepatis. As one scans down from porta hepatis to the pancreas, dilated extrahepatic ducts appear as a series of low density rings.

On CT scan upper limit of normal for the common hepatic duct diameter is considered to be 6 mm and the common bile duct 9 mm, although higher values are accepted in post cholecstectomy patients. Intrahepatic ducts more than 2 to 3 mm diameter or duct visualisation becoming confluent rather than scattered, is considered abnormal.

Advancement of CT technology including the development of spiral scanners and, more recently, multidetector row CT (MDCT) scanners and the development of three dimensional (3D) imaging software have significantly improved the ability of CT to image patients with obstructive biliopathy. Dual phase CT angiography, volume rendering, maximum intensity projection (MIP) and minimum intensity projection (MinIP) are useful for display of the vascular map and/or biliary tract.

Hepatobiliary Scintigraphy

Hepatobiliary scintigraphy generally does not compare favourably with sonography and CT. However, with improved technology and newer agents, scintigraphy has some distinct advantages in the work-up of a jaundiced patient, particularly a postoperative patient. The agents routinely used for hepatobiliary imaging are iminodiacetic acid (IDA) derivatives, which are accumulated by hepatocytes and secreted into the bile and subsequently into the small bowel.

Magnetic Resonance Imaging

At the time of initial clinical application of MR cholangiopancreatography (MRCP) over a decade ago, MRCP was regarded a new technique with questionable potential for imaging the biliary tract and pancreatic duct(4). Since that time, however MRCP has been shown to have a wide range of clinical applications (1, 5, 6). The acceptance of MR is related to technical refinements such as advances in hardware and software which have greatly improved image quality and shortened examination times. The technical refinements include development of breathing independent sequences that suppress artifacts associated with surgical clips, stents and bowel gas and allow image acquisition at section thickness of 2 to 5 mm.

MRCP is performed with heavily T_2W sequence that depicts the biliary and pancreatic duct as high signal intensity structures. MRCP can be performed as a single shot or thick slab technique or multislice thin slab technique, following which images are reformated to generate 3D images of the ductal system. MRCP offers a number of advantages compared with ERCP/ PTC. Because MRCP is a noninvasive examination, it avoids entirely the complications of ERCP that occur in upto 5% of all ERCP attempts, and include pancreatitis, gastrointestinal tract perforation and haemorrhage(7). Unlike ERCP, MRCP does not expose patients to ionising radiation or iodinated contrast

material. MRCP is also useful in the evaluation of patients who had incomplete or failed ERCP attempt and also in evaluation of patients in whom the performance of ERCP is difficult due to surgical alterations of the gastrointestinal tract. The major disadvantage of MRCP is that it is entirely diagnostic in contrast to ERCP, which provides diagnostic information as well as access for therapeutic interventions.

Endoscopic Retrograde Cholangiopancreatography is the gold standard for evaluation of pancreatic and biliary duct. However, due to a large number of advantages which MRCP offers *vis- a- vis* ERCP, it has replaced ERCP to a great extent in some institutions as a means of identifying diseases of the bile and pancreatic ducts.

However, ERCP is useful in clarifying complex ductal anatomy, providing information in the setting of an equivocal or nondiagnostic MRCP and identifying the bile duct & cystic duct leaks. Once disease has been detected with MRCP, patients may then be selected appropriately for therapy with ERCP, surgery or other radiological interventions.

BENIGN LESIONS CAUSING OBSTRUCTIVE BILIOPATHY

- Choledocholithiasis
- Benign strictures
  - Post operative/Traumatic
  - Post inflammatory
  - Mirrizi's syndrome
- Choledochal cyst.
- Primary sclerosing cholangitis.
- Bacterial cholangitis and AIDS related biliary abnormalities
- Parasitic diseases
- Infections e.g. tuberculosis
- Ampullary stenosis
- Choledochal varices
- Benign tumours of the biliary tract:

CHOLEDOCHOLITHIASIS

Choledocholithiasis occurs in about 15 per cent of patients with cholelithiasis. US is usually the initial screening modality due to its low cost and easy availability. US can detect gall stones accurately in approximately 90-100 per cent of cases(8). However, its role in the diagnosis of CBD has been reported from as low as 13 per cent to as high as 82 per cent (9, 10).

Typically US appearance of CBD calculus is an echogenic nodule with acoustic shadowing seen in a dilated CBD. If the CBD is minimally dilated or of normal calibre, acoustic shadowing is usually not seen. For evaluation of upper part of the duct, parasagittal or longitudinal scans in right anterior oblique (RAO) position are preferable, whereas good quality transverse scans are essential for the evaluation of lower part of the duct.

In a study by Bhargava S *et al*(10),on US evaluation of CBD calculi with ERCP and PTC correlation, it was observed that the factors that increased the diagnostic accuracy of US were, proper technique, dilated common bile duct, proximal position and bigger size of the calculus.

Following the initial evaluation by US, the problematic cases may be examined with CT. The reported sensitivity of CT in

detecting CBD stones varies from 45 to 90 per cent(11). Stones exhibit the same range of appearance on CT as those seen in the gall bladder. High attenuation stones can easily be seen on CT contrasted with lower attenuation of bile or the adjacent soft tissue of the pancreas. Even the impacted stone with no surrounding bile can be detected by noting that the visualized calcific nodule (stone) lies in the course of the CBD predicted from the cephalad images. However, approximately 50 per cent of the duct stones are of faint attenuation or slightly greater than surrounding bile and often similar to that of adjacent soft tissues of the pancreas. Detection of these stones requires visualization of surrounding rim or crescent of bile that outlines the intraluminal densities and allows CT diagnosis of calculi. To optimize CT visualization of these stones, thin collimation scans (3 to 5 mm) obtained at close intervals in the region of transition zone of the distal duct may be required. When a strong suspicion of CBD stone exists, oral contrast may be withheld, as contrast within the duodenum may obscure stones impacted at the ampulla of Vater. Until the advent of MRCP, many patients with suspected choledocholithiasis and a negative US or CT underwent diagnostic ERCP. Now, MRCP provides a noninvasive means of detecting bile duct stones. Recent studies show MRCP sensitivity of 90% to 100% and specificity of 92% to 100%,for detection of CBD stones, which is similar to and in most cases exceeding those of ERCP (12,13).

On cholangiography, calculi within the bile ducts are readily detected as round or faceted filling defects within the contrast column. These defects are usually mobile. If the stone is impacted in any part of the CBD, a typical convex border of the contrast column in the distal CBD is seen outlining the proximal stone margin where obstruction to flow of contrast is noted.

Air-bubbles are a common problem at cholangiography, but can usually be differentiated by their smooth, round appearance and their tendency to group together and rise to the nondependent surface as compared to stones which are usually faceted or elliptical and tend to fall at the dependent portion of the biliary tree.

BENIGN STRUCTURES

Benign strictures of the biliary tree have variety of causes including surgery and other trauma, chronic pancreatitis, gall bladder or CBD stones, duodenal ulcer, etc.

Postoperative Biliary Strictures

Majority of the strictures are the result of injury to the bile duct at the time of biliary tract surgery. ERCP and PTC are established modalities in the evaluation of CBD strictures(14). Vashisht *et* al(15) reported US evaluation of postoperative CBD strictures with comparative analysis with ERCP/PTC. The authors observed that on US the strictures were seen as :

i. Smooth tapering stenosis with proximal dilatation of CBD in 41 per cent patients.

ii. Abrupt cut off of CBD in 18 per cent patients and further,

iii. The presence of echogenic nodule without acoustic shadowing in 16 per cent of the patients.

Echogenic nodules without acoustic shadowing, however, have also been observed in patients with choledocholithiasis in nondilated or mildly dilated CBD. Of the 48 patients with biliary strictures in this study, mild to moderate dilatation of intrahepatic biliary radicles (IHBR) was observed in 4 patients only. The common bile duct showed mild to moderate dilatation (6.5-8 mm) in 40 patients. The authors concluded that in patients suspected of postoperative CBD strictures, US should be carried out as a screening procedure. In the presence of proximal dilatation of CBD with smooth tapering stenosis or sudden cut off of CBD no further investigation is required. When the findings are equivocal, the patient should be subjected to PTC or ERCP. MRCP now provides a noninvasive alternative to ERCP/PTC (Fig.1, 2). MRCP/ERCP/PTC in addition to confirming the presence of CBD stricture can also show the exact level of strictures (16). Bismuth H(17) classified

BENIGN BILIARY STRICTURES

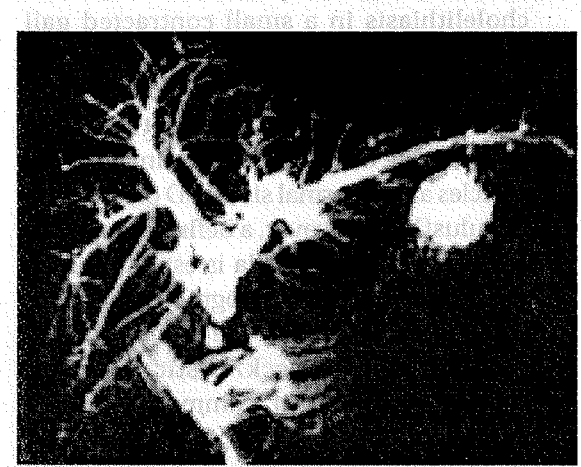

Fig. 1 : MRCP projectional image shows Type I benign Bismuth-Corlette stricture which is more than 2cm distal to the primary confluence of the right and left hepatic ducts.

←

Fig. 2 : Type IV stricture which is present at the primary confluence and involving it seen on MRCP projectional image.

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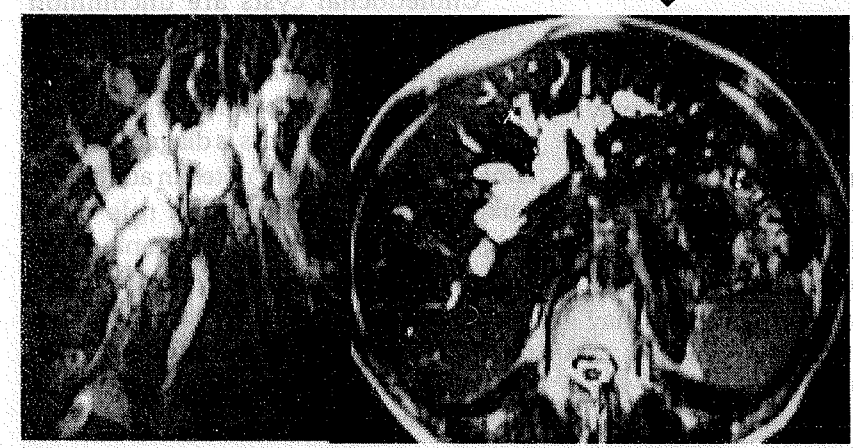

post operative bile duct strictures into five types based on ERCP/PTC.

