CONTENTS

REVIEW ARTICLE

MANAGEMENT OF PARKINSONS DISEASE : RECENT TRENDS AND CONTROVERSIES

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Summary

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by striatal dopaminergic loss with no cure available at present. Traditional drug therapy has consisted of levodopa (usually in combination with carbidopa). This drug has the disadvantage of significant motor fluctuations in the long term in the form of "on-off, "delayed on" or "no on" phenomena. These can be effectively managed by adding various other agents including MAO-B inhibitors (e.g. selegiline) and COMT inhibitors (e.g. tolcapone). Dopamine agonists have also been used as initial therapy with good results. Accurate diagnosis and individualized assessment of the risks and benefits of available antiparkinsonian medications should guide initiation of treatment for patients with early PD. In advanced cases various surgical techniques can serve as helpful adjuncts for the relief of symptoms in PD. The latest modes of medical and surgical therapy for PD are reviewed.

Introduction

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by striatal dopaminergic loss. No cure is available for this disorder at present. Recently, there has been a surge in the research regarding the pharmacological and surgical treatment of PD. The various modes of currenty applied medical and surgical treatment for PD are reviewed in this article.

Pathogenesis

At the cellular level, PD is characterized by degeneration of neuromelanin-containing dopaminergic neurons in the substantia nigra. Neuromelanin formation is the outcome of a process generally known as dopamine (DA) auto-oxidation, a chain of oxidation reactions in which highly neurotoxic DA-quinones are produced. The level of these DA-quinones, as estimated by the occurrence of their cysteinyl conjugates, is reported to be increased in the Parkinsonian substantia nigra.[1] In Parkinson's disease, loss of dopaminergic cells in the substantia nigra leads to striatal dopamine depletion. Because dopamine activates excitatory D1 receptors in the direct pathway and represses inhibitory D2 receptors in the indirect pathway, this depletion results in decreased activity of the direct pathway and increased activity of the indirect pathway and so in reduced thalamic excitation of the motor cortex .

Medical treatment

Current drug therapy can neither reverse nor completely halt the neurodegenerative process that is responsible for PD. Therapy is principally focused on controlling or improvement of functional impairment and consideration of neuroprotection to prevent or at least retard the underlying neurodegenerative process.

Drugs available

Anticholinergic agents: Nonselective muscarinic antagonists are sometimes helpful, especially in relieving tremor and rigidity. They do not affect bradykinesia.[2] Various preparations are available, including trihexyphenidyl, benztropine, procyclidine, and orphenadrine. Treatment is started with a small dose and gradually increased until benefit occurs or side effects limit further increment.

Amantadine: either alone or combined with an anticholinergic agent, is sometimes helpful for mild parkinsonism; it acts by potentiating the release of endogenous dopamine. It may improve all major clinical features of the disorder and has relatively uncommon side effects (restlessness, confusion, skin rashes, oedema, disturbances of cardiac rhythm). However, many patients derive only transient, if any, benefit from it.[3]

Levodopa: Levodopa, the metabolic precursor of dopamine, provides symptomatic benefit in most patients with parkinsonism and is often particularly helpful in relieving bradykinesia. This drug is administered routinely in combination with a peripheral dopa-decarboxylase inhibitor e.g. carbidopa.[3] There was initially concern that the early introduction of levodopa might accelerate the death of nigrostriatal neurons because of a hypothetical increase in dopamine-mediated neurotoxicity. It is

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now clear that levodopa should be introduced as soon as is warranted by the patient's clinical state, rather than postponed out of concern for this theoretical possibility.[4] Important late complications of levodopa therapy are the wearing-off effect (transient deterioration shortly before the next dose is due) and the "on-off' phenomenon (abrupt but transient fluctuations in clinical state that occur frequently during the day, without warning or an obvious relationship to dosing schedule, resulting in alternating periods of marked akinesia or greater mobility accompanied by iatrogenic dyskinesias). Other types of fluctuations also occur, especially the "delayed on"(increased time latencies from dose intake to turning "on") and "no on"(complete failure of levodopa dose to induce an "on" response)[5].

Monoamine oxidase type B (MAO-B) inhibitors: Selegiline is a selective inhibitor of monoamine oxidase B. Some studies have suggested that it may reduce oxidative damage and thus slow disease progression in PD, but the evidence for this effect is incomplete. Palhagen et al suggested using selegiline as initial monotherapy and then in combination with levodopa in the early phase of PD. They found that selegiline delayed significantly the need to start levodopa in early PD[6], Myllyla et al conducted a long term double blind study using selegiline as the primary treatment of Parkinson disease. They concluded that selegiline therapy offered beneficial long-term effects.[7] Przuntek et al carried out a 5-year study with selegiline and levodopa in combination. The early combination of selegiline and levodopa proved to be clearly superior to levodopa monotherapy.[8] But the satety of thies therapy has been questioned. The Parkinson's Disease Research Group of the United Kingdom (PDRG-UK) reported increased mortality in PD patients treated with levodopa plus selegiline compared with those treated with levodopa alone.[9] Many others, however, challenged this finding. In a meta-analysis Olanow et al reported that there was no increase in mortality associated with selegiline treatment whether or not patients also received levodopa.[10] Although theoretical reasons exist for believing that selegiline slows the progression of Parkinson's disease, this has not been shown in clinical trials. Selegiline improves the symptoms of Parkinson's disease, allowing the introduction of levodopa to be delayed in de novo patients and, later, for levodopa to be used at a lower dose. It does not lessen the long-term problems of dyskinesias and fluctuations associated with levodopa therapy. The report of an increased mortality associated with selegiline therapy awaits further evaluation.

Dopamine agonists : Dopamine agonists include ergot derivatives such as bromocriptine, lisuride, pergolide, and cabergoline and other agents, which do not possess the ergot structure such as pramipexole and ropinirole. They all are powerful stimulators of the D2 dopamine receptor, which probably underlies their therapeutic effects. They are usually prescribed in combination with levodopa when late side effects begin to occur. Results of recent wellperformed, modern clinical trials show that early use of the new dopamine agonists is able to effectively control the clinical symptoms for more than 3 years thereby offering the possibility of delaying the occurrence of levodopa-induced late motor side effects.[11,12] In previously untreated parkinsonian patients at an early stage of the disease, three comparative trials have shown that, during the first six months of treatment, the effect of ropinirole on motor symptoms almost equates to that of levodopa and is a little better than that of bromocriptine monotherapy.[13] This view is supported by Korczyn et al who found that (a) in the absence of selegiline, ropinirole is effective and superior to bromocriptine; and (b) selegiline does not affect the response in patients treated with ropinirole, but enhances the effects of bromocriptine.[14] Rascol and his colleagues reported that early Parkinson's disease can be managed successful for up to five years with a reduced risk of dyskinesia by initiating treatment with ropinirole alone and supplementing it with levodopa if necessary.[15] Printer and co-workers reported that pramipexole administration is an efficacious and well tolerated add on therapy in patients with advanced Parkinson's disease with an improvement in activities of daily living, motor function, and treatment associated complications.[16] Cabergoline appears to be effective in improving moderate motor fluctuations[17]. Apomorphine represents a significant advance in the treatment of well developed motor fluctuations in selected patients who are able to master the technique of subcutaneous[18] or intranasal administration.[19]

Selective catechol-O-methyltransferase (COMT) Inhibitors: COMT inhibitors are a new therapeutic option in the treatment of patients with Parkinson's disease. COMT inhibitors act by extending the duration of action of levodopa, thus improving the amount of time a patient can experience benefit from levodopa.[20] COMT inhibitors are only used in conjunction with levodopa. They do have a propensity to augment dopaminergic effects, such that levodopa doses might need to be adjusted downward. Other side effects of COMT inhibitors include diarrhoea and liver function abnormalities. Virtually all instances of liver enzyme abnormality and clinical liver

dysfunction seem to occur within 6 months of initiating treatment.[21] To assess the current role of tolcapone therapy in Parkinson disease, a panel of neurologists and hepatologists was convened. Consensus was reached with respect to the following: (1) Tolcapone is an effective agent in the treatment of patients with fluctuating Parkinson disease. (2) The risk of developing irreversible liver injury is negligible with appropriate monitoring. (3) It may be possible to reduce the frequency of monitoring after 6 months of treatment. (4) The requirement that tolcapone be withdrawn if liver enzymes are elevated above the upper limit of normal on a single occasion is unnecessarily restrictive. It was concluded that tolcapone, when used as an adjunct to levodopa, is an effective anti- parkinsonian agent and that less frequent monitoring after 6 months, with an action limit of 2 to 3 times the upper limit of normal, is sufficient to ensure safety in patients who are deriving benefit from the drugs.[21] Tolcapone further improves levodopa response when combined with a dual release formulation of levodopa/beneserazide.[22]

Other prospective agents: Among the promising agents that are under active investigations are glutamate antagonists, and GM I ganglioside and various neurotrophic factors. In the setting of nigrostriatal dopamine depletion, glutamatergic pathways to the striatum and basal ganglia output nuclei become overactive. Glutamate receptor antagonists may have direct antiparkinsonian actions and they may also potentiate conventional dopaminergic therapies. A role for glutamate antagonists is supported by animal and early clinical data, although the poor therapeutic index associated with the currently available nonselective, noncompetitive glutamate antagonists has prompted a search for more selective antagonists with less toxicity.[23] Steece-Collier and co-workers[24] recently demonstrated that a subunit-selective glutamate receptor antagonist has direct antiparkinsonian actions in both rodents and monkeys and it synergistically potentiates levodopa in MPTR-treated monkeys. Clinical evaluation of this agent in Parkinson's disease may be warranted. GM I ganglioside and various neurotrophic factors influence dopaminergic nigrostriatal cells, and work is in progress to develop delivery systems that will permit their use in the treatment of PD. [23]

Choice of drug(s):

In general, the goals of treatment for younger patients (less than age 60 years) are control of impairing symptoms, sparing of levodopa to minimize long-term complications, and consideration of neuroprotection.[15] The primary initial medication choices for patients under age 50 years include selegiline, amantadine, and anticholinergic agents. Patients in there fifties may require a dopamine agonist in addition to or instead of selegiline to achieve adequate symptom control. If the desired response is still not achieved, sustained-release carbidopa- levodopa should be added, followed by adjunctive amantadine or anticholinergic therapy.[26] For older patients (60 years and over), improvement of functional impairment is the primary goal. For these patients, a special concern is to avoid inducing or exacerbating cognitive impairment. Sustained-release is considered first-line treatment for these patients. A trial of immediate-release carbidopa-levodopa and then addition of a dopamine aganist can handle inadequate response when maximum levodopa doses are reached. Anticholinergic agents, amantadine, and selegiline should be avoided because of their CNS effects.[26]

Initiation of therapy :

For over two decades controversy has surrounded the initial choice of therapeutic agent for patients with early symptomatic PD. Whether levodopa or dopamine receptor agonist monotherapy in these patients is more efficacious and/or results in fewer long-term complications of dopaminergic therapy such as motor fluctuations, dyskinesias, or psychiatric disorders is unresolved. At this time, there is little evidence to support levodopa-sparing strategies or to suggest that levodopa is toxic and harmful to patients with Parkinson's disease. Weiner strongly recommends levodopa as the initial drug of choice.[27] There are argues that prolonged levodopa therapy may enhance disease process. On the other hand more than 50% of the patients develop motor flactuations.[5] Considering the occurrence of side effects with long-term levodopa therapy, Montastruc et al suggested that treatment of PD should begin with a dopamine agonist.[11,28]

Management of motor fluctuations:

More than 50% of patients with PD develop response fluctuations following prolonged treatment with levodopa.[5] Some are due to central pharmacodynamic mechanisms such as reduced striatal synthesis and storage of dopamine from exogenous levodopa and subsensitization of post synaptic dopaminergic receptors. Other fluctuations, especially the "delayed on" and "no on" are caused by peripheral pharmacokinetic mechanisms. Patients with PD, especially those with response fluctuations, have gastric atony. The reduced motility of the stomach,

combined with the poor solubility of levodopa, is the cause for the delayed and incomplete absorption of levodopa. Central pharmaco- mechanisms can be overcome and daily "on" hours increased by using either dopamine agonists, controlled release preparations of MAO-B/COMT inhibitors. Therapeutic strategies that improve levodopa absorption are needed to overcome response fluctuations that are caused by peripheral mechanisms. This can be achieved by crushing levodopa and drinking it as a suspension. Administration of crushed levodopa or levodopa/carbidopa/ascorbic acid solutions orally or through gastroduodenal or gastrojejunostomy tubes may also be helpful. Prokinetic drugs, such as prepulsid, improve absorption of levodopa by enhancing gastric motility. By passing the stomach by dopamine agonists (subcutaneous[29] or intranasal[19] apomorphine and lisuride[30] pumps) or by the novel prodrug of levodopa, i.e., levodopa ethylester, may produce dramatic rescue from incapacitating "off" states.

Surgical Treatment

Because of limited long-term efficacy of medical treatment for PD and because of advances in technology and in our understanding of the function of basal ganglia[31], certain patients with advanced PD being treated surgically. The surgical treatment modalities have been stereotaxic implantations of dopamine-producing tissues into the caudate nucleus and ventral pallidotomy of patients with PD. The implantation of cells genetically modified to express trophic factors and tyrosine hydroxylase for the synthesis of L-dopa from tyrosine has been proposed as a possible route for the treatment of Parkinson's disease.

One of the drawbacks with foetal grafts in PD is the limited outgrowth into the host striatum. In order to enhance graft outgrowth, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor-beta-I (TGF beta 1) are administered by implantation of bioactive rods. Transplantation of autologous adrenal medullary tissue has also been attempted for Parkinson's disease, with mixed results; benefit seems most likely to occur in individuals younger than 50 years of age. Another potential source of autologous dopaminergic cells is the carotid body.[32,33]

GDNF is a pleiotropic neurotrophic factor which stimulates the dopaminergic phenotype in vitro and in vivo by way of activation of the pharmacologic profile of GDNF in two well characterized animal models of PD suggests that the molecule may be useful in the treatment of neurodegenerative diseases involving dopaminergic dysfunction such as Parkinson's disease.[34]

Encapsulation of cells within polymer membranes prior to transplantation provides a novel means of achieving continuous, site specific delivery of therapeutic molecules to the CNS. The use of encapsulated dopamine secreting cells that can be transplanted directly into the striatum has particular appeal for the treatment of Parkinson's disease.[35]

Destructive neurosurgical procedures were used for some years to treat parkinsonism, but their use declined with the advent of levodopa. Unilateral posteroventral pallidotomy or thalamotomy has recently been resurrected as a therapeutic approach for relieving rigidity, bradykinesia, and tremor in patients with advanced disease in whom antiparkinsonian medication is ineffective or poorly tolerated. Samuel and colleagues[36] demonstrated that levodopa-induced dyskinesias are dramatically reduced following ventral medial pallidotomy and, according to them, constitute the principal indication for pallidotomy. Improvements in underlying parkinsonism were of smaller magnitude. Pallidotomy may also offer some patients an opportunity to increase antiparkinsonian medication. Jennifer et al[37] followed up a group of patients with advanced PD who had undergone unilateral posteroventral medial pallidotomy and observed that significant early improvements in off period contralateral signs of parkinsonism were sustained for up to 5½ years. There was a sustained significant improvement in on-period contralateral dyskinesia but not in other on-period signs of parkinsonism.