Type I Stricture more than 2 cms distal to the confluence of right and left hepatic ducts

Type II Stricture less than 2 cms distal to the primary confluence.

Type III Stricture at the primary confluence but the confluence is patent.

Type IV Stricture at the primary confluence, involving it.

Type V Stricture involving accessory duct.

Postinflammatory Strictures

Inflammatory strictures other than cholangitis can be caused by chronic pancreatitis, gall stones and penetrating or perforating duodenal ulcer.

In chronic pancreatitis, strictures occur in less than half of the patients. The most frequent configuratioin on cholangiography is about 3 to 5 cm, smooth, concentric, often tapered narrowing of the intrapancreatic portion of the CBD. An hour-glass configuration or deviation by a psuedocyst may also be seen.

Strictures associated with gall stones are often short and sometimes web like. These may be single or multiple and may involve any portion of the biliary tree. Common duct strictures may result from fibrosis secondary to an adjacent inflamed gall bladder. Other strictures may be associated with choledocholithiasis. Stones trapped proximal to such strictures may contribute to obstruction giving rise to infectious cholangitis and further stricture formation.

Postinflammatory strictures are best diagnosed and characterized by cholangiography. US, CT and MRI primarily demonstrate biliary dilatation but may also reflect the primary pathlogy leading to strictures e.g. pancreatitis, gall stones or CBD stones etc.

Mirrizi's Syndrome

Intrabiliary fistula between the gall bladder and common hepatic duct/bile duct secondary to an eroding stone located in the neck of the gall bladder or cystic duct is called Mirrizi's syndrome. US reveals cholelithiasis in a small contracted gall bladder with an echogenic nodule or calculus either in the neck of the gall bladder or adjacent common duct with mild to moderate dilatation of proximal biliary radicles and a normal size CBD distal to the calculus(18). However, a definite diagnosis of internal biliary fistula is established by ERCP/PTC. On cholangiography confluent filling defects (calculi) are seen involving the gall bladder neck/cystic duct and common hepatic duct/common bile duct.

CHOLEDOCHAL CYSTS

Choledochal cysts are uncommon congenital cysts of the bile ducts. The cysts usually manifest in childhood, and the triad of jaundice, right upper quadrant pain and a palpable subcostal mass is diagnostic. This clinical triad may not be noted in adults and imaging plays an important role in its diagnosis. Real-time ultrasonography is the preferred imaging modality for the initial investigation of patients with suspected choledochal cysts, and its usefulness in the diagnosis of choledochal cysts has been well documented(19). Todani *et al* (20) classified

choledochal cysts into five types. In type I choledochal cysts, fusiform cystic dilatation of extrahepatic CBD within the porta is seen. In type II, an eccentric fluid-filled cyst may be seen which may appear separate from the CBD as its neck may be narrow. Type III choledochal cyst or choledochocele represents localised cystic dilatation of the distal intramural duodenal portion of the CBD and is difficult to diagnose on US & CT. In type IV A, there are multiple cysts involving the intrahepatic and extrahepatic bile ducts and in type IV B, there are multiple cysts involving the extrahepatic duct only. Type V or Caroli's disease includes single or multiple intrahepatic bile duct cysts. The differential diagnosis on ultrasound includes other fluid filled structures in this region namely pancreatic pseudocysts, large right renal cyst and hepatic artery aneurysm. CT and MRI can also be useful in evaluating choledochal cysts by accurately showing the extent of involvement. Many surgeons prefer direct cholangiography in order to better appreciate the anatomical abnormality. ERCP can be performed as the next step after ultrasound, though it is not recommended as the initial procedure.

MRCP has been shown to be equivalent to ERCP in detecting and defining the morphologic characteristics of choledochal cysts and in detecting the presence of anomalous union of the pancreatic and bile ducts(21).

Reported complications of choledochal cysts include stones within the gall bladder or the cyst, pancreatitis, biliary cirrhosis, rupture of the cyst with bile peritonitis and intrahepatic abscess. Biliary tract neoplasm is also a well known complication of choledochal cyst.

PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis (PSC) is a chronic progressive hepatobiliary disorder of unknown aetiology that occurs commonly in young men. It usually presents as a chronic cholestatic syndrome and is associated with inflammatory bowel disease, most commonly ulcerative colitis in over half of the cases. The disease is also associated with other fibrosclerosing collagen diseases such as Riedel's stroma, orbital pseudotumour and retroperitoneal fibrosis.

Sclerosing cholangitis usually has no sonographic manifestations, and its US diagnosis is difficult unless biliary ductal dilatation is present. The intrahepatic ducts are seen to be involved in a patchy distribution. However, the degree of dilatation seen in PSC is minimal due to surrounding fibrotic reactioin. Thickening of either intra-or extrahepatic bile duct walls may also be seen, but this is a nonspecific finding and may be seen in other conditions like suppurative cholangitis and cholangiocar-cinoma.

Sharma *et al* (22) demonstrated on ultrasound intraluminal webs in the biliary tree in two patients of sclerosing cholangitis. This sonographic finding has not been reported so far, although bile duct diverticulae and webs have been reported on cholangiography(23). Duct wall thickening often with marked contrast enhancement, skip dilatations, mural webs and duct stenosis have also been demonstrated on CT.

Cholangiography is the most definitive imaging modality for the diagnosis of PSC. Diffuse, multifocal, short (1-2 cm in length) strictures in both intrahepatic and extrahepatic bile ducts are the most common findings. Strictures alternate with normal or mildly dilated duct segments, sometimes resulting in a beaded duct appearance. Other manifestations of PSC are short (1 to 2 mm) band-like strictures and diverticulum-like outpouching, seen most frequently in the extrahepatic bile ducts. Abrupt termination of intrahepatic branches may be seen,the so-called "pruned-tree" appearance which is due to fibrous obliteration of bile ducts. The role of MRCP in the evaluation of PSC continues to evolve. MRCP is able to depict the changes that characterise PSC including mural irregularities, strictures and diverticular outpouching. However, ERCP may still be required in early stages of the disease. It is often difficult to diagnose cholangiocarcinoma coexisting with PSC. The findings that suggest the possibility of cholangiocarcinoma include marked ductal dilatation, progressive stricture and the presence of an intraluminal polypoid mass one cm. or more in diameter.

BACTERIAL CHOLANGITIS AND AIDS RELATED BILIARY ABNORMALITIES

Bacterial cholangitis (ascending, acute, infective cholangitis) is an acute infection of the biliary tree that usually occurs in the setting of biliary tract obstruction. It occurs more commonly in benign lesions. The cholangiographic findings are variable and range from ductal dilatation to irregular angulation and ductal filling defects due to purulent material in the clinical setting of sepsis. Complication such as cholangitic abscess may be seen.

Biliary abnormalities can be found in patients with AIDS. Most frequently, gall bladder wall thickening secondary to oedema occurs. Intra-or extrahepatic bile duct dilatation may also be seen. The cause of these abnormalities is not known, but infection with HIV virus and opportunistic organisms have been implicated. Oedema of duct papilla may be the cause of biliary duct dilatation.

PARASITIC DISEASES

Although many parasites involving the gastrointestinal tract may traverse the biliary tract, significant infestation within the biliary tree with clinical symptomatology and radiographic abnormalities, is seen most commonly with Ascaris lumbricoides, and Echinococcus granulosus.

Ascaris lumbricoides

Ascaris lumbricoides normally inhabit the small intestine. These have a propensity to migrate from small intestine through the ampulla of Vater to lodge in the gall bladder and biliary tract. Ultrasound is considered the most valuable tool for evaluation of patients suspected to suffer from biliary ascariasis. On US, the worms can be recognised as tubular, nonshadowing, echogenic structures in the dilated biliary ducts. When they are alive, the movements of the worms can be seen, and it is usually possible to see a sonolucent inner tube within the echogenic tubular structure, which represents the alimentary canal of the worm. On unenhanced CT, they appear as hyperattenuating tubular structures

surrounded by less attenuated bile. In transverse sections on both US and CT, a "bull's eye" image may be seen caused by the worm inside a dilated bile duct. On cholangiography, the worms may be seen as smooth cylindrical translucencies.

Biliary Hydatid.

Hydatid disease can affect any organ of the body and the liver is involved most commonly. Rupture is an important complication of hydatid cyst of the liver. The cyst may rupture into the biliary system, peritoneal cavity and thorax. In patients with rupture into the biliary system, daughter cysts and membranes pass into the common bile duct producing surgical obstructive jaundice(24). It is now possible to make an accurate pre-operative diagnosis of hydatid disease as well as intrabiliary connections. Ultrasound, CT and cholangiography are helpful in arriving at a correct preoperative diagnosis. Doyle *et al*(25) described three different patterns of intraductal filling defects on ERCP: (i) filiform linear material in the CBD due to laminated hydatid memberanes, (ii) rounded filling defects due to hydatid daughter cysts, and (iii) amorphous debris in the CBD due to a mixture of hydatid membranes and daughter cysts.

MRCP and nuclear scan (HIDA) have also been found to be valuable in diagnosis of intrabiliary rupture of hydatid cyst(26).

RARE INFECTIONS

Tuberculosis

Hepatobiliary tuberculosis is a rare cause of biliary strictures, predominantly seen in underdeveloped countries. The most common involvement is at porta hepatis and less frequently distal common bile duct. Cholangiographic findings include irregular strictures and marked proximal dilatation or in less severe cases minimal wall irregularity or narrowing of the common hepatic duct. Dense chalky liver calcification and periportal or periductal nodal calcification suggests the possibility of tuberculosis as a cause(27). Periportal tubercular adenitis causing biliary obstruction has been demonstrated by US and CT. We came across a patient with jaundice who on US revealed peripancreatic lymphadenopathy with mild CBD dilatation (7 mm). On ERCP intrapancreatic part of CBD showed narrowing with an extrinsic impression on it. Rest of the CBD showed minimal dilatation. Fine needle aspiration biopsy of the nodes revealed tubercular pathology. Patient recovered fully on antitubercular treatment.

Other uncommon infections with Cryptococcus, Candida, Trichosporon, etc. may lead to common duct stricture and obstruction.