There is however universal agreement that surgical treatment is to be reserved for advanced PD only when there is no alternative. This view has recently been reinforced by Amano and Takakura[38] who presented their clinical experience with the use of pergolide in 55 patients with PD. Low-dose pergolide together with L-carbidopa was effective in the treatment of parkinsonism without side effects, even in advanced stages of the disease. No patients required surgical treatment.

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SEX CHROMOSOME ANOMALY : 48XXYY MALE; A RARE VARIETY OF KLINEFELTER'S SYNDROME

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Summary

We describe the chromosomal pattern of a clinically suspected Klinefelter's syndrome. The patient was 27 years of age and visited his physician because of inability to perform sexual act during married life. Clinically he has a tall man with mild gynaecomastia and normal penis; but bilateral smaller tests. Follicle stimulating (FSH) and leutinizing (LH) hormones showed high level while testosterone was low. Buccal mucosal cell analysis for Barr-Body was found negative. A diagnosis of rare variety of 48XXYY Klinefelter's syndrome was made on the basis of chromosomal analysis.

Introduction

Klinefelter's syndrome is a condition resulting from sex chromosomal aneuploidy. In 1942 Klinefelter et al[1] described the syndrome which is characterized phenotypically by gynaecomastia, testicular atrophy with azoospermia but without atrophy of Leydig cells and increased excretion of FSH. Jacobs and Strong[2] demonstrated the karyotype to be 47XXY in 1959. The 47XYY constitution was first observed in 1961 by Sandberg et al[3] in a man of normal intelligence. In 80% of cases the 47XXY karyotype is homogenous. It corresponds to the 'classic' Klinefelter's syndrome. In remaining 20%, other chromosomal variants are observed of which the most frequent being 48XXXY. Among different mosaic pattern, 47XXY/46XY, 47XXY/46XX, 47XX/46XY/45X, 47XXY/46XY/46XX have been reported. The 48XXYY is least frequent and was reported for the first time by Muldal and Ockey[4] in 1960 who designated it as 'double male'. Because of the association of the phenotypic characteristics of Klinefelter's syndrome with those of 47XXY individuals, the 48XXYY constitute a very special group.

Case Note :

A young male of 27 years came to Endocrine outpatient department of BIRDEM Hospital with the complaints of inability to perform sexual act during married life and difficulty to visualize distant object clearly for last three years. He got married at the age of 26 year but divorced at second year of conjugal life. The patient's height was 197.6cm; upper segment 84.6 cm and lower segment 113 cm. His arm span was 212 cm. The patient had no complaints of anosmia. On examination of genital organs – stressed penile length (SPL) was 17 cm, right testis having the volume of 1 ml and left testis 2 ml. Grade of pubic hair was stage-III. The breast of the patient was at the stage–II of gynaecomastia. The patient has neither chest wall deformity nor had hypertension.

Fig.-1 : *Tall young male and cunuchoid habitus.*

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Serum testosterone level was low (3.41 nmol/L; normal range 8.71-36.47 nmol/L) but serum FSH (72.32 miu/ml; normal range 1-12 miu/ml) and LH (65.48 miu/ml; normal range 2-12 miu/ml) were higher than normal. By conventional karyotyping and Giemsa banding, he was found to be a patient of phenotypically Klinefelter's syndrome with 48XXYY genotype. Barr-Body investigation from the buccal mucosal cells was negative for the patients.

He had been treated with Inj. Sustanon 1 amp. IM every weekly for 4 weekly for 4 weeks then 1 amp. IM every 2 weeks and 1 amp. IM every 1 month continuously. He used to visit the Endocrine outpatient department of BIRDEM hospital at regular interval.

Regarding family history, one male sibling is leading normal life but refused to marry for unknown reasons. According to patient's statement, his father used to take steroid inj. (inj. Decadurabolin) after the birth of his 4th son for sexual weakness. Neither his father nor his brother agreed for karyotype investigation.

Fig.-3 : *Normal penis, microtestes and PH stage-III*

Fig.-4 : *Chromosomal pattern of 48XXYY male*

Fig.-2 : *Stage-II gynaecomastia*

Discussion

48XXY karyotype has been reported earlier by other investigators, but little is known about the frequency of this genetic variant. In 1959, Jacobs and her colleagues[2] found that 12 of 197 inmates of a security institution for mental defectives had a chromosome anomaly. Seven of the 12 had a 47 XXY chromosome constitution, one had 48XXYY and another 47XXY/46XY mosaic. They also noted that height of these subjects was more than 6 feet. Casey et al[5] extended these studies and found that 21(2.2%) of 942 male patients for mental defectives were chromatin positive. Twelve of the 21 were found to have a 47XXY chromosome constitutin and 7 had 48 XXYY. Most of the XXYY individuals were usually tall. Taylor and Moores[6] found one such case in 23,200 male infants. Clinically these patients do not differ greatly from those with 47XXY Klinefelter's syndrome. The major difference between groups appear to be in stature. XXYY males tend to be taller than XXY male (as do XYY) and increase in stature is caused primarily by an increase in leg length[7].

Patients with Klinefeter's syndrome usually have gynaecomastia, testicular atrophy and sterility. The disease is most often diagnosed at the time of puberty.Gynaecomastia can appear around the age of 12-13 years. Testicular atrophy contrasts with the normal development of the penis and an otherwise normal puberty. The testicles are small, soft and often insensitive to pressure. The scrotum is normal in size and pigmentation. Usually morphology of such subjects is variable and not always a necessary diagnostic criteria. Indeed, while some subjects are longilinear, with long limbs and a gynaecoid appearance, others have a normal masculine morphology, sometimes even being of small size. Before puberty, hormonal excretions are not modified. An increase in the rate of excretion of FSH can be observed at puberty. Clinical suspicion is based only on sterility of the patient and confirmed subsequently with karyotype.

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THE ROLE OF ULTRASOUND AIDED FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) IN THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA (HCC)

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Summary

Sixty patients with suspected hepatocellular carcinoma (HCC) were included in the study. Their mean (±SD) age was 51.57 + 3.39 years, with a range of 27 to 84 years. Mean age of male patients were 53.55±2.33 years (range 27 to 84 years) and female patients were 49.94±7.32 years (range 36 to 72 years). The difference of age between two sexes were not statistically significant (P>0.05). There were 48 (80%) males and 12 (20%) females. Male and female ration was 4:1. 93.33% patients presented with short duration of illness (6 months or less) might be lack of awareness or aggressive biological behavior off HCC. Alpha-fetoprotein (AFP) was less than 10 ng/dl in 41.66% patients and greater than 500 ng/dl in 30% patients with sensitivity 58.33%. Ultrasound findings, were 55% cases had solitary space occupying lesion, 33.33% cases multiple lesions and 11.66% cases diffuse lesions. Ultrasound aided fine needle aspiration cytology (FNAC) results of 60 patients with HCC, 80% patients were true positive, 16.66% were negative and 3.33% were suspected HCC. The result shown sensitivity 92.3%, specificity 87.5%, positive predictive value 97.95% and overall accuracy 91.6%. Histopathological features shown 33.33% patients were highly well differentiated carcinoma, 22.91 % were moderately well differentiated and 43.75% were poorly well differentiated. All FNAs yielded sufficient materials for smears. No patients experience any complication. So FNAC is a simple. safe, rapid, minimally invasive, and excellent diagnostic procedure for HCC with highly sensitivity, specificity and accuracy. Negative cytological findings require subsequent percutaneous liver biopsy for further diagnosis of HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world[1] and represents about 4% of all malignancies worldwide.[2] The geographical prevalence varies considerably in different countries due to different risk factors.[3] Imaging techniques such as dynamics ultrasound[4] or computed tomography[5] provide high resolution sectional images of focal liver lesions. It is not possible with a clinical acceptable degree of accuracy to distinguish between benign and malignant lesions with any of these imaging modalities, for exact diagnosis a cytological or histological examination is necessary. There is an increase trend toward therapy basing on cytologic diagnosis.[6]

Confirmation of the diagnosis of HCC was traditionally been by coarse needle (e.g. Menghim or True cut) liver biopsy. The complications of coarse needle liver biopsy include pain, intraperitoneal haemorrhage, bile peritonitis, haemobilia and even death have been reported.[7] There is however, abundant evidence that cytology of material obtained by fine needle aspiration (FNA) is adequate for the assessment of focal hepatic lesion, especially HCC.[8,9] Fine needle aspiration cytology (FNAC) impart some practical significance to clinical practice especially in clinical management.[9] The diagnostic yield have been reported to be from 84 to 95%,[9,10] when combined with imaging modality such as ultrasound, computed tomography and MRI imaging or fluroscopy, the diagnostic yield has been reported from 85% to 100%.[11,12] The liver is easily demonstrated sonographically and lesions as small as 2 cm can be imaged, give a complete description of the tumour and its position, hence more accurate placement of the needle tip in the target lesions. FNAC is a safe, simple, rapid, minimally invasive, most accurate and sensitive procedure in the diagnosis of HCC. Negative cytological findings require subsequent percutaneous liver biopsy for further diagnosis of HCC. So the aim of this study was to evaluate the role of ultrasound aided fine needle aspiration cytology (FNAC) in the diagnosis of Hepatocellular carcinoma (HCC).

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Materials and Methods

Sixty patients with suspected hepatocellular carcinoma (HCC) were included in the study. This study was carried out from admitted patients in the Department of Gastrointestinal, Liver and Pancreatic Diseases in Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the period July 1988 to June 2000.

Informed consent was taken from each patient before entering in the study and the patients were selected randomly. Clinical diagnosis of hepatocellular carcinoma (HCC) was made on the basis of findings of a detailed history and physical examination. Complete blood count including erythrocyte sedimentation rate and biochemical tests for liver function such as serum bilirubin, ALT, alkaline phosphatase, serum total protein and albumin, prothrombin time were done in each patient. These patients then screened for space occupying lesions in the liver by ultrasound and measurement of alphafeto protein (AFP). Viral markers such as HbsAg and anti HCV were tested in all patients. Ultrasound aided fine needle aspiration cytology (FNAC) was done in every patient to confirm the diagnosis of HCC. In those with a negative result by FNAC, percutaneous liver biopsy was done. Patients were excluded from the study: i) patients with primary malignancies at other sites, ii) patients with severe coagulopathy not corrected by injection of vit K, iii) patients with a history of excess alcohol consumption, iv) female patients who were pregnant, v) patients known to have acquire immunodeficiency syndrome (AIDS) or seropositive for human immunodeficiency virus (HIV), vi) patients with a history of long term drug intake such as androgenic steroid, vinyl chloride, arsenic or exposure to thorotrast and hereditary conditions such as haemochromatosis, alpha-I-antitrypsin deficiency, hereditary tyrosinaemia etc.

Statistical analysis of data

All data were analyzed by the help of SPSS software program (Version 6.0) and expressed mean ±SD. Comparison between the groups were done by student's t test. Diagnostic tests (e.g. sensitivity, specificity, predictive value and over all accuracy) were done by standard statistical methods.

Results

A total of 60 patients were studied. There were 48(80%) males and 12(20%) females. Male and female ratio was 4:1. The mean (±SD) age of the patients was 51.57±3.39 years with a range of 27 to 84 years. The males had a mean (±SD) age 53.55±2.33 years (range 27 to 84 years) and females had 49 ±7.43 years (range of 36 to 72 years) (Table-1). The difference of age between two sexes were not statistically significant (P>0.05).

Table- I : *Age distribution (n=60)*

Age in years	No. of patients	Percentage
20-30	02	3.33
31-40	16	26.66
41-50	12	20.00
51-60	18	30.00
61-70	7	11.66
71-80	4	6.66
81-90	01	1.66

All patients had clinical features compatible with HCC i.e. wasting with hard nodular hepatomegaly. Ultrasonography and alpha-feto protein (AFP) were done in all cases as a screening investigation. Ultrasound findings in 60 patients, 55% had solitary space occupying lesion, 33.33% had multiple lesions and 11.66% patients had diffuse lesions. 65% patients were mixed echopattern, 25% were hypoechoic and 10% were hyperechoic (Table-II).

Table- II : *Showing findings in ultrasonography (n=60).*

Findings	No. of patients	Percentage
Diffuse	7	11.66
Multiple	20	33.33
Solitary	33	55.00
Hyperechoic	6	10.00
Hypoechoic	15	25.00
Mixed	39	65.00

AFP was measured in 60 patients. 41.66% patients had less than 20 ng/dl, 28.33% had 20-500 ng/dl. 20% had 500 to 1000 ng/dl and 10% had greater than 1000 ng/dl (Table-III) which have been considered by others to be highly suggestive of HCC.[8]

Table- III : *Showing alpha -fetoprotein (AFP) level in HCC (n=60)*

Range in ng/ml	No. of patients	Percentage
<20	25	41.66
20-500	17	28.33
500-1000	12	20.00
>1000	6	10.00

Cytological diagnosis was done by ultrasound aided fine needle aspiration cytology (FNAC) in 60 patients with HCC. 48(80%) patients were confirmed HCC, two (3.33%) were suspected HCC, and 10(16.66%) were negative (Table-IV), with sensitivity 92.30%, specificity 87.5%, predictive value (positive) 97.95%, and overall accuracy 91.6%. Histopathological features shown 33.33% patients were highly well differentiated and 22.91 % were moderately well differentiated and 43.75% were poorly well differentiated.

Table- IV : *Showing FNAC results of 60 patients with HCC (n=60).*

Results	No. of patients	Percentage
Positive	48	80
Negative	10	16.66
Suspected HCC	2	3.33

Twelve patients underwent liver biopsy, of which seven (11.66%) patients were true negative, one (1.66%) patient was false positive and four (6.66%) patients were false negative.

True negative patients were two metastatic adenocarcinoma, two macronodular cirrhosis, one chronic parenchymal liver disease and one hepatic lymphoma and one hepatocellular adenoma (Table-V).

Table-V : *True negative patients results (n=7).*

Results	No. of patients	Percentage
Metastatic adenocarcinoma	2	3.33
Macronodular cirrhosis	2	3.33
Chronic parenchymal liver disease	1	1.66
Hepatic lymphoma	1	1.66
Benign lesion (Adenoma)	1	1.66

All FNAs yielded sufficient materials for smears following three or less passes. Two patients experienced dizziness soon after FNA with no objective fall in blood pressure or obvious bleeding. No other complications were noted.

Discussion

Hepatocellular carcinoma is the most common primary malignant hepatic neoplasm[2] and is the fourth most common cause of death worldwide.[14] This is a dreadful disease which is mostly preventable.[15] Modern imaging techniques have improved the diagnosis at early stage. Fine needle aspiration cytology (FNAC) is an easy, safe and excellent diagnostic method for cytological diagnosis of Hepatocellular carcinoma (HCC) and impart some practical significance to clinical practice, especially in clinical management.[10,16,17]

In this study, mean (+SD) age of the patients was 51.57±3.39 years with a range of 27 to 84 years. The mean (+SD) age of male was 53.55±2.33 years (range 27 to 84 years) and female was 49.94±7.43 years (range 36 to 72 years). The difference of age between two sexes were not statistically significant (P>0.05). In great Brillant where incidence of HCC is low, the peak age is between 74 to 84 years whereas in developing countries it is the tumour[18] of younger people where it occurs between 34 to 44 years.