AMPULLARY STENOSIS

Biliary obstruction may be caused by morphologic stenosis of the ampulla of Vater or sphineter of Oddi. Although unclear, probable causes include passage of gall stones and pancreatitis. Imaging studies for the diagnosis of ampullary stenosis are frequently abnormal but not always conclusive. On US, CT and cholangiography (ERCP/MRCP) bile duct and sometimes pancreatic duct dilatation may be seen. Ultrasound before and after a fatty meal may show partial obstruction by demonstrating an increase in common duct

diameter after fatty meal. Use of hepatobiliary scintigraphy is also considered to be useful in the diagnosis. The single most valuable study for assessing the diagnosis of papillary stenosis is ERCP, where direct endoscopic inspection of the papilla is possible. Tumours of the papilla or surrounding duodenum may be identified, if present. Cholangiographic findings of common bile duct dilatation, an elongated or rigid ampullary segment and failure of the common duct to empty contrast material in 45 minutes are suggestive of ampullary stenosis. For distinguishing morphologic stenosis from functioinal spasm or dyskinesia, cholecystokinin or glucagon may be required which will relieve the spasm in functional dyskinesia.

CHOLEDOCHAL VARICES

In portal hypertension, varices of the paracholedochal veins of Petren and epicholedochal venous plexus of Saint may occur(28). Smooth, extrinsic, nodular, spiral or stenotic appearing duct abnormalities and extrahepatic bile duct obstruction caused by varices may be seen at cholangiography.

BENIGN TUMOURS OF THE BILE DUCT

Benign bile duct tumours are rare and are usually discovered as small polypoidal masses or rounded or oval nodular masses. These include papilloma, adenoma, fibroma, neurofibroma, hamartoma, lipoma and leiomyoma. Benign tumours are most frequently found in the periampullary region or in the common bile duct and are quite uncommon in the common hepatic or intrahepatic ducts.

Papillomas are usually sessile tumours with a broad base These can be multifocal but even then are confined to a small segment of the common duct.

Adenoma and leiomyoma are usually single, smooth, well-circumscribed tumours arising in the duct wall. Sonographically they are moderately echogenic nonshadowing filling defects. The lack of shadowing and relatively low echogenicity suggest a tumour rather than a stone. On CT, these are seen as soft tissue masses indistinguishable from noncalcified stones. Cholangiographically the tumours usually present as round or oval filling defects with smooth borders which do not change their position.

MALIGNANT LESIONS CAUSING OBSTRUCTIVE BILIOPATHY

- Carcinoma gall bladder
- Cholangio carcinoma
- Carcinoma Head of the pancreas

CARCINOMA GALL BLADDER

Carcinoma of the gall bladder (CaGB) is the most common biliary tract malignancy. Risk factors include cholelithiasis, chronic cholecystitis, anomalous pancreatobiliary ductal union, chronic typhoid infection and porcelain gall bladder(29). The patient is usually an elderly female complaining of pain in right upper abdomen, nausea, vomiting, weight loss and jaundice. Hard mass may be palpable. Majority of the tumours are inoperable at the time of diagnosis and average survival is only six months after the first symptom appears. The rich lymphatic and venous drainage of gall bladder allows

rapid spread to lymph nodes and widespread dissemination. The liver bed is invaded and there is local spread to duodenum, stomach and colon. Long-term survival is seen only in patients in whom the tumour is found incidentally at the time of cholecystectomy for gallstones. Histologically, majority of malignant tumours of the gall bladder are adenocarcinomas.

Ultrasound is the primary imaging modality of investigation. Three major patterns have been described on US(30). In type 1, the gall bladder is surrounded or replaced by a hypo echoic or heterogeneous mass. Cystic areas may be seen within it representing necrosis or residual bile. In type 2 there is focal or diffuse, irregular and asymmetrical wall thickening. In type 3, which is less common, a polypoidal, fungating intra luminal mass is seen. It usually has a wide base and does not move with change in the position of the patient.

Gallstones are seen in 75 per cent of the patients with Ca GB(29). Liver invasion is suggested by the lack of a distinct margin between the GB mass and the liver. Hematogenous distant metastases may also be seen in the liver. Enlarged lymph nodes may be seen at the porta hepatis, peri-pancreatic and para-aortic region. Biliary obstruction in the form of dilated intra hepatic biliary radicals and CBD may be seen because of direct extension via hepato-duodenal ligament or compression by lymphadenopathy.

On US differential diagnosis includes complicated cholecystitis and xanthogranulomatous cholecystitis. The latter is a xanthogranulomatous reaction to the intra mural extravasation of bile caused by rupture of Rokitnasky-Aschoff sinuses. It may be associated with lymphadenopathy. Reverberation artifacts can obscure anterior wall lesions. Similarly, sludge in GB may give the impression of posterior wall mass lesions. Sludge, however, is usually mobile when patient's position is changed. Also it usually is brighter than the mass lesion. Demonstration of vascularity within the GB lumen on color Doppler helps to differentiate intraluminal tumour from sludge or pus. Polypoidal form of CaGB may be confused with the noncalcified stone or benign polyp.

Commonest CT finding in CaGB is mildly enhancing mass that partly or fully replaces the GB. Direct extension to the liver is seen as an ill-defined margin initially and then as a low-density lesion in the contiguous liver parenchyma. Less common CT manifestations are irregular wall thickening and intra luminal masses. The masses exhibit mild and variable contrast enhancement and improve visualization of the intraluminal component. Ancillary findings on CT include gallstones, wall calcification, dilated biliary radicals, hepatic metastases, lymphadenopathy, ascites and extension into stomach, duodenum or colon. The lymph nodes may show necrotic center.

Cholangiography shows infiltration, encasement and obstruction of the CBD in the region of cystic duct with no filling of gall bladder. MRI shows a mass in GB fossa which is hypo intense on T1 weighted images and hyper intense on T2 weighted images.

CHOLANGIOCARCINOMA

It is an uncommon tumour. It is commoner in males with peak incidence in sixth or seventh decade. Higher incidence is associated with sclerosing cholangitis, Caroli's disease, choledochal cysts and ulcerative colitis(29). Patients usually present with jaundice, weight loss and anorexia. Local and distant metastases are uncommon even at autopsy and are found only in half of the patients. These involve regional lymph nodes, peritoneum, liver, gall bladder and diaphragm. Vascular invasion is rare and extra abdominal spread is unusual. Histologically majority of these tumours are adenocarcinomas with cuboidal or columnar epithelium and abundant fibrous stroma.

Cholangiocarcinomas can be classified as

1) Intrahepatic tumour (peripheral lesions).
2) Hilar lesions (the most common location) referred to as Klatskin tumour and
3) Distal ductal tumour

Cholangiocarcinoma may occur in between these general locations(31). Morphologically scrrihous infiltrating neoplasms causing focal biliary strictures without evidence of a mass are seen most commonly. Exophyic bulky masses are seen usually in intrahepatic peripheral location and polypoidal intraluminal ductal lesions are seen in the distal duct. The tumour stroma is composed of two major elements, fibrous tissue and mucin producing glandular tumour and these tissues dramatically influence the CT and MR imaging appearances of the tumours.

Intra-hepatic (Peripheral) cholangiocarcinoma

Peripheral intrahepatic carcinomas are usually large at presentation because they are rarely symptomatic early in their course. Imaging features are non specific and it can not be reliably differentiated from primary or metastatic carcinoma of the liver. Sonographic findings are non specific and may be seen as hypo or iso echoic masses, which may be homogenous or heterogeneous. On contrast enhanced CT scan, the tumour shows mild and peripheral enhancement. On multiphase CT scan, the enhancement is peripheral and delayed. The central area of the tumour, which contains fibrous tissue, does not enhance during early phase but becomes hyperdense during the delayed phase, 4 to 20 minutes after injection, a feature which may help to differentiate it from HCC(28,32). On MRI, intrahepatic cholangiocarcinoma is seen as irregular, heterogeneous mass with low signal intensity (SI) on T1 weighted images and high SI on T2 weighted images. Mild to moderate rim enhancement is seen on contrast enhanced MRI.

Hilar cholangiocarcinoma (Klatskin tumour)

The most common location is either at the confluence of right and left hepatic ducts, or the proximal common hepatic duct, and has been termed Klatskin tumour. On imaging biliary dilatation is seen with or without patency of the confluence. Both US and CT are equally accurate in demonstration of this finding, however, CT is superior to US in identification of the tumour. Morphologically the tumour is of

three types; infiltrating, exophytic or polypoid. Infiltrating type is the most common.

Ancillary CT findings in cholangiocarcinoma include infiltration of the liver, GB, pancreas or duodenum and lymphadenopathy involving peri pancreatic or celiac group of nodes.

MRI and MRCP have an important role in evaluation of cholangiocarcinoma. MRCP is more accurate than ERCP in determining anatomical extent of biliary obstruction and cause of jaundice(16,33,34). In addition to MRCP, cross sectional MR imaging is also a valuable tool. Spoiled gradient echo, fat sat T1 weighted sequences obtained at 2 to 5 minutes after gadolinium injection have been found to be more consistent in demonstrating the tumour. Cholangiocarcinoma is seen as a moderately enhancing lesion with this technique(35). MR (T2WI) shows proximal hilar cholangiocarcinoma as moderately high signal intensity thickened duct wall or ill defined tumour mass. The intensity of the thickened duct wall is higher then adjacent liver, but is of lesser intensity then intraluminal bile. On cholangiography (MRCP/ERCP), the Klatskin tumour is seen as an irregular stricture at the confluence, with prestenotic dilatation. Either one or both of the hepatic ducts may be obstructed in addition to the common duct (Fig.3&4). Diagnostic PTC and ERCP are now less commonly employed for cholangiocarcinoma.

MALIGNANT STRICTURES

Fig. 3 : MRCP projectional image shows Bismuth-Corlette Type II stricture involving the primary confluence. There is no communication with right and left hepatic ducts.

Fig. 4 : Oblique projectional MRCP image shows Bismuth-Corlette Type IIIB stricture involving the primary confluence and extending up to the secondary confluence on the left side.

Hilar cholangiocarcinomas are graded according to Bismuth classification(36). Type I lesion involves common hepatic duct only; type II lesion involves right and left hepatic ducts at the confluence. First order branches are involved of either (type III) or both (type IV) of the hepatic ducts.

Distal duct cholangiocarcinoma

The least common location for cholangiocarcinoma is the distal duct. Ultrasound demonstrates biliary dilatation proximal to an abrupt obstruction. Site of the lesion will determine the GB distention. Demonstration of mass is rare, so it becomes difficult to differentiate it from benign strictures. In absence of history of previous surgery, cholangiocarcinoma should be suspected when abrupt obstruction of distal duct is seen without visualisation of a mass or calculus and the pancreas is normal. If the mass is seen near head of the pancreas, carcinoma of the pancreas is more likely. The bile duct at the level of obstruction in cholangiocarcinoma is narrowed if the process is primarily desmoplastic and widened if there is an obstructing intra luminal mass.

CT manifestations of cholangiocarcinoma include biliary dilatation till the level of obstruction and less commonly, demonstration of a mass. The diagnosis is suggested by abrupt cut-off without a mass or calculus. Diffuse, enhancing wall thickening may be seen. If a mass is seen, it is hypodense in pre contrast scans and shows delayed enhancement on post contrast scans. Rarely, a peripheral ring enhancement pattern is seen(37).

On cholangiography, distal cholangiocarcinoma may reveal obstructive, stenotic or protuberant lesion. Commonest is the obstructive lesion, which appears as U or V shaped occlusion with nipple, rat tail, smooth or irregular termination. The stenotic type is seen as a rigid, narrowed lumen with irregular margins and pre stenotic dilatation. The protuberant type is seen as nodular or polypoidal intra-luminal filling defects attached to the wall. The Diffuse sclerosing type of cholangiocarcinoma causes widespread strictures of both intra and extra hepatic ducts resembling sclerosing cholangitis. Clues for the correct diagnosis of cholangiocarcinoma include absence of diverticuli and more severe disease in extra-hepatic ducts and prominent dilatation of the ducts.

Periampullary carcinoma :

Periampullary carcinoma is the term used to describe tumors at the ampulla. These may arise from the pancreatic duct, terminal bile duct or duodenum at the amupulla of Vater. Because of their strategic location, these tumours produce jaundice early and hence are detected when very small. Consequently, these tumours have much better prognosis and up to third of the resected patients survive for more than five years. On imaging, the biliary obstruction till the level of ampulla, with or without dilatation of pancreatic duct is seen (Fig. 5a to c). Demonstration of the mass is uncommon.

PERIAMPULLARY CARCINOMA

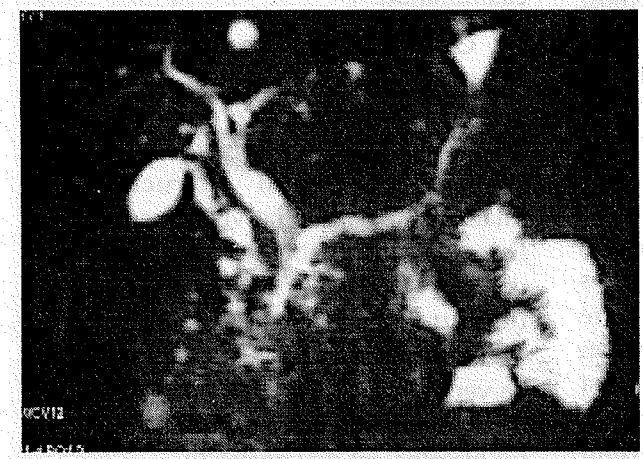

Fig. 5(a) : MRCP (thick slab) image shows dilatation of central intrahepatic biliary radicles, common bile duct as well as MPD.

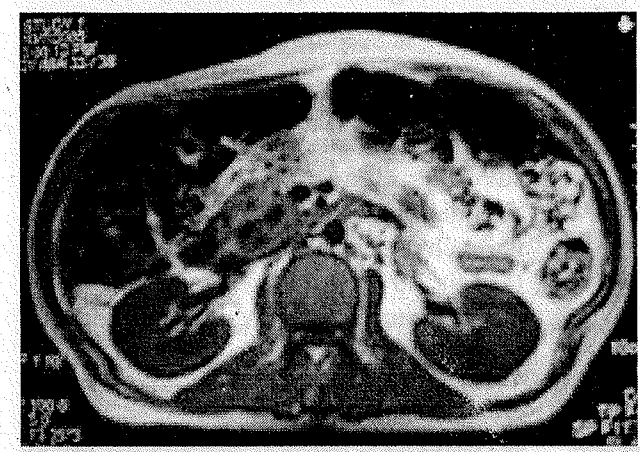

Fig. 5(b) : Axial T_1W (FLASH) image shows the dilated CBD and MPD (double duct sign)

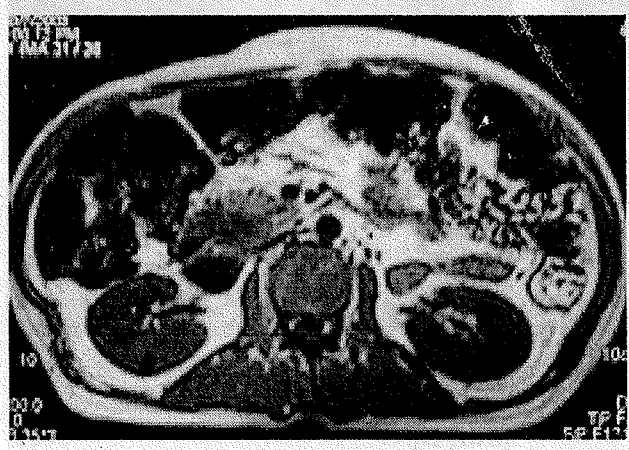

Fig. 5(c) : Axial T1W (FLASH) image, caudal to the above image shows.helerogenous mass in periampullary location.

Majority of duodenal adenocarcinoma are polypoidal. Papillary region is the most common site of origin(38). These tumours often produce biliary obstruction and present with jaundice.

CARCINOMA HEAD OF THE PANCREAS

Carcinoma of the head of the pancreas is an important cause of malignant lesions leading to biliary obstruction. The commonest tumour of the pancreas is an adenocarcinoma of ductal origin and most adenocarcinomas arise in the head. Obstruction of the CBD and concurrent neighbouring pancreatic duct frequently occurs. The typical pancreatic cancer is a solid scrrihons tumor which has a decreased vascular perfusion compared to the normal pancreatic tissue

In patients with carcinoma head of the pancreas, widening of C loop with spiculated duodenal wall with fixity of the duodenal folds and Frostberg's reverse 3 sign may be seen on barium meal examination.

On ultrasonography pancreatic carcinomas are usually hypo-echoic as compared to normal parenchyma. Necrotic tumours may show heterogenous echopattern. Ductal obstruction and dilatation may also be visualized. Colour Doppler is now being used extensively for detection of vascular invasion of pancreatic tumours(39). For small <2 cms solid tumours endoscopic US has been found to be very useful. Perhaps its real advantage lies in its ability to obtain accurate tissue for histological diagnosis(40).

CT has become a widely used imaging technique for the evaluation of patients with suspected neoplastic pancreastic disease. Dynamic contrast enhanced spiral CT offers several advantages with increased attenuation difference between pancreatic parenchyma and the hypovascular mass (Fig.6a to c) and between the liver parenchyma and metastenses. In addition

CARCINOMA HEAD OF THE PANCREAS

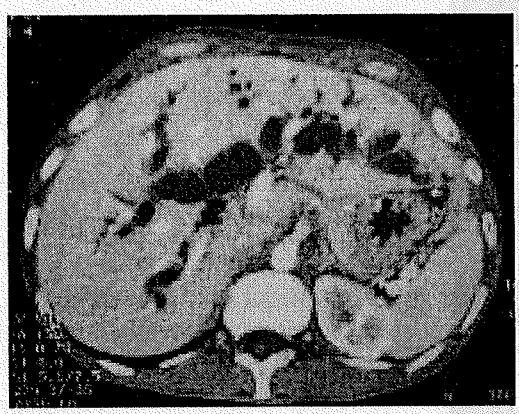

Fig. 6(a) : Axial CT image shows gross dilatation of IHBR in both lobes of liver.

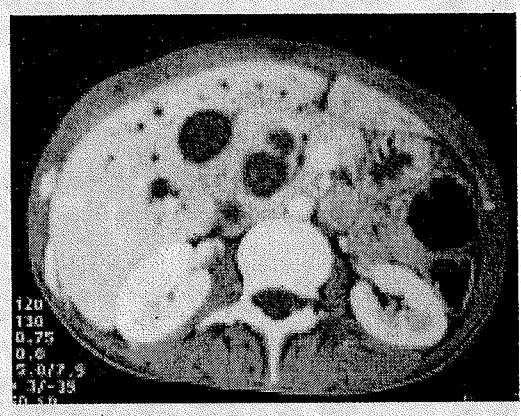

Fig. 6(b) : CT section, at a more caudal level, shows the double duct sign (dilated CBD & MPD).

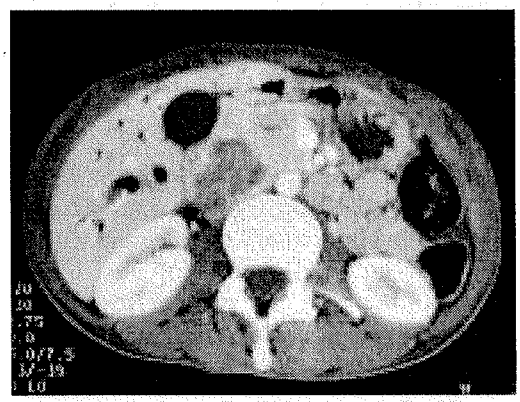

Fig. 6(c) : Heterogenous mass seen in the head of pancreas.

pancreatic and biliary ductal anatomy is better defined because of adequate enhancement of the parenchyma while intense enhancement of the blood vessels facilitates assessment of vascular involvement.

MRI is especially suited for the detection of small, nonorgan deforming pancreatic ductal adenocarcinoma, and detection of islet cell tumours. Ductal adenocarcinoma appears as an area of abnormally decreased signal on T_1W images and as a poorly enhancing hypovascular mass on contrast enhanced MR scan due to its desmoplastic fibrotic composition. MR is superior to helical CT for determination of local tumour extension and nearby organ invasion.

MRCP has been shown to be accurate in identifying the presence and level of neoplastic obstruction of the pancreatico-biliary tract. In addition MRCP performed in conjunction with a conventional abdominal MR and when necessary MRA, yields a comprehensive examination that permits not only diagnosis but also staging of malignant neoplasms of the pancreatico-biliary tract.

OTHER MISCELLANEOUS TUMORS OF BILIARY TRACT

Miscellaneous tumors of biliary tract include biliary cystadenocarcinoma, carcinoids, lymphoma, villous tumour, embryonal rhabdomyosarcoma and secondaries.

Biliary cystadenocarcinomas are rare biliary tract neoplasms that arise from intra hepatic and less frequently, extra hepatic biliary tree. Majority occur in women. On sonography, multiloculated cystic mass is seen. Mural nodules and fluid-fluid levels may also be seen. CT demonstrates low-density intrahepatic lesion with internal septations and mural nodules. Each loculus may have a different CT density(36).