In our study, 30(50%) patients were under 50 years of age, 48(80%) patients were male and 12(20%) patients were female. Male and female ratio was 4:1. This figure is consistent with male predominance all over the world. It is not clear whether male predominance of HCC reflects increased susceptibility of men to the tumour or their greater exposure to environmental risk factors (i.e. HBV infection, smoking and alcohol drinking) for HCC.[19] In this study shown, 93.33% patients presented with short duration of illness (6 months or less). This might be lack of awareness or aggressive biological behavior of HCC in Bangladesh that needs further large scale study.

AFP levels greater than 500 ng/ml are found in about 70 to 80 percent of patients with HCC and in serum greater than 1000 ng/ml or progressive rising levels are highly diagnostic marker of HCC, with relatively high specificity and sensitivity and has been used expensively in the clinical setting.[20] In this study, 30% patients shown AFP greater than 500 ng/dl with sensitivity 58.33%.

Ultrasound is the most practical, cheap, useful, noninvasive and primary diagnostic method of choice for the detecting focal lesions within the liver. It can detect space occupying lesion even less than 3 cm in diameter with sensitivity 90-92% and specificity of 93%.[21] However with smaller or more discrete lesions ultrasound guidance is essential to target abnormal areas of the liver for accurate needle tip puncture. In this study, 50% patients had solitary space occupying lesion, 33.33% had multiple lesions and 11.66% had diffuse lesions. Sixty five percent patients had mixed echopattern, 25% patients had hypoechoic and 10% had hyperechoic.

Ultrasound directed fine needle aspiration (FNA) has been shown elsewhere to be a safe, highly sensitive and specific procedure for differentiating HCC and benign lesions,[22-24] with a sensitivity of 93 to 95 percent and a specificity of 87 to 100 percent.[25,26] In this study ultrasound aided fine needle aspiration cytology (FNAC) was done in 60 patients presented with HCC. 48(80%) patients were true positive, two (3.33%) patients were suspected HCC and 10(16.66%) patients were negative with a sensitivity 92.3%, specificity 87.5%, positive predictive value 97.95% and overall accuracy 91.6%. Histopathological features shown 33.33% patients were highly well differentiated carcinoma, 22.91% were moderately well differentiated and 43.75% were poorly well differentiated. All FNAs yielded sufficient materials for smears following three or less passes. Ultrasound aided fine needle aspiration cytology (FNAC) is a very simple, safe, rapid, inexpensive, minimally invasive most accurate diagnostic method for cytological diagnosis of HCC.

Tsou MH et al[27] studied 88 HCC patients, FNA diagnosis was made in 84 cases with an accuracy of 96.6%, sensitivity 93.3% and specificity of 100%. Pintu et al[28] did FNAB and described sensitivity was 91 % and specificity was 100%. Bru et al[29] described this procedure with 69% sensitivity and 93% specificity. Bret et al[30] shown sensitivity of 84%. Fornari F et al[31] ultrasound aided FNAC was done in 481 patients with a sensitivity of 93.2%, specificity of 100% and overall accuracy of 95%.

The advantage of ultrasound aided FNAC is an easy, safe and reliable technique is to enable a doctor in a peripheral hospital, who may not posses the training or resources to perform liver biopsy, or to deal its complications to be able to confirm the diagnosis of HCC without having to send patients over long distances to referral centres.

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TRIAL OF RECOMBINANT INTERFERON ALPHA-2A IN CHRONIC VIRAL HEPATITIS B INFECTION IN BANGLADESHI PATIENTS

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Summary

Hepatitis B virus is a DNA virus causing either acute or chronic hepatitis which may lead to cirrhosis and carcinoma. The incidence of HBV infection is reported to be high in Bangladesh. Until recently treatment of this infection was disappointing. At present interferon is the most promising and only established therapy. In Bangladesh, two small studies done so far revealed a high response rate. This study was designed to include a larger no of patients and to see the response rate in our population.

This was an open trial of chronic HBV infected patients with HBeAg antigenaemia. This study comprised of a total number of 20 patients studied in the department of gastroenterology, IPGM&R, Dhaka from April '96 to January '98. There were 17 males and 03 females with age ranging from 15-65 years. All the patients were thoroughly assessed clinically, biochemically, serologically and in some cases histologically. All the patients were treated with 4.5 MU of recombinant interferon alpha- 2a given intramuscularly thrice in a week for 6 months. The patients were followed monthly for 6 months during therapy. Only 03 patients (15%) had lost HbeAg and none (00%) had lost HbsAg after completion of therapy at 6 month.

Introduction

Hepatitis B virus is a DNA virus causing either acute or chronic hepatitis. Chronic hepatitis may lead to liver cirrhosis and hepatocellular carcinoma with considerable morbidity and mortality[1].

WHO estimates that 2000 million people have been infected by HBV world wide. Of these, some 350 millions are chronically infected of whom 25% are at risk of serious illness and eventual death from cirrhosis and hepatocellular carcinoma[2].

Asia is a hyperendemic area for HBV infection and in Bangladesh it is also reported to be high. In consideration of high prevalence and fatal consequences the prevention and treatment of this disease is of high priority world wide. HVB vaccine can prevent HBV infection but until recently treatment of this disease was disappointing. At present interferon is the most promising and the only established therapy. Although interferon is effective, but it is far from absolute. The response to interferon also varies according to patients ethnic origin and Asian especially Chinese tend to have low response and there are also pretreatment variables e.g., age, sex etc. influencing the response.[3,4]

In Bangladesh, two small studies done so far revealed a high response rate of 57% and 55% for HbeAg clearance and 7% and 9% for HbsAg clearance respectively at the end of therapy at six months.[5-6]

As the treatment is costly and not universally effective - cost-effectiveness of this highly expensive therapy is a critical factor to be considered in the perspective of our poor socioeconomic status. This study was designed to include a larger number of patients and to see the response of interferon in our population.

Materials and methods

This study was carried out in the department of Gastroenterology, IPGM&R Dhaka from the period of April 1996 to January 1998. Any patient of chronic hepatitis B of either sex had been considered for enrollment into this study. A total of 20 (twenty) patients were selected after proper screening and the inclusion criteria were-

- All patients have to be Bangladeshi with age range between 15 and 65 years.
- Must be positive for HbeAg
- Must be able and likely to attend regularly for follow up and treatment.
- Patients were included irrespective of their ALT levels.

Patients were excluded from the study if there were

- any evidence of decompensated liver disease either clinical or laboratory.
- history of bleeding oesophageal varix.

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- thrombocytopenia, leukopenia etc.
- any psychiatric illness
- history of interferon therapy in the past and
- pregnancy in case of female.

Patients fulfilling the entry criteria have been admitted in gastroenterology unit. Detail general medical history, including history of hepatitis and concomitant diseases and medications were recorded. Complete physical examination along with all required laboratory investigations were done in all patients.

Detail discussion about the cost effectiveness, side-effects, importance of regular injection and follow up were done with all patients and legal guardians. Only properly motivated patients were selected for this study. Initial few injections were given in hospital and the patients were advised to report monthly or immediately if any adverse events occur.

Injection Roferon-A, a preparation of recombinant interferon alpha-2a, 4.5 MU thrice in a week intramuscularly was given for six months.

All patients were seen routinely in every month for any adverse events.

Following laboratory investigations were done monthly

- Hb%, ESR, TC, DC of WBC
- Total platelet count
- Serum ALT level.

After the end of therapy at six month along with the above serum HbsAg and HbeAg were done.

All the data were processed and analyzed by using computer based SPSS programme. Only P value < 0.05% was considered significant.

Result

After proper screening a total of 20 patients were included in this study. Out of 20 patients 17 were males and 03 were females. Twelve (12) patients were within age range of 15- 30 years (60%), 03 patients were within age range of 31- 50 years (15%) and 05 patients were more than 50 years of age (25%). (Table-I).

Table - I : *Age and sex distribution of patients*

Age (Years)	No.	%	Sex	N0.	%
15 -30	12	60	Male	17	85
31- 50	03	15	Female	03	15%
> 50	05	25	-	-	-

Way of clinical detection of chronic hepatitis B infection :

Majority of the cases were detected during routine check up, mostly for vaccination or overseas employment. Some cases were detected when they were investigated for some clinical problems, e.g., upper gastrointestinal bleeding, anorexia, weight loss and other cases were due to persistence of the viruses following acute hepatitis. Out of 20 cases, 14 (70%) were detected during routine screening, 01 (05%) were detected during investigation for upper gastrointestinal bleeding, 03(15°%) cases were the sequelae of acute viral hepatitis and 02 (10%) were detected to have chronic hepatitis B virus infection as they were suffering from anorexia, weight loss, weakness, fatigue etc. (Table-2).

Table-II : *Way of detection of chronic HBV infection*

Way of detection	No.	Percentage
Routine screening	14	70
Persistence of acute infection	03	15
Anorexia, weight loss, weakness etc.	02	10
Upper gastrointestinal bleeding	01	05

Duration of infection :

The exact duration of infection could not be ascertained accurately in most of the cases, as exact time of introduction of infection was not possible to decide. Here, the duration of infection was recorded from the day of first detection of infection. Most of the cases were detected within one (01) year prior to therapy. Out of 20 cases 15 (75%) cases were detected within 12 months. Five (25%) cases were detected for more than 2 years prior to therapy (Table-III).

Table-III : *Duration of infection prior to therapy*

Duration	No.	Percentage
01 month – 12 months	14	70
12 months – 24 months	01	05
> 24 months	05	25

Response to interferon :

Out of 20 patients, only 03 (15%) patients lost HbeAg and none became negative for HbsAg after completion of therapy at six months. So complete response was not seen in any patients. Seroconversion to anti-Hbe was not seen at that stage (Table-IV).

Table-IV : *Rate of response*

Markers	Negative	Positive	Percentage of response
HbeAg	03	17	15%
HbsAg	00	20	00%

Sex distribution of responders :
All the three patients responded to interferon were male, none was female. So 100% of the responders were males.

Table-V : *Sex distribution of responders*

Sex	No.	Percentage
Male	03	100
Female	00	00

Pretreatment ALT values and its changes in different groups of patients :

The median ALT values were seen in both responders and non-responders. The pretreatment ALT values and values on every months of therapy were seen and compared. The serial ALT values are graphically shown for two groups of patients, revealing different types of curves. (Fig.-1,2).

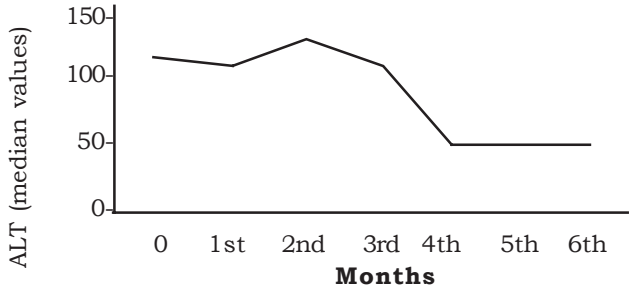

Fig.-1 : *ALT values changes in responders group*

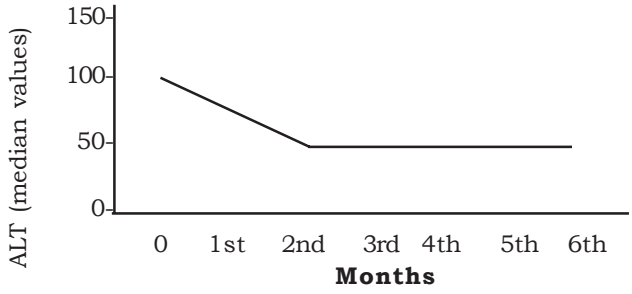

Fig.-2 : *ALT values changes in non-responders group*

The pretreatment median ALT values was little higher in responders than in non-responders, 115U/l versus 105U/L, so also are the other values in the initial 04 months of treatment, but no values is found to be significant when statistical analysis is done by Mann-Whitney U test. (Table-VI).

Table-VI : *Value* changes in different groups*

Time	Responders (n=3)	Non-responders (n=17)	P-value
Pretreatment	115 (69, 136)	105 (32, 235)	0.711
1st month	108 (68, 359)	84 (31, 146)	0.189
2nd months	123 (66, 151)	61 (11, 286)	0.264
3rd months	99 (99, 99)	59 (29, 342)	0.414
4th months	105 (41, 169)	60 (32, 381)	1.0
5th months	39 (39, 39)	61 (25, 371)	.329
6th months	38 (39, 38)	63 (37, 238)	.158

+ P-value <.05 is significant (M-W U test)
* Values are median values and range

Discussion

Viral hepatitis is the commonest liver disease occurring sporadically round the year. Of the viral hepatitis, hepatitis B remains a global problem and most prevalent also. Asia is a hyperendemic area (8% or more) for HBV infection. It is estimated that Chinese account for about 80% of the world HBV carriers.[2]

Bangladesh is a small developing country in South Asia where virus related liver diseases are important causes of morbidity and mortality The prevalence of HBV infection is found to be high in Bangladesh.[7-16] In one study 7.2% of healthy individuals[15], 7.5% of healthy job seekers[17], and 19- 20% of professional blood donors were found to have HBV infection.[18] In another study 9.7% of healthy persons, 18.8% of blood donors and 15% of day labourers were found to be infected.[19] Cirrhosis of liver comprises 2.6% of our hospital admission and in one study 61% of the cirrhosis of liver were found to be due to HBV infection[16].

In consideration of high prevalence and fatal consequences, the prevention and treatment of this disease is of high priority. HBV vaccine can prevent HBV infection, but treatment of this infection is still disappointing. At present, interferon (INF) is the most promising single agent for this chronic viral hepatitis.

It is observed that response to INF varies according to patients ethnic origin and Asian patients specially Chinese patients tend to have reduced response.[3,4]

The exact rate of response to INF in Bangladeshi patients is not known, as no large controlled study has been done so far. A study done by M Hasan et al.[5] on 14 patients revealed that, 08 patients (about 57%) became negative for HbeAg and 01 (about 07%) become negative for HbsAg at the end of therapy and all remained negative during the follow-up time of 7.4 months. One patient was withdrawn from the trial because of side effects and no other had any significant adverse reaction . Another study by M Khan et al. on 11 patients revealed that 06 patients (about 55%) became negative for HbeAg and 01 (09%) became negative for HbsAg on completion of therapy.[6]

In this study, a total of 20 patients were included of which 17 were male and 03 were female. So, most of the patients (85%) were male. Male female ratio was 5.6:1. In a study by M. Khan et al, 10 patients were male out of 11 and similar number was also found in study by M. Hasan et al.[5,6] Similar observation is found in most of the Western studies and studies in China.[4,20-22]

The small number of female patients may be due to many factors. Due to social and religious ground females are less exposed to screening test, at the same time less privileged to receive treatment in hospital. So the disparity in sex prevalence in this study does not reflect the true picture of general population. However, males are six times more likely to become carriers than females.

Age of the patients in this study ranges from 15-65 years, of which 60% are in the range of 15-30 years. In most of the studies in home and abroad, the age ranges are almost similar.[5,6,20-22] The reason why most of the patients are young may be due to the fact that this is the productive period of life and mostly cared for.

Most of the patients (70%) in this series were detected by routine screening for vaccination or overseas employment. History of acute viral hepatitis with persistence of infection was found in 15% of the patients. This is also consistent with general clinicopathological behaviour of the virus and the liver disease produced by it. The patients with subclinical or mild acute infections are more likely to progress to chronicity than those with severe symptoms and more than two-thirds of chronic hepatitis B patients have never experienced symptoms.[23] Fifteen percent (15%) of the patients had history of acute viral hepatitis is also consistent with the general behaviour of the virus. About 10% of the acute infection go on to develop chronicity.[2] In one meta analysis of 10 controlled trials about 27% had found to have history of acute viral hepatitis[20].

Fifteen percent (15%) of the patients lost HbeAg and none (00%) lost HbsAg at the end of therapy. M. Hasan et al[5] found a response rate of 57% and 7%, whereas M. Khan et al.[6] found 55% and 9% respectively. This great discrepancy may be due to small no of cases in all series, non homogeneous nature of the population and selection criteria and incidental selection of cases with long duration of infection. In Indian study, HBV-DNA and HbeAg clearance was found in 50% in contrast to 4.8% ($P<.05$) in control at the end of 6 months[24]. In studies performed on Chinese patients, the response varied from 00% to around 20%.[4,25] The studies performed in other countries showed loss of HbeAg in 19- 42% of the patients and loss of HbsAg in 00-20% of the patients.[26,27]

The observation in this study is in conformity with the fact that Asian and Chinese patients are less responsive to interferon but differs greatly from previous observations in our country and our neighbouring country, India.

Pre-treatment ALT level and ALT level changes after treatment showed higher pre-treatment value in responders than in non-responders, although this is not significant statistically ($p=.711$). The ALT change curve of the responders although not typical of usual response, but is simulating that. On the other hand, the curve in the nonresponders is totally different (Fig.- 1,2). However, ten percent (10%) patients may not show typical changes.[22]

Conclusion

Hepatitis B virus infection is the main etiological factor for chronic liver diseases and is a common clinical burden in Bangladesh. The treatment of this infection is far from absolute and the only therapy established till today is interferon. There are reports of variability of response to interferon in these patients in different population groups and Asian ethnics are proposed to be less responsive. At the same time the drug is very costly and toxic. This study was undertaken to find out the response of our patients with chronic hepatitis B infection to interferon. This was an open trial of chronic HBV infected patients with HbeAg antigenaernia. The study comprised of a total number of 20 patients, studied in the department of gastroenterology, IPGM&R, Dhaka from April '96 to January '98. There were 17 males and 03 females with age range from 15-65 years.

The patients were thoroughly assessed, appropriate laboratory tests, viral serology, biochemical liver function tests were done in all patients and fiver biopsy was done in some patients for histological diagnosis. All patients were treated with 4.5 MU of interferon alpha-2a given intramuscularly, thrice in a week for 6 months. 'Me patients were followed at monthly interval for 6 months during therapy.

Three (15%) patients had lost HbeAg and none had lost HbsAg after completion of therapy. The study shows that the response of chronic HBV infected patients in Bangladesh is less than those reported from Western countries, but similar to those reported from China. However, response rate is also less than those seen in other studies done previously in Bangladesh.

Over past 10 years a wealth of clinical trials have established the usefulness of this family of cytokines in the treatment of chronic hepatitis B and its role is beyond question at present. However, the drug is not universally effective, moreover it is very costly and toxic.

So. considering all these disadvantages, the therapy to be cost-effective and precise, the following recommendations are offered

—A well organized controlled study with a large number of patients to find out the exact rate of response to eliminate the dilemma still prevailing in this aspect.

— Controlled studies of different variables influencing the response of interferon, so that the patients with the probability of high response rate could be selected to make the treatment cost-effective in the perspective of our poor economic status.

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ACUTE UPPER GASTROINTESTINAL BLEEDING – AN ENDOSOCPIC STUDY

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Summary

Acute upper gastrointestinal (GI) bleeding is one of the common emergency admission to the hospital in our country. Correct diagnosis is very important for proper management. Upper GI endoscopy has an important role in this situation. We studied 135 patients (male –115, female-20) admitted in SSMC and Mitford Hospital, Dhaka with a diagnosis of haematemesis and /or melaena. Endoscopy was performed between 24 to 72 hours of hospital admission. Age range of the patients were between 10 to 70 years. Most of the patients were between 20 to 40 years of age. Upper GI endoscopy revealed duodenal ulcer disease in 59 (43%), gastric ulcer in 18 (13%), oesophageal varix in 17 (12.5%), gastric erosions in 12 (9%), carcinoma stomach in 6 (4.5%) and duodenal and oesophageal varix in 2 (1.5%) patients. No causes were found in 14 (10%) of cases. Duodenal ulcer disease was found to be the leading cause of upper GI bleeding in this series followed by gastric ulcer, oesophageal varix and gastric erosions. History of nonsteroidal anti-inflammatory drugs (NSAIDs) was present in 22 cases. Carcinoma of the stomach was uncommon causes of upper GI bleeding found in this study. Emergency upper GI endoscopy is a rapid, safe and accurate method of diagnostic procedure for patients with acute upper GI bleeding.

Introduction

Peptic ulcer is the most common cause of acute upper gastrointestinal bleeding that accounts for about half of the patients. Patients bleed more commonly from duodenal ulcer than gastric ulcer.[1-8] More than 100,000 patients are estimated to bleed from peptic ulcer in the United States each year.[9] The use of Non-steroidal anti-inflammatory drugs (NSAIDs) is probably the most important risk factor identified for the development of bleeding in patients with peptic ulcer disease.[10-13] Endoscopy should be performed as soon as the patient is haemodynamically stable and adequate support personnel are present to ensure proper monitoring. Most studies comparing fibreoptic endoscopy and barium radiography in patients with acute upper gastrointestinal bleeding shows that endoscopy provides a substantial higher diagnostic yield.[14]

In the developed countries mortality from bleeding ulcer remained constant at 6 to 8 percent over the past three decades despite medical and technologic advances.

Acute upper gastrointestinal haemorrhage in the form of haematemesis and meleana is the common emergency admission to the hospital and causes significant number of deaths in our country. The aim of our study is to find out the aetiology, clinical pattern and to assess the usefulness of upper GI endoscopy in the diagnosis of acute upper GI bleeding.

Materials and Methods

One hundred thirty five patients age ranged from 11 to 70 years were studied. One hundred fifteen cases were male and 20 cases were female (Table-I-II).

Patients were referred from the Medical Ward of SSMC and Mitford Hospital with a diagnosis of haematemesis and melaena. These patients were clinically assesed by recording pulse, blood pressure, temperature, respiratory rate, pallorness, nacked eye examination of stool and vomitus. After ressucitation patients were subjected to some routine investigations : Hb%, blood count, blood grouping, liver function test in suspected liver disease patients, serum urea, creatinine, electrolyte, HbsAg. Those who were HbsAg positive were excluded. The upper GI endoscopy was performed in the morning after about 12 hrs fasting without sedation within 24 to 72 hours of hospital admission. No complication was noted in any patients.

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Results

The total number of patients and their sex and age range are shown in Table I-III.

Table-I : *Total number of patients with upper GI bleeding*

	Number	Percentage
Total patients	135	100
Male	115	85
Female	20	15

Table-II : *Age range of the patients*

Age (years)	No. of patients	Percentage
11-20	16	12
21-30	38	28
31-40	40	30
41-50	23	17
51-60	15	11
> 60	3	2

Presenting symptoms are shown in Table-III

Table-III

Symptoms	No. of patients	Percentage
Upper abdominal pain	98	73
Haematemesis and melaena	82	61
Melaena	32	24
Haematemesis	21	16

Endoscopic findings of the study subjects are shown in table-IV. Most common diseases diagnosed endoscopically were duodenal ulcer disease (43%) followed by gastric ulcer (13%), rupture oesophageal varix (12.5) and gastric erosions (9%).

Table-IV : *Endosocpic diagnosis in patients with upper GI bleeding.*

Name of the disease	No. of patients	Percentage
Duodenal ulcer (DU)	59	43
Gastric ulcer (GU)	18	13
Oesophageal varix (OV)	17	12
Erosive gastritis	12	9
Bleeding disorders	6	4.5
Carcinoma stomach	6	4.5
DU and GU	2	1.5
GU and O. varix	2	1.5
No causes identified	14	10

Nonsteroidal anti-inflammatory drugs (NSAIDs) induce upper GI bleeding was found in 22 (16%) cases. 66 (49%) patients were smoker and 4 (3%) patients were alcoholic (Table-V).

Table-V : *Predisposing factors related with upper GI bleeding*

	No. of patients	Percentage
Cigarette smoking	66	49
NSAIDs	22	16
Alcohol ingestion	4	3

Discussion

Peptic ulcer accounts for about 50% of all patients who have upper GI bleeding. Patients more commonly bleed from duodenal ulcer than from gastric ulcers-a two fold or greater difference in most series.[1-8] Early and accurate diagnosis is essential for a better prognosis. Upper GI endoscopy has an important role in this condition. It has been shown that upper GI endoscopy is the initial investigation of choice in acute upper GI bleeding which provides a higher diagnostic accuracy than barium radiography in different series.[15,16] The aetiology, clinical pattern and the role of endoscopy in the diagnosis and management of upper GI bleeding has not yet been well studied in this country.

In the present study 135 patients were included of which 115 patients were male and 20 were female. Most of the patients (58%) presented within 21-40 years of age. Sixty one percent of patients were presented with haematemesis and melaena, 24% of patients with melaena and 16% of patients with haematemesis which is consistent with other study.[17]

In this series causes of acute upper GI bleeding was identified in 90% of cases with the help of upper GI endoscopy. Similar result was also found in other studies.[15,16] In 14 (10%) patients no cause was identified possibly due to delayed endoscopy.

Peptic ulcers was found in 53% of patients in the present study which is almost similar with other studies.[1-8,19] Among peptic ulcers, duodenal ulcer was the commonest cause which was found in 44% of patients and gastric ulcer in 13% of patients. Other causes identified endoscopically includes oesophageal varix 12%, erosive gastritis 9%, carcinoma stomach 4.5%, bleeding disorders 4.5%.

Nonsteroidal anti-inflammatory drugs induced acute upper GI bleeding was found in 22 (16%) of patients which is consistent with other studies.[18,19]

In conclusion, duodeanl ulcer disease is the most common cause of acute upper gastrointestinal bleeding and gastric erosions and ruptured oesophageal varices are the next two common causes in our country. NSAIDs is an important risk factor for bleeding ulcers. Bleeding disorders should also be kept in mind specially during the epidemics of dengue fever in our country. Emergency upper GI endoscopy is a rapid, safe and accurate method of diagnosis.

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GALL STONE DISEASE IN A RURAL BANGLADESHI COMMUNITY

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Abstract

Background : The prevalence of gall stone disease is not known in Bangladesh. The aim of this study was to assess the prevalence and associated factors of gall stone disease in a rural community in Bangladesh. Methods: A population sample of 1332 persons of ages above 15 years was invited to participate in the screening study. Of these, 1058 subjects responded after tertiary invitation giving a response rate of 80%. Each subject was asked to answer a questionnaire and underwent an upper abdominal ultrasound examination. Results: Gall stone disease (current cholelithiasis and history of cholecystectomy) was detected in 5.4% of subjects. The prevalence was higher in females (7.7%) than in males (3.3%) (P=0.002). From age group <30 years to age group > 50 years the prevalence rates increased from 0.9% to 10% (P= 0.0124). Majority (71.9%) of the subjects with gall stone disease were asymptomatic. Out of 52 obese subjects 25 (48.1%) had gall stone disease in contrast to 3.2% (1006/32) of subjects in the non-obese group (P=0.000). Six subjects (1.5%) in the lower socioeconomic class; 5.7% in the middle class and 13.4% of subjects in the higher class group had gall stone disease (P= 0.000). Occupation was negatively associated with gall stone disease. Conclusion: Results of the present study shows a definite relationship between age, sex, obesity economy and an increase prevalence of gall stone disease. But occupation do not show a definite relationship with galls tone disease.

Introduction

Data about the prevalence of Gall Stone Diseases (GSD) around the world are discrepant. In recent years, sonographically based epidemiologic studies investigating the prevalence of GSD have been reported for a number of nations[1-4]. The introduction of real time diagnostic ultrasond has provided a simple, noninvasive method for the diagnosis of GSD, which with a sensitivity of upto 95.5% makes the performance of prevalence studies on large population[5] feasible.

The prevalence of GSD varies in different parts of the world. The prevalence rate in Sweden is 15%[6], in Mexico 14.5%[7]; whereas in Italy it is 5.9%[8]. Among the Asian countries Taiwan continues to have the highest prevalence. 20% may be the average figure for the whole country[9]; whereas the prevalence rates in Japan and Thailand are 3.2% and 3.5% respectively[10-11]. In India the prevalence rate is (0-8.1%) in males and (2-29.1%) in females[4]. Available data suggests that the incidence of GSD has been rising sharply in recent decades[12,13] and it is not yet known whether a plateau has been reached.

Much has been learned in recent years about the factors associated with gall stone (GS) formation, however, the reasons for the rising incidence of gall stone remains unknown. Epidemiological data suggests that environmental factors, diet in particular may be important[14]. Certain factors like, age, sex, obesity and symptoms has been studied extensively but the relationship of socioeconomic condition and occupation with GSD have not been studied so much.

In clinical practice, GSD is also common in Bangladesh. Surprisingly, no study has yet been performed to know its prevalence in Bangladesh. This study was initiated to find out the prevalence of GSD in a defined rural population and to identify its relation with age, sex, obesity, occupation, socioeconomic condition and symptoms.

Materials and Methods

Study population: Two villages apparently typical of Bangladesh, about 4 km from Khulna city were selected for the study. The area can be reached by foot or rickshaw and consists of a settled agricultural community. All the subjects of 15 years and above were included in the study. Prior consent was taken

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from each subject before the screening procedure. Hospitalized patients were excluded. Two volunteers were employed to bring the subjects. Subjects who failed to attend after three attempts were excluded.

Methods

Ultrasonography (USG) was used as the screening procedure. Two experienced sonologists were involved in USG examination. Before the study, both the sonologists were cross-checked in a double blind manner in examining 20 subjects and the results were similar. With the subjects in the fasting state, scanning was done by a scanner equipped with a 3.5 MHz transducer (Aloka SSD 1100, Aloka Co. Ltd, Japan) in supine and erect positions. The examination was carried out in three planes; longitudinally, diagonally and in cross-section. Five to ten subjects were examined daily. In doubtful cases, the scan was repeated after further preparation. Criteria for the diagnosis of GSD were: (1) one or more echogenic structures in the gall bladder lumen with distal shadow; (2) one or more echogenic structures in the gall bladder without distal shadow, which on the basis of examination in several planes or attempted mobilization, could be differentiated from a gall bladder septum, the valve of Heister or a Polyp; (3) a strongly echogenic structure in the region of the gall bladder with distal shadow in cases of limited or no visualization of the gall bladder lumen and (4) history of cholecystectomy for GSD and absence of gall bladder in USG examination.

A preformed questionnaire was filled in before USG examination by a trained interviewer. The questionnaire included name, age, sex, occupation, height, weight, socio-economic condition, history of biliary colic and history of cholecystectomy for GSD. Only billiary colic was considered as symptom of GSD. All cholecystectomized patients were symptomatic.

Obesity was defined as a body mass index (BMI) of 25 kg/m2 or greater. Socio-economic condition was assessed according to percapita income per month and stratified into high (>US$ 72.7/month), middle (US$ 36.5-72.7/month) and low socioeconomic group (<US$ 36.5/month).

The study was carried out between September 1994 to September 1995.

Statistical analysis: The univariate data analysis was performed using the Pearson Chi-Square test with continuity correction and Fisher's exact test. To account for associations between the independent variables, a multiple logistic regression analysis was carried out.