Embryonal rhabdomyosarcoma (Sarcoma botryoides) is the second most common cause of obstructive jaundice in children past infancy; first being choledochal cyst. Average age of onset is four years with rapid progression and death. It grows along the wall of CBD beneath the mucosa with polypoidal intraluminal projection. US and CT show dilated intra hepatic ducts and a soft tissue mass in the region of CBD. On cholangiography, grape like filling defects are seen in dilated CBD.

LIVER DISEASES AFFECTING THE BILIARY TRACT

Diseases of hepatic parenchyma may affect the intrahepatic biliary tree causing obstructive biliopathy. Bile duct changes are

better evaluated by cholangiography, whereas the underlying liver disease may be demonstrated on US, CT and MRI. Parenchymal liver masses (neoplasms, cysts, abscesses) may cause displacement or focal or generalised narrowing of intrahepatic ducts and dilatation of proximal intrahepatic ducts.

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Trends in sexually transmitted infections in India

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ABSTRACT

Sexually transmitted infections (STI) are a significant health problem and there has been a steady increase in the STI's worldwide and in India. The pattern of STI's varies not only in different parts of the world but also even in different parts of India. Viral STI's are emerging to be the most common STI's in India a trend which has been identified in the developed countries.The syndromic approach to treatment where common etiologic agents responsible for a group of symptoms are treated with or without investigations, has revolutionized the treatment of STI and now all doctors and paramedics can treat STI following simple flow charts. The interaction of sexually transmitted pathogens and HIV are other significant changes in the field of ST which has modified clinical symptoms, investigations and treatment of STI.

INTRODUCTION

Sexually transmitted infections (STI) are a major public health problem and have a tremendous impact on the national health. The epidemiology of STI in a given community is very dynamic and with better methods of diagnosis, treatment and prevention and the emergence of HIV there has been a continuous change in the pattern of the various STI. However there is a paucity of a reliable surveillance system in most of the developing countries including India and most data are derived from tertiary care centers and do not reflect the actual burden of disease in the community. Lack of adequate laboratory infrastructure at the primary and secondary care levels hinders the confirmation of diagnosis and most of the data available is syndromic. In India because of the stigma associated with STI, patients prefer to get treated from traditional healers, quacks, pharmacists and private practitioners rather than governmental hospitals and fail to get notified. There is also considerable variation in the prevalence of various STI in different parts of the country. But there is a definite need for demographic data for designing control pro-

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grams as well as for evaluating existing programs.

Global trends

According to WHO estimates, 333 million cases of new curable STI occur, of which 150 million were in south and southeast Asia and 65 million occurred in sub-Saharan Africa(1).

In the U.S, the CDC estimates that 19 million new infections occur each year, 50% of which occurs in the young, aged 15 – 24 years(2). The changes in sexual behavior – decrease in the age at first intercourse, increase in lifetime partners, concurrent relationships, decrease in safe sex practices among homosexual men, prostitution and sex in exchange for drugs contributing to the rise.

In developed countries, the prevalence of STI has steadily increased. The viral STI and genital chlamydial infections are on the rise in most developed countries. The emergence of HIV infection and the subsequent behavioral, social and psychological changes, and better treatment of bacterial STI has contributed to the increasing prevalence of viral STI.

Indian scenario

In India, 50 million new curable STI are estimated to occur in adults every year. In the 1970s, syphilis and chancroid were the main causes of genital ulcer disease (GUD),

Table 1: Trends in STI in STI Clinic Attendees in Tertiary Hospitals of India

S. No.	Region	Syphilis	Chancroid	LGV	Donovaniosis	Genital Herpes	HPV	Gonorrhoea	NGU
1.	Delhi(4) 1955-61	7.3	22.5	0.6	0.25	-	-	15.9	4.9
	Delhi(5) 1965-78	54.9	5.9	0.9	1.2	2.5	2	1.9	-
	Delhi(6) 1989-95	14.3	23.9	1.6	1.4	11.8	9.2	12.2	3.7
	Delhi(7) 1995-99	15.6	11	0.45	0.48	11.8	9.3	11.6	7.4
2.	Chandigarh(8) 1977-85	10.4	12.2	0.6	6.3	11.4	21.4	16.9	4.1
	Chandigarh(9) 1985-92	8.7	8.1	0.9	1.6	19.7	25.2	5.3	4.1
	Chandigarh(10) 1995-96	2	3	6	0.5	21	7	3	6
3.	Rohtak(11) 1992-94	30.2	22.1	0.97	1.45	10.6	18.1	12.9	4.7
	Rohtak(12) 1995-96	7.4 - P 17.5 - S	14.5	0.67	0.8	11.1	21.5	12.6	6.7
	Rohtak(13) 1995-2000	24	10.9	0.2	0.86	16.9	19.4	16.2	4.8
4.	Ahmedabad(14) 1993-94	22.2 - P 28.7 - S	7.6	0.58	2	8.2	7.2	5.05	-
	Ahmedabad(15) 1998-99	28.9	9.6	-	1	27.9	9.1	12.7	1.5
5.	Pondicherry(16) 1982-90	18	10.6	8	8.2	14.1	11.9	11.9	0.8
6.	Kurnool(17) 1992-96	14.4	2.8	9.7	-	14	11.3	11.7	19.1

while viral GUD such as genital herpes were uncommon. But, from the 1980s there has been a steady rise in the viral STI and a relative fall in the traditional, treatable bacterial STI. Data from the tertiary care hospitals(Table 1) in India have shown an increase in the viral STI similar to the changes in developing countries and genital herpes has emerged as the most common STI at Chandigarh (21% in 1995-96) and a close second most common STI in Ahmedabad (27.9% in1998-99) and Delhi(11.8% in 1995-99). At the premier hospitals (Table 2), herpes genitalis and condylomata acuminata together constitute

Table 2: Prevalence of STI in premier Institutes of India

STI	AIIMS	PGI(9)	JIPMER
HPV	24.6	25.2	19.8
Genital herpes	24.2	19.7	28.3
Syphilis	14.0	8.7	25.9
Gonorrhoea	10.1	5.3	6.2
NGU	5.8	4.1	0.5
Chancroid	6.3	8.1	4.5
Donovanosis	1.1	1.6	4.8
Multiple	10.1	5.7	—

44.9% to 48.8% of the total STI. However in the district hospitals, studies in 1994 and 1998 have shown that the common STI still continue to be chancroid and gonorrhea (Table 3). In a population based study conducted in Tamil Nadu, 47.3% of individuals had reported genital symptoms. The prevalence of STI was as high as 15.8% and that of classic STI was 9.7%. Vaginal discharge was the most common STI syndrome

Table 3: Prevalence of STI in District Hospitals of India

STI	Tezpur(18)	Portblair	Srinagar(19)
	(1994)	(1994)	(1998)
Chancroid	35.0	21.0	28.8
Gonorrhoea	17.8	19.0	11.7
Syphilis	14.6	25.0	20.8
LGV	10.2	-	9.7
HPV	9.2	9.0	11.3
HG	5.0	7.0	4.1
NSU	3.3	0.7	2.6
Donovanosis	0.013	-	0.22

and was found in 41.5% of women. In men, scrotal swelling (2.1%) and urethral discharge (0.2%) were most common(3). In a study conducted in Varanasi, where 1500 male students were screened, 3.93% were found to have an STI.

Syphilis

In developed countries, the prevalence of syphilis has fallen steeply because of improved access to health care, effective control programs and efficacious treatment. However, its prevalence is still high in some developing countries especially in Africa and an estimated 12 million cases occur globally every year.

In the U.S., the prevalence rates of syphilis are on the rise especially in men because of increased MSM which is more prevalent in the urban areas. In 2004, syphilis was 5.6 times more common in blacks although the racial gap seems to be narrowing with significant increasing rates in white men and declining rates in African Americans.

In India, syphilis incidence rates are on the decline in most of the tertiary care hospitals. In a tertiary hospital in Delhi, the cases of syphilis declined from 61.2% to 9.1% from 1954 to 1994 although the total STD cases had increased eight times. In a population based study in Tamil Nadu its prevalence was as low as 0.3%. However, prevalence of 8% was found in tribals of Gadchiroli, Maharashtra suggesting that pockets of high prevalence still persist. In a study of 494 cases of syphilis from Trivandrum, latent, secondary and primary syphilis accounted for 54%, 38.4% and 6.3% of cases respectively. The seroprevalence of VDRL reactivity among blood donors in various parts of the country ranged from 0% in Lucknow, 2.8% in Delhi, 3.62% in Tirunelveli and 7% in Bihar. The VDRL positivity rate among antenatal women was 2.9% and 3.4% in Aligarh and Delhi respectively. It is estimated that yearly 344,000 to 516,000 newborn are at risk of exposure to syphilis at birth which is more than the mother to child transmission of HIV.

Chancroid

Chancroid is an uncommon STD in most developed countries. Its incidence has progressively declined although chancroid outbreaks have occurred in New York (1997) and New Orleans (1990-1992). In India, the incidence rates have ranged from 1.6% in Patiala to as high as 51.9% in Mumbai. The rates in district hospitals are still high at 35%, 21% and 28.8% at Tejpur (1994), Port Blair (1994), and Srinagar (1998) respectively. But there has been a steady decline in its rates in tertiary care hospitals in Delhi from 22.5% in 1955-1961 to 11% in 1995-1999; in Chandigarh from 12.2% in 1977-1985 to 3% in 1995-1996; in Rohtak from 22.1% in 1992-1994 to 10.9% in 1995-2000. The declining rates are probably due to easy availability and indiscriminate use of effective antibiotics at the primary care level.

Donavanosis

Donovaniosis is endemic in India, Papua New Guinea, aborigines of Australia, South Africa and Brazil. Racial and ethnic predispositions seem to be associated with the disease. In India, it is common in the states of Tamil Nadu (4.7%), Pondicherry (8.2%), Andhra Pradesh (1.12%), Orissa (7.5%), and Andhra Pradesh (1.12%). The prevalence of the disease is showing a steady decline.

Lymphogranuloma venereum (LGV)

LGV is endemic in West, Central and East Africa, India, Southeast Asia, South America, Papua New Guinea, and the Caribbean Islands. It is less commonly encountered in developed countries. In India, its incidence varies from 0.15% to 9.74% from different parts of the country. Localized epidemics along with HIV infection have been reported.