Results

Subjects: Total number of subjects of ages above 15 years were 1332. Of these, 1058 subjects could be examined with a response rate of 80%. Five hundred and forty four (51.4%) were males and 514 (48.6%) were females. Male, female ratio was 1.05:1.

Age: The subjects were divided into three groups: < 30 years, (31-50) years and > 50 years. Majority of the subjects were in the age group of (31-50) years (Table-I). The number of subjects with GSD increased with age. Four subjects (0.9%) had GSD in group < 30 years, 42 (8%) in 31-50 years group and 11 (10%) in > 50 years group (Table-I), and the difference is significant between all groups (P = 0.000). Logistic regression analysis also confirmed this (P = 0.0124).

Table-I : *Relationship of GSD with age, sex, obesity and economy.*

	GSD				
	Present (n = 57)		Absent (n = 1001)		
Variables	No.	%	No.	%	P*
Age (Year)					
< 30	4	0.9	422	99.1	
(30-50)	42	8	481	92	
> 50	11	10	98	90	0.0124
Sex					
Males	18	3.3	526	96.7	
Females	39	7.7	475	92.3	0.002
Obesity					
Obese	25	48.1	'27	51.9	
Non-obese	32	3.2	974	96.8	0.000
Economy					
Lower class	6	1.5	373	98.5	
Middle class	30	5.7	498	94.3	
Higher class	21	13.4	130	86.6	0.000

·Multivariate Logistic regression analysis.

Prevalence : GSD [current gall stones (45) and history of cholecystectomy (12)] was present in 57 (5.4%) subjects. The frequency in female was 7.7% (514/39) and in male 3.3% (544/18) (Table-1). Thus the prevalence was 2.3 times greater in females than in males (P= 0.002). Logistic regression analysis also revealed significant difference (P= 0.009). Out of 57 subjects with GSD, 36 (63.1%) had multiple and 21 (36.9%) had single stones.

Obesity : Out of 52 obese subjects 25 (48.1%) had GSD and out of 1006 non-obese subjects, 32 (3.2%) had GSD (Table-I). The differences are statistically significant (P= 0.000). Logistic regression analysis also showed obesity as an independent risk factor (P= 0.000). The frequency of GSD in female obese subjects was 59.4% (32/19) and in non-obese female was 4.1% (482/20). The differennce is statistically significant (Chi = 45.6, P = < 0.001). The frequency in male obese subjects was 30% (20/6) and in non obese subjects was 2% (524/12). The difference is statistically significant (Chi = 136.7, P < 0.001).

Symptoms: Sixteen (28.1%) subjects were symptomatic and 41 (71.9%) were asymptomatic. No significant difference was observed regarding symptoms between males and females (P= 0.548). Increased number of subjects (36/13) in the multiple stones group were found to be symptomatic than in the single stone group (21/3) [P= 0.000].

Economy :

Six subjects (1,5%) in the low socioeconomic group had GSD. On the contrary, 30 (5.7%) and 21 (13.9%) subjects in the middle and higher class groups respectively had GSD (Table-I). The difference is statistically significant (P= 0.000). Logistic regression analysis also showed that economy is an independent risk factor for GSD.

Occupation:

Table II shows the relationship of GSD with occupation. Logistic regression analysis failed to confirm occupation as an independent risk factor.

Table- II : *No of subjects with GSD in relation to occupation.*

Stone	Occupation						Total
	Serv	Cult	H/W	Busi	St	Dep	
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	
Present	15 (5.9)	5 (2.0)	32 (7.11)	5 (8.2)	0 (0)	0 (0)	57(5.4)
Absent	240(94.1)	249 (98)	417 (92.9)	56 (9.8)	19 (100)	20 (100)	1001 (94.6)
Total	255 (100)	254 (100)	449 (100)	61 (100)	19 (100)	20 (100)	1058 (100)

P = 0.039 (Chi-Square test)

Serv- Service, Cult- Cultivator, H/W- House-wife, Busi- Businessman, St-Student, Dep- Dependant.

Discussion

The prevalence of GSD in this study was 5.4%. This result is higher than that found in some Asian countries like Japan,[10] Thailand,[11] close to data reported for Germany,[15] and Italy,[8] and much lower than that of France,[16] Mexico,[3] Norway[18] and Sweeden.[6] Among the Asian countries, Taiwan continues to have the higher prevalence (20%).[9] This difference in the prevalences may be due, on the hand, to ethnic and geographical differences as shown in the study by Diehl et al[17] and on the other, to difference in age distribution of the subjects. Very high and very low prevalence of GSD have been reported for certain ethnic groups.

Prevalence of GSD in our study population increases from 1.4% in subjects (15-30) years old to 8.1% in those above 40 years of age. This trend (P = 0.000) has been reported for all sonographic surveys.[1-4,18-20] This is also confirmed in our study by logistic regression analysis (p = 0.01). A lack of correlation between increased prevalence of GSD and age has been described by Maclure in a sonographic follow up study on 88837 health workers.[21] However, Maclure conducted the study only on middle aged women.

We found a higher overall prevalence in women (7.6%) than in men (3.3%). The difference is statistically significant (P-0.002). Logistic regression analysis also showed the presence of a significant positive association been sex and GSD (P= 0.004). Our result is in agreement with other published data.[2,6,,8,18,22-23] A few studies however did not confirm this data[24-25]. In the present study the authors observed a positive association between increased BMI and prevalence of GSD, which proved statistically significant even at logistic regression analysis (P<0.000). This observation is supported by the data of other European sonographic surveys.[26-29] However, some non-European studies did not confirm the association between obesity and GSD.[4,10,30-31]

The contradictory findings of various studies may be interpreted as evidence that over-weight may especially play an important etiologic role in the formation of GS with high cholesterol content, which represents the most common stone type in Europe,

but are seldom seen in Asian populations. The association of obesity and the increased risk of biliary concretions may be due to a relative decrease in the secretion of phospholipids and bile salts as compared with cholesterol in bile of obese persons.[32] Relative decreases in phospholipids and bile salts increases the lithogenic index of bile which may promote the precipitation and crystalization of cholesterol, providing a nidus for GS formation.

Most patients with GSD are asymptomatic, as were 71.9% of our patients. These data are similar to previous European studies.[1,2,19,33,34] Carmen et al[35] observed a greater tendency of GS to cause symptoms in women than in men. However, no sex difference was observed regarding symptoms in our study (P0.548). Sixty three percent of patients had multiple stones and 37% had single stones. This result is in agreement with other studies[36,37]. Increased number of patients with multiple stones were symptomatic than that of patients with single stone. The difference is statistically significant (P= .000).

Significantly increased number of patients in the high socioeconomic group had GS (P= .000) Logistic regression analysis also confirmed this. Possible explanation of this result may be that the subjects of this group in general usually take much fatty diet than the low income group. Some studies[20,38] have found positive correlation between serum triglyceride and low HDL cholesterol level and increased risk of GSD. Persons in this group do also less physical activity. Moderate physical activity (both occupational and non-occupational) reduces the risk of GSD[39]. However, studies in Danish population showed no significant association between the level physical activity and GSD[40].

No valid conclusion can be drawn from this study regarding the relationship of occupation with GSD. We did not study the relationship of dietary and environmental factors with GSD.

In conclusion, whether factors, such as socioeconomic condition, occupation, diet and environmental factors play a role in the etiology of GSD remains to be answered in the frame work of future larger studies.

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A CONTROLLED CLINICAL STUDY OF CIPROFLOXACIN IN THE RETREATMENT CASES OF TUBERCULOSIS

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Summary :

A total of seventy four patiens who fulfilled the inclusion and exclusion criteria were enrolled for the study, of which 38 belonged to group A (3HRZECS/9HREC) and 36 belonged to group B (2HRZES/1HRZE/5HRE). Among them 70 (94.6%) patients, 35 from each group i.e. 92.1% from group A and 97.2% from group B completed the total retreatment of 12 months and 8 months respectively and total 4 (5.4%) patients were defaulters, of which 3 (7.9%) and 1 (2.8%) were from group A and group B respectively. Out of 70 patients who completed the retreatment 64 (91.4%) patients i.e. 33 (94.3%) from group A and 31 (86.6%) from group B were cured. The difference between the groups was not significant (x^2 = 0.729 and p = 0.637).

Introduction :

The problem of tuberculosis is worldwide and is a major health problem in Bangladesh. Irregular and inadequate treatment is the most common cause for the development of drug resistance. Drug resistance transforms a curable disease into a life threatening condition. The number of drugs effective against tuberculosis is limited. Moreover, second line antituberculosis drugs are less effective, more expensive, not easily available, must be used for prolonged period and may cause serious adverse effects.[1,2] Considering the increasing prevalence of drug resistance and shortcomings of the existing drugs WHO gives high priority for surveillance of drug resistance, development of new drugs and screening antituberculosis activity of the existing antimicrobial drugs.[3] Although, tuberculosis is a major health problem in Bangladesh, its management was not well organized until a few years back.[4] Management of tuberculosis is a difficult problem particularly in the developing countries, like ours. The difficulties include need for multiple drugs, which are costly and have to be continued for a pretty long time. The major reasons for premature stoppage of drugs are poor briefing before the commencement of therapy, financial constraints, apparent clinical improvement, illiteracy, ignorance and unavailability of some drugs in remote areas[5]. So far known, there is no study involving ciprofloxacin in the retreatment of tuberculosis has yet been done in Bangladesh. Therefore, this study was planned and designed to observe the efficacy and safety of ciprofloxacin and at the same time to observe and compare the efficacy and safety and the defaulter rate between the two regimens of group A: 3HRZECS/ 9HREC and group B: 2HRZES/1HRZE/5HRE.

Materials and Methods

Place and period of study: Patients were collected from medicine out patient department (MOPD) of Bangabandhu Sheikh Mujib Medical University (BSMMU) Shahbagh Dhaka. The study was carried out from January, 1998 to March, 2000.

Selection criteria:

Patients presenting with the clinical features suggestive of tuberculosis and having history of previous treatment with antituberculosis drugs for at least one month or more were included as follows:

1. Relapsed case: Patients previously successfully completed treatment of tuberculosis with subsequent appearance of symptoms and / or patients who became smear positive again after having been treated for tuberculosis and declared cured after the completion of their treatment.

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2. Defaulters: Patients previously treated for tuberculosis for more than one month but discontinued or interrupted the treatment for more than two months for any reason e.g symptomatic improvement, adverse effects of drugs, poverty or ignorance but returned with clinical features of tuberculosis or bacteriologically confirmed tuberculosis.

3 Treatment failure: Patients previously treated with antituberculosis drugs for two months or more with no improvement or initial improvement followed by deterioration or patients who began treatment for smear positive tuberculosis and who remained or became smear positive again at five months or later during the course of treatment.

Diagnosis of tuberculosis was established with at least one of the following criteria:

1. Bacteriological confirmation i.e positive smear for AFB or positive culture for mycobacterium tuberculosis.

2. Clinical diagnosis of tuberculosis supported by investigations eg suggestive radiological changes in chest, positive tuberculin test, suggestive histological or fine needle aspiration cytology (FNAC) findings in lymph node.

Exclusion criteria:

1. Age less than 15 years.

2. Pregnancy.

3. Patients suffering from diabetes mellitus, renal disease, liver disease or any other acute and / or serious illness or any debilitated condition.

Study design and drug regimens :

After preliminary selection each and every patient was explained elaborately about the seriousness and the importance of the disease and the necessity of so costly retreatment with so many drugs for so prolonged a period. Patients who convincingly agreed to undergo such a retreatment were finally selected for retreatment and enrolled in a randomized fashion strictly following a random sampling numbers table. If the selected patients corresponded to the odd number in the random table they were enrolled in group A but if the selected patients corresponded to the even number in the random table they were enrolled in group B. Of course, the first number was selected from the table by the lottery system. Subsequently, from that number, in the column onwards, guided the enrollment either in group A or B. Finally, seventy four patients were selected, of which 38 were in group A and 36 were in group B (n_1=38, n_2=36, N=74).

Group A: Total of 12 months regimen (3HRZECS/ 9HREC) was given to these patients. They had 6 drugs e g isoniazid, Rifampicin, pyrazinamide, ethambutol, ciprofloxacin and streptomycin for the initial 3 months (intensive phase) then 4 drugs e.g isoniazid, rifampicin, ethambutol and ciprofloxacin for subsequent 9 months (continuation phase).

Group B: WHO proposed treatment regimen (2HRZES/ 1HRZE/5HRE) of total 8 months duration was given to these patients. They had 5 drugs e.g isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin for the initial 2 months and 4 drugs e g isoniazid, rifampicin, pyrazinamide and ethambutol for subsequent 1 month and 3 drugs e.g isoniazid, ritampicin and ethambutol for next 5 months.

The dose of the drugs:

H = Tab isoniazid : 300mg or 5mg/kg body weight /day.

R = Tab rifampicin : 450mg, if the body weight is < 50kg or 600mg, if the body weight is > 50kg or 10 mg/kg body weight /day

Z = Tab pyrazinamide: 20mg/kg body weight /day.

E = Tab ethambutol : 25mg/kg body weight /day for the first 3 months (intensive phase) and then 15mg/kg body weight /day for subsequent period (continuation phase).

S = Inj streptomycin: Ig intramuscularly if age is <40 years and body weight is > 45kg or 0.,75g if age is > 40 years and body weight is < 45kg.

Pretreatment assessment :

Detailed history and thorough physical examination was done in each and every patient prior to the commencement of the retreatment. The following investigations e g TC, DC, Hb% ESR, X-ray chest PA view, tuberculin test, routine and microscopic examination of urine, serum bilirubin, SGPT (ALT), blood urea, serum creatinine, blood glucose 2 hours after breakfast, serum uric acid were done in every patient. Sputum smear for AFB and sputum for mycobacterium culture and sensitivity were done after proper decontamination and concentration by indirect proportion method in patients presenting with features suggestive of pulmonary tuberculosis. Lymph node biopsy and / or FNAC were done in patients presenting with lymphadenopathy .

Follow up and assessment of treatment:

After commencement of the retreatment the first follow up was done at about 15th day. Subsequently

follow up was done at the 1st, 3rd, 6th and 8th month. In addition, at the end of 12th month final follow up was done for A group of patients and post treatment follow up was done for B group of patients. During each visit patients were asked about fever, cough, expectoration, well-being, appetite and body weight and size of or presence or absence of lymph node were recorded. Any presence and / or development of anorexia, nausea, vomiting, abdominal discomfort, itching, rash, urticaria, vertigo, deafness, myalgia, arthralgia were noted. Visual acquity, field of vision, colour vision were tested and opthalmoscopy was done in patients who were on ethambutol. Patients who developed any side effects were managed accordingly. In relevant patients sputum smear for AFB and sputum for mycobacterial culture was done.

Statistical data analysis:

All data were analyzed by using SPSS program (SPSS for MS Windows release 6.1) and expressed as mean ± SD or in frequency or percentage unless mentioned otherwise. Comparison between groups was done by Students t-test, Chi square (x^2) test, Fisher's test and Kruskal one way ANOVA as applicable. Level of significance was expressed as P values. P values < 0.05 were considered as significant.

Results

Out of total of 74 patients, 38 belonged to group A and 36 belonged to group B. Among them 70 (94.6%) patients, 35 from each group i.e 92.1 % from group A and 97.2% from group B completed the retreatment and 4 (5.4%) were defaulters, of which 3 (7.9%) and 1 (2.8%) were from group A and group B respectively. As shown in table -I mean age (mean ± SD) of the total patients and of each group was 34 ± 13 years. The majority of the patients were below 40 years of age, ranging from 16 to 70 years. There was no significant age difference between the groups. Out of total 74 patients 47 (63.5%) were male and 27 (36.5%) were female. Male to female ratio of total patients was 4.7 : 2.7 and that of group A and group B were 2.5 : 1.4 and 2.2 : 1.4 respectively as shown in table -1. Sex difference between the groups was not statistically significant.

AS shown in table -lI, out of total 74 patients, 70 completed the retreatment and 64 (91.4%) patients i.e 33 (94.3%) from group A and 31 (86.6%) from group B were cured. The difference between the groups was not significant (x^2 = 0.792 and p = 0.673). 2 (5.7%) patients from group A and 4 (11.4%) patients from group B did not respond to treatment. 3 patients out of 4 who did not respond to treatment of group B regimen subsequently responded to group A regimen. The rest 3 failed cases responded to therapy with addition of ehtionamide and suitable adjustment of other drugs as required. Out of total 74 patients 4 (5.0%) patients dropped out, of which 3 (7.8%) were from group A and only 1 (2.7%) was from group B. The difference between the groups was not significant (x^2= 0.947 and p= 0.615).

Response to therapy of patients of pulmonary tuberculosis is shown in table -III. Out of total 74 patients, 51 (70.8%) had pulmonary tuberculosis without tuberculous lymphadenitis, of which 26 (68.4%) were from group A and 25 (69.4%) were from group B. Out of the 51 pulmonary tuberculosis patients 24 (100%) from group A and 21(87.5%) from group B were cured from the disease and 3 (12.5%) from group B had no cure. Out of the 51 pulmonary tuberculosis patients 3 were defaulters , of which 2 were from group A and only 1 was from group B.

Response to therapy of tuberculous lymphadenitis patients is shown in table -IV. Out of total 74 patients, 20 patients had tuberculous lymphadenitis, of which 9 (81.8%) from group A and 7 (87.5%) from group B were cured from the disease and 2 (18.2%) from group A and only 1 (12.5%) from group B had no cure and only 1 patient was defaulter from group A and none from group B. Out of the total 74 patients only 3 (4.1 %) patients had both pulmonary tuberculosis and tuberculous lymphadenitis as a common feature and all of them were in group B and were cured from their disease.

As shown in table -V, out of total 74 patients only 2 (2.7%) developed nausea, 3 (4.1 %) developed arthralgia and 6 (8.1%) developed vertigo around the end of the first month. All the adverse effects disappeared after the third month except 1 (1.4%) who had nausea which disappeared within a few days. All of the three patients who were given ethionamide, due to failure of either group A or group B regimen, developed anorexia.

Table-I : *Mean age and male to female ratio of the patients (n=74)*

Character	Total (n=74)	Group A (n=38)	Group B (n=36)	p
Age (Mean ± SD)	43 ± 13	43 ± 13	43 ± 13	0.864
Male : Female	4.7:2.7	2.5:1.3	2.2:1.4	0.810

Group A : 3HRZECS/9HREC Group B : 2HRZES/2HRZE/5HRE

Table-II : *Overall response to the retreatment regimens (n=70)*

Outcome	Total (n =70)	Group A (n=35)	Group B (n=35)	x2	P
	n (%)	n (%)	n (%)		
Cured	64 (91.4)	33 (94.3)	31 (88.6)	0.729	0.,673
Not cured	6 (8.6)	2. (5.7)	4 (11.4)		
Total	70	35	35		

Group A : 3HRZECS/9HREC Group B : 2HRZES/2HRZE/5HRE
4 patients were defaulters : 3 from group A and 1 from group B

Table-III : *Outcome of the retreatment of pulmonary tuberculosis patients (n=48)*

Outcome	Total (n =48)	Group A (n=24)	Group B (n=24)	x^2	P
	n (%)	n (%)	n (%)		
Cured	45 (93.7%)	24 (100)	21 (87.5)	3.200	0.234
Not cured	3 (6.3)	0	3 (12.5)		
Total	48	24	24		

Group A : 3HRZECS/9HREC Group B : 2HRZES/2HRZE/5HRE
3 patients were defaulters : 2 from group A and 1 from group B

Table-IV : *Outcome of the retreatment of tuberculus lymphadenitis patients (n=19)*

Outcome	Total (n =19)	Group A (n=11)	Group B (n=8)	x^2	P
	n (%)	n (%)	n (%)		
Cured	16 (84.2)	9 (81.8)	7 (87.5)	0.112	1.000
Not cured	3 (15.8)	2 (18.2)	1 (12.5)		
Total	19	11	8		

Group A : 3HRZECS/9HREC Group B : 2HRZES/2HRZE/5HRE
1 patient was defaulter from group A and none from group B

Table-V : *Adverse effects of drugs during retreatment (n=74)*

Duration	01 month				03 month			
Adverse effects	Total	Group A	Group B	p	Total	Group A	Group B	p
Nausea	2 (2.7)	1 (2.7)	1 (2.8)	1.000	1 (1.4)	0	1. (2.8)	0.486
Pruritus	3 (4.1)	3 (7.9)	0	.240	0	0	0	-
Arthralgia	3 (4.1)	2 (5.3)	1 (2.8)	1.000	0	0	0	-
Vertigo	6 (8.1)	4 (10.5)	2 (5.6)	0.675	0	0	0	-

Group A : 3HRZECS/9HREC Group B : 2HRZES/2HRZE/5HRE

Discussion

Throughout the world tuberculosis is the leading infectious killer disease of the mankind[6,7] but there was no cure for tuberculosis till the introduction of chemotherapy in 1950. Then the incidence of tuberculosis declined dramatically specially in developed countries[8,9] and it became the disease of the poor and developing countries[9] but the disease reemerged with new ferocity of MDR-TB both in developed and developing countries.[6,7,10-14] Although the drug resistance in tuberculosis is a spontaneous phenomenon but the development of MDR-TB is a man made phenomenon[13] and may be due to inadequate and irregular treatment.[6,15] So, to overcome this man made phenomenon of the development of MDR-TB adequate and regular

treatment of tuberculosis must be ensured. Furthermore, defaulters, relapsed and failed cases should be retreated more rigorously with proper regimen for adequate period to prevent the spread of the resistant mycobacteria in the community.

In this study the retreatment was successful in 64 (86.4%) of the patients, of which 33 (94.3%) were from group A and 31 (86.6%) were from group B. The difference was not statistically significant between the two groups. The success rate of this study corresponds with the success rate of many other studies using different regimens. Chowdhury et al[16] of Bangladesh Rural Advancement Committee (BRAC) found treatment successful in 81% and 86% in two of their studies respectively. In China Murrey et al[17] found 91.8% successful treatment outcome in their series. In Japan Mori et al[18] found 78.4% success rate. In other studies successful treatment outcome was as follows: 77% in Tanzania, 87% in Malavi, 78% in Mozambique and 78% in Nicaragua.[19]

In the present series 4 (5.4%) patients were defaulters. This defaulter rate is more or less close to the defaulters rates of the other studies. In BRAC studies 3.1% and 1.6% of patients were defaulters[16]. In China[17] and Japan[18] 0.6% and 7.2% patients were defaulters respectively. But in other studies the defaulters rate were still higher e g Bhuiyan et al[20] found 35.7%, Faiez et al [21] found 35% , Alam et al[5] found 37.4% and Khan et al[22] found 51% defaulters. Similar findings were also reflected in the half yearly report of Chankharpool TB clinic in the year 1998.[5,23] Most of these defaulters were illiterate, poor and floating. Moreover, symptomatic improvement may be the cause for this defaulting. Similar view was also held by Alam et al[5] who, in their study furthermore, reported that the patients were defaulters due to poor briefing prior to initiation of therapy, which indicates that adequate prior briefing can reduce the defaulter rate to a great extent. However, financial constraints (34.8%) and ignorance of the patients (12.9%) were also reported to be important causes of drug defaulting. Adverse effects (8.9%) and unavailability of the drugs (3.6%) are less common cause of drug defaulting[5]. Defaulters are very dangerous in the society because they avoid treatment or undergo treatment irregularly and inadequately and so, harbour organisms and thereby become responsible for the development and spread of normal as well as drug resistant mycobacteria in the community.

Although in the present series there was no statistical significant difference, between the outcome of two groups of drug regimens but 3 out of 4 patients who failed to respond to group B regimen responded while given group A regimen. It may indicate that ciprofloxacin was effective in those patients. In fact, ofloxacin and ciprofoxacin are fluroquinolone antimicrobial drugs that are proving useful in the retreatment regimens[8]. They have shown excellent activity in studies in animals and have clinical efficacy when used for the retreatment. At one centre ofloxacin or ciprofloxacin were extensively used for eight years and remarkably good tolerance and little toxicity were noted despite long term high dose administration[8]. Although specific usefulness of these two drugs has never been studied, they are preferable to the other oral retreatment medications with regard to the antimycobacterial activity and safety. Resistance to fluoroquinolone drugs has developed when the drugs were in inadequate regimens and in inadequate doses. Fluoroquinolones, such as ciprofoxacin, ofloxacin and sparfloxacin, have potent in-vitro activities against mycobacterium tuberculosis[24-26] including the vast majority of multi-drug resistant clinical isolates. Fuoroquinolones have been demonstrated to have clinical efficacy against tuberculosis in combination with other antituberculosis agents[28, 29]. However, resistance to ciprofloxacin and ofloxacin appeared in clinical isolates of mycobacterium tuberculosis and in some cases such resistance has been shown to emerge during treatment of patients infected with fluoroquinolones susceptible strains[30].

Adverse effects of anti-tuberculosis drugs vary from very mild to very severe one. But in the present series very mild adverse effects were noted during the study period. Only nausea (2.7%), pruritus (4.1%), arthralgia (4.1 %) and vertigo (8.1%) were noted. Out of these adverse effects only vertigo required to withdraw streptomycin from the regimen about one month after commencing the retreatment in 8.1 % of patients but without affecting the outcome of the regimen. Although, like other anti-tuberculosis drugs ciprofloxacin may give rise to so many adverse effects but unlike other second line anti-tuberculosis drugs these are very rare despite high dose long term administration[8].

Conclusions

WHO regimen, which is the same as the group B regimen of the present series as well as categorey 2 regimen of the National Tuberculosis Control Program (NTP), is quite effective still now and can be used as an useful retreatment therapy specially in a situation like ours where susceptibility tests are not readily, widely or dependably available.

Ciprofloxacin can be used, in a suitable combination with other drugs, as a very useful anti-tuberculosis

therapy even on long term basis in the retreatment cases of MDR-TB. The drug is well tolerated and is associated with little or negligible adverse effects despite long term high dose administration. But the use of ciprofloxacin should be kept reserved for the documented MDR-TB or for the cases in which WHO retreatment regimen has failed, in a situation where susceptiblity tests are not available. But one should not forget the fact that the potential pressure for fluoroqunolone resistance is considerable in developing countries, where tuberculosis is highly endemic and patients are frequently treated with fluoroquinolones for enteric or other infections.

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EFFICACY OF VEGETARIAN DIET IN RHEUMATOID ARTHRITIS: A CONTROLLED STUDY WITH 24 WEEKS FOLLOW UP

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Summary

The efficacy and tolerability of vegetarian diet was compared with those of methotrexate (MTX) in an open random controlled, prospective clinical trial. 87 patients were included but 78 patients had completed the trial with a 24 weeks follow-up. Forty patients were assigned to the MTX group and thirty-eight patients to the vegetarian group but only 21 patients in Vegetarian diet group could complete the 24 weeks trial. After 12 weeks of treatment 17 patients from Vegetarian diet group were switched over to other disease modifying anti-rheumatic drugs. Statistically significant improvement was observed in all most all clinical and laboratory parameters in MTX & Vegetarian group except ESR & Hb% in vegetarian group. Compared with the MTX group, the improvement was less marked in vegetarian group. In the outcome measurement we found that 36(90%) cases were responder in MTX group in comparison to 11(28.95%) in the vegetarian group. The response rare was significantly higher in MTX group than in the vegetarian group (p<0.0001). Shorter duration of illness, low initial ESR and lower initial functional class were found to influence the outcome favourably.

Introduction

Rheumatoid arthritis (RA) is the commonest form of inflammatory arthritis that affects about 1-3% of the population[1-5]. Nearly 90% of patients with aggressive disease become clinically disabled within 20 years. The severity of RA emphasizes the need for an effective management plan which has led to a move towards using disease modifying anti-rheumatic drugs (DMARDS) early in the disease[6-9]. Non-pharmacological therapics such as dietary modification are undervalued. It has been reported that intake of vegetarian diet, diet with low total protein and dietary supplementation with fish fatty oil are associated with decreased disease activity of RA[10-11]. The rersults of all these studies were promising. Establishing the value of diet would provide a means for planning an easier country based dietary approach for management of RA. However, all these studies suggested the need for a comparative study to establish the efficacy of vegetarian diet in the management of RA.

Materials and methods

This open, randomized, prospective, clinical trial was conducted in the rheumatology clinical, Banghabandhu Sheikh Mujib Medical University, Dhaka from July 1997 to June 1998. All RA patients attending the Rheumatology clinic were consecutively included in the study. The disease was considered active if at least three of the following five criteria were present: six or more joints tender or painful on motion, three or more swollen joints, morning stiffness > 60 minutes, ESR >28 mm and Hb% < 13.8 g/dl in men and < 11.8 g/dl in women. Patients younger than 16 years, with disease duration less than three months, with history of intake of steriod within one month prior to the study, with known hypersensitivity to methotrexate (MTX), pregnant women or women contemplating pregnancy, lactating mothers, with serious concomitant or systemic diseases including hepatic, renal or respiratory insufficiency and with leukopenia (leukoeyte count < 3.5 x 10^9/L) or thrombocytopenia (platelet count < 150 x 10^9/L) were excluded from the trial.

The subjects were randomly assigned to the study or the control groups. The subjects in the study group

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were given a flexible diet chart, which excluded food items of animal origin excepting cow-milk. This was low protein, vegetarian diet of 2250 kcal per day. The subjects in the control group were given MTX 7.5 mg in single dose on one day of every week with folic acid 5 mg on the following day. The dose of MTX was raised to 10 mg/week at the end of the second month and by 2.5 mg/week at monthly intervals thereafter in case there was no response. All the subjects were given NSAIDs *ad lib*. The subjects were advised to take famotidine 40 mg bd while taking NSAIDS. The subjects were followed up at two weeks intervals during the first four weeks and then at four weeks intervals for further 20 weeks. For assessment of adverse effect of the treatment, the subjects were interrogated carefully and routine urinalysis, complete blood counts and transaminases were done at each visit. Following variables were used for assessment of outcome: tender joint count, swollen joint count, joint tenderness index, joint swelling index, duration of morning stiffness, functional class, patient's assessment of pain on a 10 point numerical rating scale (NRS), patient's global assessment of disease activity on a five point scale, physician's global assessment on a five point scale and ESR. WHO/ILAR response criteria was used to define positive response to treatment. It was defined as improvement in five of following six criteria: decrease of tender joint count, swollen joint count and ESR by at least 20% and decrease of patient's assessment of joint pain, patient's global assessment and physician's global assessment of disease activity by at least 30%. The same assessor (PMI) assessed all the subjects.