Gonorrhoea

It is a common STD in most parts of the world although its incidence has steadily declined in the developed countries. In U.S, African Americans remain the group most heavily affected by gonorrhoea. In India, a steady decline has been noted in Delhi and Chandigarh but a marginal increase has been reported from Rohtak and Ahmedabad (Table 1). Its prevalence in gynaecological OPD in Amritsar (1995) and

Chandigarh (1986) is 0.8% and 1.8% respectively. The steady decline is attributed to easy availability and indiscriminate use of effective antibiotics at the primary care level and growing awareness of AIDS in the Indian population.

Chlamydial Infection

Genital chlamydial infection is an STD of epidemic proportion. It causes upto half of all non-gonococcal urthritis and at least one third of acute epididymitis in men. In women, it is responsible for upto half of all mucopurulent cervicitis and 20%-40% cases of pelvic inflammatory diseases with risk of subsequent infertility or ectopic pregnancy. Chlamydia is the most commonly reported infectious disease in the U.S. with 2.8 million new cases occurring every year(2). In Europe, its prevalence varies from 2.6% to 51.5% among women attending various health clinics. In U.K a prevalence rate of 3% to 4% and 3% to 7% was found in family planning and general practice clinic attendees respectively. The increasing rates are attributed to enhanced screening and more sensitive diagnostic tests given the frequent asymptomatic nature of the infection. In India, an incidence rate varying from 1.5% to 19% has been reported from STD clinic attendees from various parts of the country. In Delhi, 26 (21.3%) of 122 pregnant women were positive for Chlamydia trachomatis and it correlated with low birth weight, still birth and prematurity. It is estimated that if 1:10 women carry Chlamydia, 1.77 million newborn are at risk of chlamydia infection and associated risks per year. In women attending gynaecological OPD in Delhi, a prevalence of 41% and 36% was found in vaginal discharge and infertility respetively. High risk factors identified in India include low socioeconomic factors, multiple sexual partners and use of intrauterine devices and protective factors are higher age group and use of oral and barrier contraceptives.

Genital Herpes infection

Genital Herpes is the second most prevalent STD worldwide and the commonest cause of genital ulcer disease in the developed countries. The large population reservoir of undiagnosed cases with risk of transmission and the risk of perinatal transmission pose significant public health implications. In U.S, about one in five persons over age 12 and approximately 45 million people are infected with HSV-2 and upto one million new HSV-2 infections are transmitted annually. In India there has been a significant increase in the proportion of viral STD especially genital herpes with incidence rates varying from 4.11% to 27.9% in STD clinics from various parts of the country. In Chandigarh the incidence increased from 11.4% in 1977-85 to 21% in 1995-96. In Ahmedabad, the incidence increased from 8.23% in 1993-94 to 27.9% in 1998-99. In Delhi also the incidence increased from 8.23% in 1993-94 to 27.9% in 1998-99. The increasing incidence may be attributed to decreasing bacterial STD due to better treatment at the primary level with effective antibiotics.

Human Papilloma virus (HPV) infection

Genital HPV is the commonest viral STD in the developed world, with an estimated 30 million new cases diagnosed annually worldwide. The incidence of genital warts has increased from 13/100,000 in

1950 to 106/100,000 in 1978 in Minnesota. In India, the incidence of genital warts ranges from 2% to 25.2% in STD clinic attendees. The data from different parts of the country show varying trends. In Delhi, in 1955-61, no case was reported; the incidence in 1965-78 was 2% and increased in 1995-99 to 9.3 %. Similarly in Ahmedabad there has been a slight increase from 7.17% in 1993-94 to 9.1% in 1998-99. However the incidence rates have been declining in Chandigarh from 21.4% in 1977-85 to 7% in 1995-96.

Trends in treatment: Syndromic approach

The syndromic approach is based on treating patients of STD on the basis of group of symptoms or syndromes as given below:

STD Syndrome	Possible Causes
• Urethral discharge	Neisseria gonorrhoeae (N. gonorrhoeae) Chlamydial trachomatis (C. trachomatis) D to K
• Genital ulcer disease	Treponema pallidum, Haemophilus ducreyi, C. trachomatis (L1, L2, L3)
• Vaginal discharge	Trichomonas vaginalis, Candida albicans, Gardnerella vaginalis N.gonorrhoeae, C. trachomatis, Anaerobes
• Lower abdominal pain	N. gonorrhoeae, C. trachomatis, anaerobes
• Inguinal Bubo	H. ducreyi, C. trachomatis (L1, L2, L3)
• Scrotal swelling	N. gonorrhoeae, C. trachomatis, viruses and surgical conditions
• Ophthalmia neonatorum	N. gonorrhoeae, c. trachomatis

Advantages of the Syndromic Approach

- Easy and effective and both medical and paramedical can treat STI
- Treatment available at all rural and peripheral centers
- Prompt treatment as no waiting for test results
- Early treatment prevents spread of STI including HIV/AIDS
- Cost effective

Criticisms of the Syndromic Approach

- The syndromic approach is not a scientific procedure.
- Syndromic diagnosis is too simple for a physician to use, and approach does not use a provider's clinical skills and experience.
- Some physicians still feel better treating according to clinical diagnosis in the absence of lab facilities and then, if symptoms do not improve, treating for another cause.

- The syndromic approach wastes a lot of drugs.
- It promotes the development of antibiotic resistance.
- Simple laboratory tests should be included.

Critical appraisal of Syndromic Approach:

- Revalidation has been done in urethral discharge syndrome in men
- Poor results in vaginal discharge syndrome especially Chlamydial cervicitis given the asymptomatic nature of infection.
- Effective in the management of genital ulcer disease
- Data on individual disease is no longer available as laboratory confirmation is not done
- Needs to be reassessed at regular intervals for its effectiveness, according to the changes in the epidemiological patterns in the region and monitoring for drug resistance.

The flow charts for syndromic treatment are available at websites of WHO and NACO (www.who.int/reproductive-health/stis/training.htm and www.nacoonline.org/prg_sche_targetint.htm)

STD and HIV

The NACO has estimated that at least 5.21 million persons have been infected by HIV by 2005. The prevalence of HIV in individual states in 2004 was tabulated (table 4.). Andhra Pradesh has the highest positivity rates in both STI cases and antenatal women. Among the high risk groups, highest positivity was seen in female sex workers in Mumbai with 44.7% positivity and 22% positivity in intravenous drug abusers in Manipur.

Table 4: NACO Prevalence of HIV positivity in 2004

	ANC	STI
Andhra Pradesh	2.25%	16.4%
Tamil Nadu	0.5%	8.4%
Delhi	0.38%	7.98%
Goa	1.13%	15.8%
Karnataka	1.25%	12.0%
Mumbai	1.12%	15.65%
Manipur	1.5%	22.0%

The natural history, manifestations and treatment of STI is altered by concurrent HIV infection in several ways as given below:

Syphilis

Clinical finding

Primary lesions	Painless ulcer becomes painful due to super infection; giant chancre
Secondary lesions	Lues maligna-secondary syphilis with vasculitis manifested by fever, malaise, headache, nodules, indurated plaques with or without hyperkeratoses and or ulceration, sclerosis

Course of the disease	Shorter latent period with rapid progression to tertiary disease within first year of infection, i.e meningovascular syphilis
Serological response to syphilis	Limited or absent antibody response to syphilis with repeatedly negative reagin and treponemal antibody testing in serum or CSF
Diagnosis	In the absence of negative serological tests dark field microscopy, biopsy of the lesion, direct fluorescent antibody staining of material from lesion may be helpful
Treatment	Greater likelihood of treatment failure, relapse without re-exposure despite adequate treatment
Chancroid	
Clinical finding	Genital ulcers tend to be larger and persist longer-Multiple inguinal buboes. Frequent occurrence of giant and phagedenic ulcer.
Treatment	Less responsive to standard therapy, 3 to 4 fold higher failure rate with single dose therapy with azythromycin and ceftrizxone
Herpes genitalis	
Clinical findings	As immunosuppression progresses lesion may persist or progress to chronic enlarged painful ulcers with raised margin, ulcer may bleed.
Treatment	Higher dose and longer period treatment with acyclovir, 400mg orally 5 times daily until complete clinical resolution or 5 to 10 mg/kg IV 8 hourly until clinical healing.
Granuloma inguinale	
Clinical findings	Lesion may be larger, extensive, pseudobubo formation which may burst producing ulceration, slow response to treatment
Treatment	Doxycycline 100mg orally bid or erythromycin 500 mg orally qid for 2-3 weeks.
LGV	
Clinical findings	Acute inflammation with bilateral inguinal bubo which may burst into ulceration
Treatment	Same regiment (doxycycline, 100mg orally bd or erythromycin, 500mg orally qid for 14 days, but prolonged therapy may be required.

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Prevalence of gram negative organisms isolated from blood culture and their antimicrobial susceptibility pattern : A five-year retrospective study from a tertiary referral hospital

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Summary

Early diagnosis and proper management of septicaemia can bring down the mortality and morbidity substantially. Current study was aimed at the bacteriological profile of septicaemia cases and their antibiotic susceptibility pattern with special reference to gram negative isolates for planning strategy and management of these cases. The aim of the study was to determine the prevalence of gram negative blood culture isolates and their susceptibility pattern to the commonly used antibiotics. From January 1998 to December 2002, total 4968 cases of clinically suspected samples for bacteremia were processed and susceptibility to commonly treated antibiotics were analyzed according to National Committee for Clinical Laboratory Standard (NCCLS) criteria. *Pseudomonas* spp. were found to be most prevalent (27%) followed by *Salmonella* Typhi (23%), E.coli (14%), *Citrobacter* spp. (12%) *Acinetobacter* spp. (11%) *Klebsiella* spp. (7%), *Proteus* spp. (3.5%) , *Enterobacter* spp. (2.5%) and *Edwrdsiella* spp. (0.1%).

A combination of cefoperazone/sulbactam was found to be the most potent antimicrobial agent. Other antibiotics like ceftriaxone, amikacin, gentamicin and ciprofloxacin were also effective compared to the other drugs tested invitro. For the effective management of bacteremia cases, study of the bacteriological profile with their antibiogram plays a significant role. If facilitates the proper treatment and prevents the possible spread of multi drug resistant bacteria.

Key-words : Gram negative bacteria, bacteremia, septicemia, drug resistance.

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INTRODUCTION

Micro organisms present in the circulating blood, whether continuously or intermittently, are a threat to every organ in the body. Microbial invasion of the blood stream can have serious implications, including shock, organ failure, disseminated intravasular coagulation and death (1). In spite of great advances in antimicrobialn therapy, life support measures and early detection of risk factors, septicaemia continues to be a major cause of mortality and morbidity among people world wide. Blood stream infections are know to be the most common infections in all age groups. A very wide spectrum of organisms has been described for the cases of septicaemia and this spectrumis subject to geographical alterations. More over the organisms isolated are ofter resistant to multiple antibiotics which makes the treatment more difficult and complicated.