Statistical analysis: Inter-group analyses of differences between discrete and continuous variables were done by chi square test with Yates' correction and student's t test respectively. Comparison between pre-and post-treatment values of different continuous outcome variables was done with paired t test. Logistic regression analysis was performed to estimate the influence of different variables on the outcome of treatment.

Results

A total of 87 patients were enrolled in the traial. Till 12th week of the trial, nine patients dropped out; 4 from the vegetarian and 5 from the MTX groups. Finally, 78(89.66%) patients completed the trial, 40 belonged to MTX group and 38 to vegetarian group. Data of these 78 patients were used in all subsequent comparative analysis.

The age of the patients ranged from 18-70 years and the mean (SD) was 40.1 (13.2). Sixty were female and eighteen were male. Rheumatoid factor was positive in 79.5% and positive family history of RA was found in 20.5% of subjects. Baseline characteristics and activity indices are shown in table-1. The study and control groups were nearly identical with respect to demographic, clinical and laboratory variables (Table-2). The two groups differed significantly only in sex component.

Only 21 patients in vegetarian group could complete 24 weeks trial and the remaining 17 patients were switched over to another DMARD. Three subjects in the MTX group dropped out during this second phase of the study. All paired analyses were done with these 21 and 37 subjects in the vegetarian diet and MTX groups respectively to assess the results at the end of 24 weeks. All the clinical parameters in the vegetarian diet group improved after 24 weeks but there was no significant improvement in the NSAID score, HB% and ESR (Table-3). All the clinical and laboratory parameters were improved significantly from baseline in MTX group after 24 weeks of treatment (Table-4).

Most commonly occuring side effects in MTX group were nausea and anorexia in 64.29% cases followed by giddiness in 39.29% cares (Table-V). Vegetarian diet group showed no side effects.

Table- I : *Baseline characteristics of patient's*

Parameters	Patients (n- 78)
Age (Years): Mean ± SD	40.12 ± 13.21
Range (year)	18 – 70
Duration of illness (Yr) Men ± SD	4.09 ± 4.29
Sex (F/m)	6 – 18 (3.3:1)
Rheumatoid factor + ve (n-62)	79.55%
Family H/O of R/A (n- 16)	20.51%
Corticosterids previously used (n-15)	19.2%
Past H/O of being treat for rheumatic fever (n-16)	20.51%
Number of involved joints	16.22 ± 7.63
Number of swollen joints	11.40 ± 7.12
Joints swelling index	18.14 ± 10.58
Number of tender joints	16.10 ± 13.60
Patient's assessment of pain (NRS)	6.82 ± 2.31
Physician's global assessment of disease activity	3.21 ± 0.71
Duration of morning stiffness (mins)	119.49 ± 54.44
Functional class	2.72 ± 0.45
ESR (mm/1st hour)	77.81 ± 29.49
Hemoglobin (gm/dl)	10.66 ± 1.32

Table- II : *Comparison of baseline characteristics of the two groups of patients*

Variable	Veg (n-38)	MTX (n-40)	P
Age in years (M ± SD)	40.24 ± 11.80	40 ± 14.57	NS
Sex (F/M)	34/4	26/14	0.01
Duration of illness in years	4.46 ± 5.03	3.75 ± 3.49	NS
No. of swollen joints	11.11 ± 5.43	11.68 ± 8.49	NS
Joint swelling index	17.37 ± 8.13	18.88 ± 12.54	NS
No. of tender joints	15.26 ± 5.84	16.90 ± 9	NS
Joint tender index	26.05 ± 10.14	29.47 ± 16.18	NS
Joint pain (NRS)	6.92 ± 3.00	6.72 ± 1.40	NS
Patient,s global assessment of disease activity	3.59 ± 0.93	3.82 ± 0.90	NS
Physician's global assessment of disease Activity	3.10 ± 0.60	3.30 ± 0.79	NS
Morning stiffness (mins)	117.11 ±54.57	121.75 ± 54.61	NS
Functional class	2.71 ± 0.46	2.72 ± 0.45	NS
ESR (mm in 1st hr)	75.13 ± 28.69	80.35 ± 30.37	NS
Total leucocyte count	9492 ± 2099	9950 ± 1877	NS
Neutrophil count (%)	64.86 ± 8.45	64.27 ± 9.22	NS
Haemoghobin (gm/dl)	10.86 ± 1.27	10.47 ± 1.36	NS

Table- III : *Treatment response in vegetarian group after 24 weeks (n-21).*

Parameter	Week O	Week 24	P Value
No. of swollen joint	10.81 ± 4.84	5.67 ± 5.96	<0,001
Swelling index	16.90 ± 6.68	8.29 ± 8.66	<0.001
No. of tender joints	15.38 ± 5.74	10.05 ± 8.30	<0.01
Joints tender index	25.38 ± 9.94	15.67 ± 14.01	<0.01
Patient's assessment of pain	7.33 ± 3.80	4.52 ± 3.22	<0.001
Patient's global assessment of disease activity	3.05 ± 0.59	2.48 ± 1.12	<.001
Physician's global assessment of disease activity	3.05 ± 0.59	2.48 ± 1.12	<0.01
Morning stiffness (mins)	114.29 ± 55.82	53.10 ± 51.42	<0.001
NSAID Score	43.49 ± 110.55	13.67 ± 31.8	NS
Functional class	2.67 ± 0.48	2.0 ± 0.78	<0.001
ESR	66.52 ± 22.23	58.94 ± 37.10	NS
Haemoglobin	11.05 ± 0.95	11.67 ± 1.46	NS

Table-IV : *Treatment response in MTX group after 24 weeks (n= 40)*

Parameter	Week 0	Week 24	P Value
No. of swollen joint	12.08 ± 8.70	0.27 ± 0.87	<0.001
Swelling index	19.35 ± 12.92	0.49 ± 1.74	<0.001
Number of tender joints	17.49 ± 9.12	0.43 ± 1.12	<0.001
Joint tender index	30.38 ± 16.51	0.57 ± 1.59	<0.001
Patient's assessment of pain (NRS)	6.72 ± 1.42	0.43 ± 0.99	<0.001
Physician's global assessment of disease activity	3.32 ± 0.78	1.11 ± 0.39	<0.001
Patient's global assessment of disease activity	3.84 ± 0.90	1.10 ± 0.40	<0.001
Morning stiffness (mins)	123.51 ± 56.12	3.65 ± 7.61	<0.001
Functional class	2.73 ± 0.45	1.05 ± 0.23	<0.001
ESR (mm in 1st hr.)	81.46 ± 30.85	31.24 ± 21.64	<0.001
Haemoglobin (gm/dl)	10.52 ± 1.38	12.33 ± 1.40	<0.001

Table- V : *Side effects observed in MTX group*

Side effect	MTX group (n-28)	Per value
Nausea/anorexia	18	64.29%
Dizziness	11	39.29%
Headache	8	28.52%
Raised ALT	8	28.52%
Pain abdomen/Heart burn	5	17.86%
Oral ulcer	3	10.71%
Vomiting	3	10.71%
Muscle cramps & myalgia	3	10.71%
Distaste	2	7.14%
Itching	2	7.14%
Burning extremities	2	7.14%
Burning urine	2	7.14%
Diarrhoea	2	7.14%
Pnumonitis	1	3.57%
Jaundice	1	3.57%
Herpes zoster	1	3.57%
Gynecomastia	1	3.57%
Sleep disturbances	1	3.57%

WHO/ILAR response criteria were followed to measure the outcome of treatment. In MTX group there was maximum number of responder (90%), where as in vegetarian group the responder was 28.95% (Table-VI).

Table-VI : *Outcome of treatment WHO/ILAR response criteria.*

Group	Responder	Percentage	P value
MTX (n-40)	36	90	
Vegetarian (n-38)	11	28.95	0.00024

*by chi-square test with Yate's correction

Logistic, regression was performed with a view to correcting for the effects of probable confounding variables (Table-VII). The difference between the two treatment groups remained significant at multivariate analysis.

Table-VII : *Logistic analysis, Variable in the equation*

Variable	**B**	**Sig**
Age	-0.05696	0.0867
Duration	-0.2911	0.0177
ESR	0.0439	0.0150
Functional class	3.3651	0.0138
Morning stiffness	-0.0082	0.2776
No. of swollen joints	0.1399	0.4511
No. of tender joints	-0.1036	0.8424
Pain (NSR)	-0.3120	0.2481
Pt. Global assessment of diseases activity	0.3965	0.3991
Phy global assessment of disease activity	-0.7975	0.4059
Rheumatoid factor	0.0009	0.4770
Sex	.7919	0.1814
Swelling index	.0047	0.9729
Tender index	.0267	0.7114
Treatment group	-2.0113	0.0003

Shorter duration of illness, low initial ESR and lower initial functional class was found to influence the outcome favorably (Table-VIII).

Table- VIII : *Multivariate analysis*

Vegetarian Group	Responder	Non-responder
Duration of illness	3.80 5.50	4.88 4.78
Initial ESR	66.40 25.82	80.83 29.56
Initial Functional Class	2.47 0.52	2.87 0.34
MTX Group		
Duration of illness	3.39 3.43	5.43 3.49
Initial ESR	76.64 31.45	97.86 16.94
Initial Functional class	2.70 0.47	2.86 0.38

Discussion

The present study was designed to see the efficacy of the Vegetarian diet on the activities of RA in comparison with MTX, an established DMARD. In the present study there was 60 female and 18 male with a ratio of 3.3:1 which can be compared favourably with the ratio of 2.63:1 in the series of Alam et al [12] and 3.14:1 in the series of Kremer et al[13]. The average age of the patients of MTX group was 40(15.57) years and in the vegetarian diet group was 40.24(11.80) compared to 53.9 and 59.5 years in MTX group in the series of Kremer[14] and O'Dell et al[15] and 50 years in group in the series of Haughen and Kjeldsen-krangh et al[16]. Baseline characteristics between the two groups did not differ significantly as in the study of kjeldsen-krangh et al[16]. There was marked improvement of the clinical parameters of the disease activity after 24 weeks of MTX treatment i.e. no. of swollen joints (12.08 to 0.27), joint swelling index (19.35 to 0.49), no. of tender joints (17.49 to 43), joint tenderness index (30.38 to 0.51), patient's assessment of pain (3.84 to 1.10), patient's global assessment of disease activity (3.84 to 1.10), physician's (3.32 to 1.11) global assessment of patient's activity, duration of morning stiffness (124 mins to 4 mins), functional class (2.73 to 1.05) which were statistically significant at 0.001 level. The laboratory variables were also showed significant improvement from baseline in MTX group at 0.001 level which was consistent with that of kremer,[14] Wimblatt et al [17] and Bologma et al[18]. In the Vegetarian group, 17 patients were switched over to another DMARD because of poor improvement of the disease activity after three months of treatment. Remaining 21 patients completed 24 weeks of treatment and showed significant improvement in most of the clinical parameter at 0.01 level i.e. no. of swollen joints (10.81 to 5.67), swelling index (16.9 to 8.,29), no. of tender joints (15.38 to 10.05), joint tenderness index (25.38 to 15.67), patient assessment of pain (NRS) (7.33 to 4.52), patient's global assesment of disease activity (3.57 to 2.62), physician's global assessment of disease activity (3.05 to 2.48), morning stiffness in mins (114 to 53), functional class (2.67 to 2). These findings were consistent with those of Nenonen et al[19]. In the Laboratory variables there was no significant improvement from the baseline. Intention to treat analysis was done and when we compared Vegetarian group with that of MTX group in respect to the response of treatment after 24 weeks most of the clinical and laboratory parameter showed statistically significant changes ($p < 0.001$) in favor of MTX group. In MTX group the maximum weekly dose of MTX was 15mg in 30% of patients followed by 10mg in 22.5% of patient's. The dose schedule compared favourably with the observation by Tugwel[20] and Kremer et al[21] respectively. In MTX group 70% cases developed side-effects. Gasto-intensinal (64.29%) and CNS (39.29%) side effects were the highest. Raised aminotransferace (ALT) up to two folds was noted in 28.52% of patients but it subsided despite continuation of the drug which was consistent with that of Weinblatt et al[17]. Only one subject developed jaundice with marked rise in transaminanse and the drug had to stopped altogether. Most of the side-effects in the MTX group were reported within 8 weeks of therapy and they were mild and transient. In the Vegetarian group no side-effect was observed. However majority of the patients disliked the strict dietary regimen. We followed the WHO/ILAR response criteria[22] and found 36(90%) patients were responder in MTX group in comparison to 11(28.95%) patients responder in Vegetarian group; the difference was statistically significant ($P<0.001$). In multivariate analysis MTX was also found superior to Vegetarian diet group. Logistic regression was done including compounding variables because the two groups differed significantly in their sex component. However the difference between MTX group and Vegetarian diet group remained significant even after logistic regression ($P<0.0003$). Short duration of illness, lower initial ESR and lower initial functional class were fond to influence the outcome favourably in all patients pooled together as well as MTX and Vegetarian groups separately. There was an ineresting observation that 16 of our patient's in MTX group undergone complete regression (fulfilling ARA remission criteria).

Conclusion

From the present study it can be concluded that

- Vegetarian diet is middly effective, safe and possibly less acceptable in controlling the disease activity in RA.
- the efficacy of Vegetarian diet is less marked than that of MTX. So, the Vegetarian diet may be advocated as a supplement to an established DMARD. A less regorous dietary regimen may also be tasted in future trial.

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ORIGINAL ARTICLES

IODINE STATUS IN MULTINODULAR GOITRE

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Summary

Multinodular goitre is one of the common thyroid disorders. Iodine-deficiency is considered as the prime factor for the development of simple diffuse goitre, which eventually leads to multinodular goitre. A cross-sectional study based on a thyroid clinic, IPGM&R, Dhaka, was designed during the period from January 1996 to December 1997 to find out iodine status of the patients with multinodular goitre in comparison with healthy controls. Measurement of urinary iodine concentration (UIC) from casual sample was undertaken as an important parameter to find out the association of iodine deficiency in the patients with multinodular goitre. Sixty-six patients with multinodular goitre and sixty healthy controls comprising a total of 126 subjects were enrolled in the study.

The mean (±SD) UIC of the patients in the age group of 30-39 years was significantly lower than that of the controls. The mean (±SD) UIC were also less than those of controls in other age groups, but the differences were not statistically significant. May be the fourth decade is the most vulnerable part of life for the development of multinodular goitre. In the present study, mean serum T_3, T_4, and TSH were within normal limits but mean T_3/T_4 ratio was raised in patients with euthyroid multinodular goitre. The mean (± SD) UIC in the patients and controls were 10.41 (±1.83) and 11.35 (± 1.76) µg/dl, respectively, and the difference was statistically significant. In 75.76 percent cases, UIC was below 11.64 µg/dl (median urinary iodine level of the controls). But none had urinary iodine concentration below 5 µg/dl. The median value of UIC of the patients and controls were 10.02 and 11.64µg/dl, respectively. It appears that the patients with multinodular goitre had been suffering from iodine deficiency in comparison with control subjects. Therefore, it can be deduced that the patients with multinodular goitre had been suffering from iodine deficiency for long time and have been taking iodized salt after the development of goitre, with consequent rise in UIC, but not up to the level of control values.