For the last five years gram negative bacteria were the most common blood stream pathogens. The current incidence of gram negative bacteremia has been estimated between 70,000 to 3,30,000 cases per yers, with most estimates over 200,000(2). This represents approximately 1% to 3% of all hospitalised patients. Mortality rates among patients who are appropriately treated range from 10% to 38%. Patients who are granulocytopenic or inappropriately treated my have mortality rate that approaches 100%. More over fatalites among patients infected with gram negative bacilli are higher than those among patients who have gram positive cocci as causative agents of their bacteremia (3-6).

Early studies were made in this regard inIndia, where there was an overall predominance of gram negative organisms from blood culture (7-9). An emergence of methicillin resistant *staphylococcus aureus* and vancomycin resistant *Enterococci* in blood culture was also reported (10). Thus it is te urgent need of the hour to know te antimicrobial susceoptibility pattern of the blood isolates.

Early diagnosis and proper management of septicaemia can bring down the mortality and morbidity substantially. So our study was aimed at the bacteriological profile of the septicaemia cases and their antimicrobial sensitivity pattern with special reference to gram negative isolates for planning strategy and management of these cases.

MATERIALS AND METHODS :

In our study, from January 1998 to December 2002, specimens were taken to estimate the prevalence of the blood culture isolates with special reference to gram negative organisms and to determine the antibiogram of the isolated organisms.

The study includes 4968 cases of clinically suspected bacteremia. Blood samples (5 ml) were collected from each patient using proper aseptic precautions and inoculated immediately in to 50 ml of Brain Heart Infusion both (Hi media laboratories, Mumbai) with 0.025% sodium polyanethol sulfonate as anti coagulant. After overnight incubation at 37°C, subculture was made on to MacConkey agar and Blood agar. The subculture was repeated on 7th day if first subculture was negative. The isolate obtained was further processed as per standard procedure to identify the pathogen (11,12). Anti microbial susceptibility testing was performed by Kirby-Bauer disc diffusion method as per the NCCLS recommendations (13). The antibiotics used were

ampicillin (10μg), amoxycillin/clavulanic acid 20/10 μg), cefalexin (30μg), cefuroxime (30μg), ceftazidime (30μg), ceftriaxone (30μg), gentamicin (10μg), tobramycin (10μg), amikacin (30μg), netilmicin (30μg), ciprofloxacin (5μg), chloramphenicol (30μg), tetracycline (30μg), trimethoprim/sulfomethoxazole (1.25/23.75μg), piperacillin (100μg), carbenicillin (100μg) [Hi media] and cefoperazone/sulbactam (75/30μg), [Pfizer].

The drug used for the member of *Enterobacteriaceae* other than *Salmonella* Typhi were ampicillin, amoxycillin/clavulanic acid, cefalexin, cefuroxime, gentamicin, ciprofloxacin, tetracycline, ceftriaxone, tobramycin, amikacin, netilmicin and cefoperazone/sulbactam. For *Salmonella* Typhi, tobramycin, amikacin, netilmicin and cefoperazone/sulbactam were replaced by chloramphenicol and trimethoprim/sulfomethoxazole. For *Pseudomonas* spp. and *Acinetobacter* spp. ceftriaxone, ceftazidime, gentamicin, tobramycin, amikacin, netilmicin, ciprofloxacin, cefoperazone/sulbactam, carbenicillin and piperacillin were used. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate and susceptible according to NCCLS criteria (13).

RESULTS :

A total of 4968 samples were studied from January 1998 to December 2002 and a total of 1050 (21%) were pathogenic isolates. Out of which 487 (46%) were found to be gram positive orgtanisms which included *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Enterococcus faecalis* and *Streptococcus* spp. From 530 (50.5%) samples gram negative organisms and from 33 (3%) samples fungi (*Candida* spp.) were isolated. Gram negative organisms included mainly members of *Enterobacteriaceae* family and non fermenting gram negative bacilli.

Pseudomonas spp. was found to be most prevalent (27%) followed by *Salmonella* Typhi (23%), *E.coli* (14%), *Citrobacter* spp. (12%), *Acinetobacter* spp. (11%) *Klebsiella* spp. (7%), *Proteus* spp. (3.5%), *Enterobacter* spp. (2.5%) and *Edwardsiella* spp. (0.1%).

For *Pseudomonas* spp. combination of cefoperazone/sulbactam proved to be the most effective (82%) followed by amikacin (64%) while for *Acinetobacter* spp. ceftazidime and amikacin showed 58% sensitivity.

For *Salmonella* Typhi ceftriaxone (92%), ciprofloxacin (72%) and gentamicin (66%) were the most effective, whereas amoxycillin/clavulanic acid and cefuroxime also showed moderate activity. In other members of the *Enterobacteriaceae*, the combination of cefoperaxone/sulbactam (72%) was the most potent drug, followed by ceftriaxone (68%), amikacin (62%) and netilmicin (52%).

A total of 35 isolates [*Pseudomonas* spp. (15 nos.), *Citrobacter* spp. (1 no.)] were resistant of all the antibiotics tested invitro.

DISCUSSION :

For the effective management of bacteremia cases, study of the bacteriological profile with their antibiotic sensitivity pattern plays a significant role. In the current study a low blood culture isolation rate (21%) might be due to several reasons, e.g. administration of antibiotics before blood collection or the possibility of infection with anaerobes, which can not be ruled out and being a tertiary referral hospital, partially treated patients were usually admitted. The

rate of isolation could be improved if blood is collected after withdrawing all antibiotics for 72 hours. Similar rate of isolation have also been seen in previous studies (8).

In previous studies, gram negative bacilli constituted the majority (80%) of the total isolates and *Pseudomonas* spp. and *Klebsiella* spp. were more dominate among all followed by *E.coli* and negative bacilli (8,9).

In our study, gram negative organism formed 50.5% of the total pathogenic isolates, among them *Pseudomonas* spp. and *Salmonella* Typhi were more prevalent. Prevalence of *Salmonella* Typhi is quite unlike the previous studies conducted in this country where the prevalence has been found to be ranging from 9% to 12% (9). Frequency of the third major pathogen *E.coli* (14%) remains similar to that of the earlier studies (7,8,14). Our study shows that *Pseudomonas* spp. and *Salmonella* Typhi were dominant throughout the study period, though there was an increase of *Citrobacter* spp. and *E.coli* infection in the year 2000. High frequency of *Pseudomonas* spp. indicates nosocomial blood stream infection possibly due to interventions like prolonged vascular catheterization. Similarly, high prevalence of *Salmonella* Typhi throughout the study period indicates its endemicity. The alarming rate of prevalence is a cause of serious concern and needs public awareness regarding hygiene and sanitation.

In the current study, among the antibiotics used singly for susceptibility testing for gram negative isolates, ceftriaxone was the most effective against *Enterobacteriaceae*, whereas for nonfermenters like *Pseudomonas* spp. and *Acinetobacter* spp. amikacin was more active. However the combination of cefoperaxone/sulbactam put up for all gram negative isolates showed the highest activity among all antibiotics used for these isolates.

The present observation that ceftriaxone was most effective invitro against *Enterobacteriaceae* family has been well documented by other authors as well (8, 15-18). A similar susceptibility pattern for *Salmonella* Typhi was observed in the previous studies with high activity of ceftriaxone (88%) follwed by ciprofloxacin (79%) (19). For *Pseudomonas* spp. and *Acinetobacter* spp. higher efficacy of amikacin was evidenced by other too (20, 21).

In our study the gram negative isolates did not show high susceptibility to any single antibiotic tested in vitro. This may be due to indiscriminate use of the drugs, genetic background of the isolates and due to some environmental factors which lead to the occurrence of the resistant organism in this region. So a combination of two or more drugs is recommended to cover the broad range of possible pathogens which may be difficult to distinguish clinically. This may prevent the emergence of resistance as they may have additive or synergistic antimicrobial activity (22).

Brill reported the first case of bacteremia in 1899 (1) where *Pseudomonas aeruginosa* was the causative organism, hundred years later, the *Pseudomonas* spp. continues to be the extremely important causes of blood stream infection in this region. It is highly alarming that there is an increase in resistance of the blood isolates against commonly tested antibiotic and none of the single antibiotic could prove to be effective

if used empirically. The high frequency of drug resistance can be avoided by using drug to which most organism are susceptible. In our study cefoperazone/sulbactam, ceftriaxone gentamicin, ciprofloxacin showed to be more effective, so the combined therapy of these drugs will be good alternative to treat blood stream infections.

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Table 1. Invitro antimicrobial susceptibility of Pseudomonas spp. and Acinetobacter spp.

Antibiotic	Organisms			
	Pseudomonas spp.		*Acinetobacter spp.*	
	N*	%†	N	%
Cetftriaxone	17	12	11	19
Ceftazidime	77	54	34	58
Gentamicin	74	52	18	31
Tobramvcin	80	56	32	55
Amikacin	91	64	34	58
Netilmicin	78	55	28	48
Ciprofloxacin	70	49	26	45
Cefoperazone/sulbactam	116	82	30	52
Carbenicillin	69	48	23	39
Piperacillin	71	50	18	31

* No. of susceptible organism.
† Approximate percentage of susceptible organism.

Table 2. Invitro antimicrobial susceptibility of Enterobacteriaceae family.

Antibiotic	Organisms													
	E. coli		Klebsiella Spp.		Proteus spp.		Enterobacter spp.		Citrobacter spp.		Edwardsiella spp.		Salmonella Typhi	
	N	%	N	%	N	%	N*	%†	N	%	N	%	N	%
Amplicillin	20	27	5	14	5	28	2	15	14	21	0	–	51	47
Amoxycillin/clavulanic acid	21	28	5	14	6	33	0	–	15	23	0	–	70	57
Cefalexin	22	29	5	14	5	28	3	23	8	28	1	100	54	44
Cefuroxime	25	33	1	3	5	28	3	23	10	15	1	100	68	56
Ceftriaxone	52	69	22	61	14	78	10	77	41	64	1	100	114	92
Gentamicin	37	49	14	38	10	55	6	96	24	37	1	100	81	66
Tobramycin	32	43	21	58	12	67	10	77	27	42	1	100	–	–
Amikacin	40	53	27	75	14	78	9	69	37	58	1	100	–	–
Netilmicin	37	49	21	58	12	67	8	61	30	47	1	100	–	–
Ciprofloxacin	27	36	18	50	9	50	9	70	24	37	0	–	88	72
Tetracycline	8	10	3	8	0	–	0	–	5	8	0	–	–	–
Cefoperazone/sulbactam	–	–	32	89	16	89	11	87	29	45	1	100	–	–
Chloramphenicol	–	–	–	–	–	–	–	–	–	–	–	–	36	30
Trimethoprim/Sulfomethoxazole	–	–	–	–	–	–	–	–	–	–	–	–	50	41

*No. of susceptible organism.
=Approximate percentage of susceptible organism.

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ADDENDUM

In the article entitled "Hemophilia and allied disorders care in India : A story of dismay and success" by Dr. Dipika Mohanty, which was published in the Annals of the National Academy of Medical Sciences (India), 42(2), 147-156, 2006, the figures and tables were inadvertantly left out. These are now being included. The error is regetted.

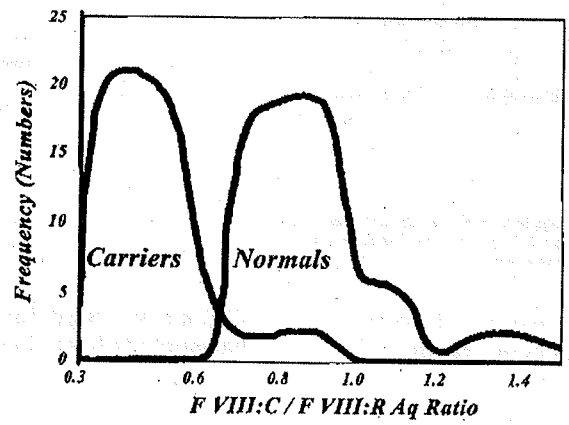


Fig. 1: Ratios of F VIII:C and VWF:Ag levels in carriers of haemophilia A and normal females.

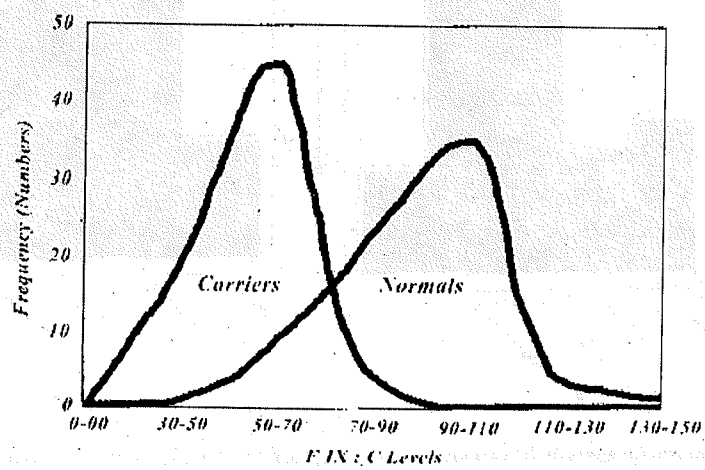
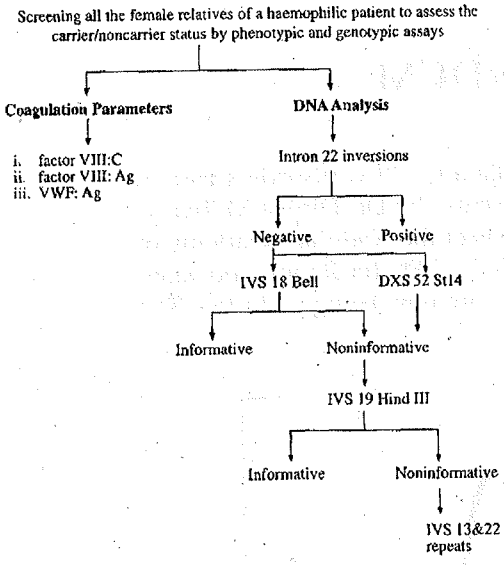


Fig. 2: F IX:C levels in carriers of haemophilia



The coagulation parameters are only supportive of genotyping techniques. The ratio of <0.7 for factor VIII:C and VWF:Ag is considered as a probable carrier of haemophilia A which would be subsequently confirmed by DNA techniques.

Fig. 3: Strategy for carrier detection in haemophilia A families at our centre.

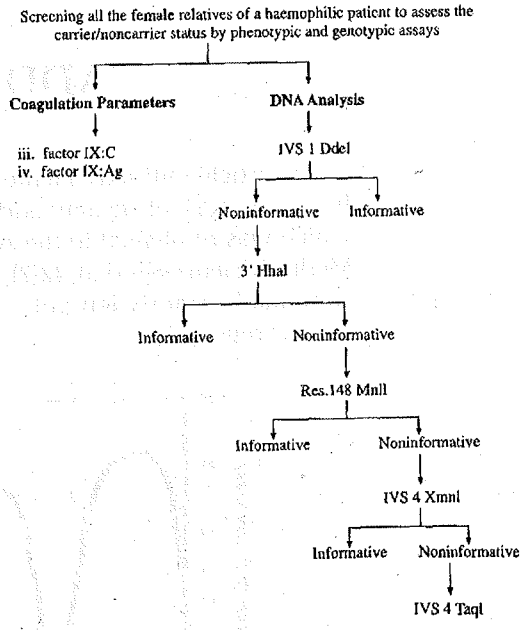


Fig. 4: Strategy for carrier detection in haemophilia B families at our centre.

Fig. 5: Informativeness of the various markers of: A) Factor VIII B) Factor IX genes used in carrier detection and antenatal diagnosis of haemophilia families.

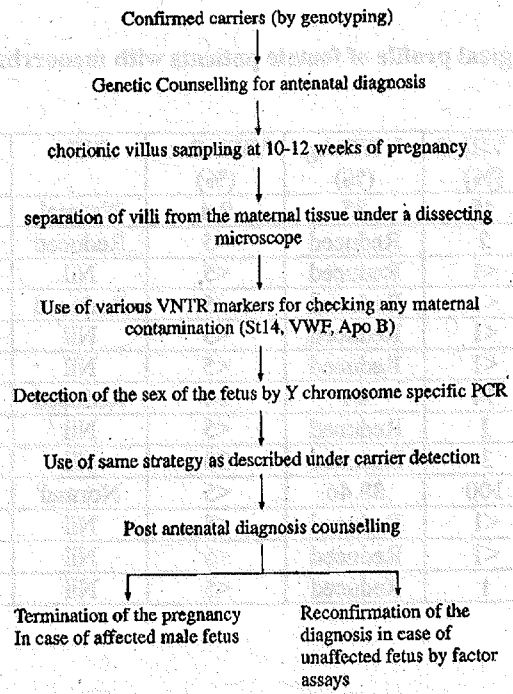


Fig. 6: Strategy for antenatal diagnosis in haemophilia families at our centre.

Table1: Spectrum of bleeding manifestations in 54 cases of VWD investigated.

Type of bleeding	Number of patients
Bleeding on trauma	43
Spontaneous bleeding	8
Epistaxis	21
Ecchymoses	18
Petechiae	4
Purpura	0
Gum bleeding	24
Bleeding post dental extraction	8
After circumcision	1
Post operative	1
Hemarthrosis	5
Hematuria	1
Hematemesis	1
Menorrhagia	8
Post delivery / umbical cord	0
GI bleeds	3

Table 2: Hematological profile of female patients with menorrhagia.

Pt.No.	BT (mins)	F VIII:C (%)	VWF:Ag (%)	VWF:Rco (%)	RIPA	Inheritance pattern
1.	8	45	85	9.4	Normal	Aut. Dom.
2.	7	2	Reduced	<5	Reduced	Aut. Rec.
3.	15	<1	Reduced	<5	Nil	Aut.Rec.
4.	11.25	<1	Reduced	<5	Reduced	Aut.Dom.
4a.	12	<1	Reduced	<5	Nil	Aut.Rec.
5.	>19	<1	Reduced	<5	Nil	Aut.Dom.
5a.	12.5	34	44	24	Reduced	Aut.Dom.
6.	>15	1	Reduced	<5	Nil	Aut.Rec.
6a.	6	3	Reduced	90	Nil	Aut.Rec.
7.	4.5	100	88.46	<5	Normal	Aut.Dom.
7a	ND	<1	Reduced	<5	Nil	Aut.Dom.
7b.	ND	<1	Reduced	<5	Nil	Aut.Dom.
8.	>15	1	Reduced	<5	Nil	Aut.Rec.

Table 3: Mean levels of F VIII:C, VWF:Ag, VWF:RCo in the three types of VWD.

Type of VWD	F VIII:C (%)	VWF: Ag (%)	VWF: RCo (%)
1	22 ± 28.14	28.83 ± 38.27	33.71 + 50.26
2	24.51 ±28.51	69.93 ± 38.25	22.18 + 33.13
3	<1%	<5%	<5

Table 4: Multimeric pattern of von Willebrand factor in various types of VWD.

Type (No. of Pts.)	1 (7)	2A (1)	2B (1)	2M (2)	2N (2)	3 (25)	Acquired VWD (1)
High Mol. Wt.	Absent	Absent	Absent	Present	Present	Absent	Absent
Intermediate	Absent	Present	Present	Present	Present	Absent	Absent
Low Mol. Wt.	Absent	Present	Present	Present	Present	Absent	Absent

Table 5: Spectrum of VWD.

Type	1	2	3	Acquired	Unclassified
%	12.96	29.62	42.59	1.85	11.11

Table 6: Allele frequencies of VWF1 and VWF2 polymorphic markers of VWF–intron40 of the VWF gene in 300 controls and 25 patients.

VWF1					VWF2				
VNTR $(ATCT)_n$	No. of chromosomes analyzed		Frequency (%)		VNTR $(ATCT)_n$	No. of chromosomes analyzed		Frequency (%)	
	C	P	C	P		C	P	C	P
14	31	2	5.17	4	8	10	0	1.67	0
13	87	4	14.5	8	7	31	1	5.17	2
12	116	15	19.33	30	6	75	7	12.5	14
11	73	4	12.17	8	5	107	8	17.83	16
10	24	0	4	0	4	140	15	23.33	30
9	14	3	2.33	6	3	105	9	17.5	18
8	112	11	18.67	22	2	76	3	12.67	6
7	128	9	28.33	18	1	56	7	9.33	14
6	15	2	2.5	4					
Heterozygosity	C = 83%				Heterozygosity	C = 81.6%			
Overall Heterozygosity = 93%.									

Key : C- Controls; P- Patients.