Introduction

Multinodular goitre is one of the common thyroid disorders and is responsible for long-term morbidity and mortality.[1]

Evolution of multinodular goitre occurs usually from endemic or sporadic simple diffuse goitre. The increase in thyroid size resulting from excessive replication of benign thyroid epithelial cells is a slow process evolving over many years, starting with a diffuse initial enlargement, which frequently becomes multinodular.[2] With increasing duration, some follicles become involuted, where others enlarge with colloid accumulation and fibrotic areas sometimes separate hypertrophic from atrophic and involuted areas resulting in the appearance of multinodularity.[2] In an endemic area, the goitre is presumed to result from environmental factors, such as iodine deficiency or the presence of goitrogens in the food chain which inhibit thyroid hormone formation.[2] In several studies, simple diffuse goitre, nontoxic solitary nodule, simple multinodular goitre and toxic multinodular goitre, were considered as the "Iodine-deficiency related disorders".[3-5] In the study of Alam et al[6] these iodine-deficiency related disorders comprised 65.28 percent of 1858 thyroid cases. Nearly all longstanding simple diffuse goiters transform into multinodular goitre, [7] which conforms to the concept that iodine deficiency-related simple diffuse goitre and nontoxic solitary nodule progress through simple multinodular goitre to toxic multinodular goitre.[8-10]

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Etiologically, iodine-deficiency is considered as the prime factor for the development of simple diffuse goitre which eventually leads to multinodular goitre and the association of genetic factors and dietary goitrogens and possibly other environmental factors contribute to the severity of the condition and influence its clinical expression.[11]

In areas of nonendemicity, the etiology of nontoxic multinodular goitre is usually indeterminate.[12] But stimulatory effects of extrathyroid growth factors, like IGF-I and EGF on thyroid cell growth have been reported. In addition, TSH receptor-directed antibody that have only a growth-stimulating effect have been described.[2] In nonendemic or sporadic goitre, mild dietary iodine deficiency, slight impairment of hormone synthesis, increased iodide clearance of the kidneys, and the presence of thyroid-stimulating immunoglobulins have been variously suggested.[13]

The incidence and prevalence of multinodular goitre in Bangladesh is unknown because no study has yet been performed in large scale among the general population. Out of 2629 subjects seen in the thyroid clinic, Institute of Postgraduate Medicine and Research (IPGM&R), between January 1994 and June 1995, 1858 had some form of thyroid disorders: 8.40 percent had simple multinodular goitre and 3.13 percent had toxic multinodular goitre.[6]

Bangladesh is a zone of endemic goitre.[14] Because iodine deficiency is considered to be a prime factor for the development of endemic simple diffuse goitre and its consequences, so, estimation of urinary iodine level is important to find out the etiology of endemic goitre and its consequences like simple multinodular and toxic multinodular goitre. Other biochemical parameters, like serum T_3, T_4, TSH,T_3/T_4 ratio, radioactive iodine uptake (RAIU) are only the indirect evidences of iodine deficiency.

Approximately 90 percent of ingested iodine is excreted in urine. Keying iodine excretion to creatinine excretion has not proved to be helpful because of variation in creatinine excretion with nutritional status, so that their ratio is no longer used.[15] It is not generally possible to obtain 24-hour samples of urine, measurement of urinary iodine level in a casual sample is an excellent surrogate.[15]

Therefore, measurement of urinary iodine concentration (UIC) from casual sample was undertaken as an important parameter to find out the association of iodine deficiency in the patients with multinodular goitre in this series.

Materials and Methods

This cross-sectional study based on a thyroid clinic, IPGM&R, Dhaka, was designed to find out iodine status of the patients with multinodular goitre in comparison with healthy controls. Patients with thyroid disorders from different parts of the country attended the clinic and thyroid patients were also referred to the clinic.

Sixty-six patients with multinodular goitre and sixty healthy controls comprising a total of 126 subjects were included in the study.

The patients were recruited from this clinic and were investigated in INM, Dhaka, and also in the Department of Pathology, IPGM&R, Dhaka. Urinary iodine level was measured at the Institute of Nutrition and Food Science (INFS), University of Dhaka. The study period was from January 1996 to December 1997.

The patients with multinodular goitre, irrespective of functional status, attending the thyroid clinic during the stipulated period were included in this study. Multinodularity was confirmed by ultrasonography in each case.

Patients with any nonthyroidal illness that may affect the iodine absorption and urinary iodine excretion were excluded.

Healthy controls matched with age, sex, weight for comparison of urinary iodine concentration were recruited from the relatives of the patients, students, doctors and friends. Visible or palpable goitre in subjects were not included as healthy controls. Nonthyroidal illness that may affect the iodine absorption and urinary iodine excretion were also excluded.

The purpose of the study was clearly explained to each of the subjects and they were recruited only after they had given their consent.

Detailed history was taken from each patient with multinodular goitre irrespective of thyroid status, including age, sex, residence, family history and duration of goitre, drug history, history of intake of goitrogens and also other symptoms related to thyroid disorders, etc. according to study protocol.

Through physical examinations, including examination of the thyroid gland, cervical lymph nodes, eyes, etc. were done in each case.

Besides routine examination of blood and urine, and x-ray chest in relevant cases, the following investigations were carried out in each patient:

a) Serum T_3, T_4 TSH estimation

b) Ultrasonogram(USG) of the thyroid

c) Radioactive iodine uptake (RAIU)

d) Thyroid scintiscan

e) Fine needle aspiration cytology (FNAC)

f) Estimation of urinary iodine concentration in the casual urinary sample.

Observation and Results

Findings of this study were evaluated from different perspectives based on multinodular goitre with functional status, age, sex, habitat, urinary iodine concentration and FNAC. However, urinary iodine concentrations were highlighted all through. Sixty control subjects and 66 patients belonged to the homogeneous state in respect to age, sex, weight, pulse rate and blood pressure.

Table-1 shows that the Mean (± SD) age of the controls and patients were 40.13 (± 13.84) and 39.18(± 14.41) years, respectively. There were 10 male and 50 female in the control group and 8 male and 58 female patients in the study group. The mean (± SD) weight, pulse rate and blood pressure in the controls were 45.00 (± 11.02) kg, 89.00 (± 8.50) per minute and 126.00 (± 9.50) /77.00 (± 4.50) mmHg and those of the patients were 44.00 (± 12.78) kg, 91.00 (± 9.50) per minute and 128.00 (± 11.50)/79.00 (± 5.60) mmHg respectively.

Table-II shows that there were 45 (68.18%) euthyroid, 7 (10.61%) hypothyroid and 14(21.21%) hyperthyroid patients in this series.

Table-III shows the Mean (±SD) serum T_3, T_4, T_3/T_4 and TSH levels of patients with different thyroid functional status.

Age of the controls varied from 13 to 69 years and that of the patients varied from 15 to 70 years. Majority of the patients (28.8%) was in the age group of 30-39 years, followed by 22.7 percent in the age group 40-49 years (Table-IV).

Table-V shows that majority of the euthyroid patients (33.33%) was in the age group of 30-39 years. Most of the patients (42.85%) with hypothyroid status were in the second decade and that of hyperthyroid status (35.71%) in the age group of 40-49 years.

Table-VI showed the difference of Mean (± SD) UIC among the control age groups as well as aTable-V shows that majority of the euthyroid patients (33.33%) was in the age group of 30-39 years. Most of the patients (42.85%) with hypothyroid status were in the second decade and that of hyperthyroid status (35.71%) in the age group of 40-49 years.

mong the patient age groups was insignificant. There was only significant difference of Mean (± SD) UIC being observed between controls and patients (F =62.00, P =0.0095) in the age group of 30-39 years.

Table-VII shows that the Mean (± SD) UIC in healthy controls and in the patients with multinodular goitre were 11.35 (± 1.76) and 10.41 (± 1.83) mg/dl, respectively. The difference was statistically significant (P=0.004). The median value of UIC in controls and patients were 11.64 and 10.64 and 10.02 mg/dl, respectively.

Table-VIII shows that among the three functional groups, the Mean (± SD) age of the hyperthyroid patients was highest and that of the hypothyroid patients the lowest. The difference of mean (± SD) age was statistically significant (F=3.7205; P=0.0133) among the patients with different thyroid functional status.

Table-IX shows that the difference of Mean (± SD) UIC between male and female was neither significant neither in the controls nor in the patients. However, significant difference of the mean (± SD) UIC was observed only between the female patients and the female controls (t =2.57; P =0.012).

Table-X shows that the Mean (± SD) duration of goitre was longest in hyperthyroid and shortest in hypothyroid patients. The difference of mean (± SD) duration of goitre among different thyroid status was statistically significant (F=37.6300 ; P=0.0000).

The Mean (± SD) RAIU in 2 hours and 24 hours among different thyroid functional status showed statistically significant difference (F=28.1671; P=0.000, and F=54.4293; P=0.000) (Table-XI).

Table-XII shows that the difference of the UIC between euthyroid and controls was statistically significant (t=3.09, P=0.003). The UIC of hypothyroid and hyperthyroid patients did not differ significantly from the control. No significant difference of Mean (± SD) UIC was found among the patients of different thyroid functional status (F= 0.7606, P= 0.4716).

Table-XIII shows that among the 66 patients, 75.76 percent patients had UIC below the median UIC of controls (11.64mg/dl).

Table-XIV shows that UIC was <11.64 mg/dl (median value of UIC of controls) in 36 (80%) euthyroid, 4 (57.14%) hypothyroid and 10 (71.43%) hyperthyroid patients.

Table-XV shows that the UIC did not correlate significantly with variables,such as age, duration of goitre, serum T_3,T_4, T_3/T_4 ratio and TSH, FNAC findings and thyroid functional status. But RAIU in 2 hours and 24 hours significantly correlated with UIC (r= -0.3502; P= 0.004, and r= -0.4476; P= 0.000).

Table-I : *Characteristics of the studied subjects.*

Parameters		Healthy control (n=60)	Patients (n=66)
Age (years) (Mean± SD)		40.13± 13.84	39.18± 14.41
Sex	Male	10	8
	Female	50	58
Weight (kg) (Mean± SD)		45.00± 11.02	44.00± 12.78
Pulse (per minute) (Mean± SD)		89.00± 8.50	91.00± 9.50
Blood pressure (mmHg) (Mean±SD)			
	Systolic	126.0± 9.5	128.0±11.5
	Diastolic	77.0± 4.5	79.0± 5.6

Table-II : *Distribution of multinodular goitrous patients by thyroid functional status (n=66).*

Thyroid functional status	Number	Percentage
Euthyroid	45	68.18
Hypothyroid	7	10.61
Hyperthyroid	14	21.21

Table-III : *Serum T_3, T_4, T_3/T_4 ratio, TSH in the patients with multinodular goitre by thyroid functional status.*

Parameters	Thyroid status		
	Euthyroid	Hypothyroid	Hyperthyroid
T_3	1.80± 0.46	1.54± 0.56	5.05± 2.59
T_4	112.52± 21.57	43.56± 17.16	215.80± 51.75
T_3/T_4	0.020± 0.01	0.040± 0.01	0.0148± 0.01
TSH	1.51± 1.26	53.82± 17.94	0.15± 0.13

Values quoted as Mean± SD

Normal values:

T_3 = 0.80-3.16 nmol/L
T_4 = 64.5-152.0 nmol/L
T_3/T_4 = 0.015
TSH = 0.3-6.0 mIU/L

Table-IV : *Age distribution of the studied subjects.*

Age group (years)	Controls (n=60)	Patients (n=66)
	No. (%)	No. (%)
10-19	5(8.3)	6(9.1)
20-29	9(15.0)	9(13.6)
30-39	14(23.3)	19 (28.8)
40-49	16 (26.7)	15(22.7)
50-59	8 (13.3)	9 (13.6)
$\geq$ 60	8 (13.3)	8 (12.1)

Table-V. *Distribution of age by thyroid functional status.*

Age group (years)	Euthyroid	Hypothyroid	Hyperthyroid
	No (%)	No. (%)	No. (%)
10-19	3(6.67)	3(42.85)	0
20-29	6(13.33)	2(28.57)	0
30-39	15(33.33)	1(14.29)	3(21.43)
40-49	9 (20.00)	1 (14.29)	5 (35.71)
50-59	7 (15.56)	0	3(21.43)
≥ 60	5 (11.11)	0	3 (21.43)
Total	45	0	3 (21.43)

Table-VI : *Urinary iodine concentration (UIC) in controls and patients by age distribution.*

Age groups (years)	Urinary iodine concentration (mg/dl) (Mean±SD)		F value	P value
	Control	Patients		
10-19	11.41±0.76	10.25± 1.84	6.00	0.0973
20-29	11.69± 0.40	11.38± 2.14	36.50	0.7237
30-39	12.09± 1.82	10.33± 1.60	62.00	0.0095*
40-49	11.53± 1.87	10.42± 2.04	73.50	0.1046
50-59	9.98± 1.06	9.78± 2.25	35.00	0.9231
60+	10.82± 2.50	10.08± 1.04	29.00	0.7518
F	2.2423	0.6553		
P	0.0631	0.6586		
LSD (at P<0.05 level)	1.1819	1.3097		

Analysis by one-way ANOVA (along column)

Analysis by Mann-Whitney U test (along row)

*Significant difference

Table-VII : *Urinary iodine concentration in the controls and the patients with multinodular goitre.*

Subjects	Urinary iodine concentration (mg/dl)	
	Mean± SD	Median
Control (n=60)	11.35 ± 1.76	11.64
Patients (n=66)	10.41±1.83	10.02

t =2.94; p= 0.004 (Significant difference)

Analysis by Student's unpaired 't' test.

Table-VIII : *Mean (± SD) age of the patients in relation to thyroid functional status.*

Functional status	Age in years (mean± SD)
Euthyroid	39.42 ± 13.16
Hypothyroid	24.71 ± 11.18
Hyperthyroid	45.64 ± 13.96

F	3.7205
P	0.0133*
LSD (at P< 0.05 level)	6.0544

Analysis by one-way ANOVA (along column)

*Significant difference

Table-IX : *Urinary iodine concentration in controls and patients by sex.*

Sex	Urinary iodine concentration (mg/dl) (Mean± SD)		t value	P value
	Controls	Patients		
Male	11.70 ± 1.37	10.79 ± 1.28	1.45	0.166
Female	11.28 ± 1.76	10.35± 1.89	2.59	0.012*
t	0.70	0.62		
p	0.489	0.535		

Analysis by Student's unpaired 't' test

*Significant difference

Table-X : *Duration of goitre in relation to thyroid functional status.*

Functional status	Duration of goitre (years) (Mean± SD)
Euthyroid	8.11± 7.15
Hypothyroid	6.14± 6.74
Hyperthyroid	14.21± 8.13

F	37.6300
P	0.0000*
LSD (at P< 0.05 level)	3.7228

Analysis by one-way ANOVA (along column)

*Significant difference

Table-XI : *RAIU in 2 hours and 24 hours in different thyroid functional status of the patients with multinodular goitre.*

Thyroid status	RAIU in percentage (mean±SD)	
	2 hours	24 hours
Euthyroid	5.84± 1.43	15.07± 6.43
Hypothyroid	9.86± 14.65	15.71± 14.81
Hyperthyroid	13.50± 13.06	24.29± 17.45
F	28.1671	54.4293
P	0.000*	0.000*
LSD (at P<0.05 level)	3.8380	5.390

Analysis by one-way ANOVA (along column)

*Significant difference

Normal values:

RAIU in 2 hours = 5- 15%

RAIU in 24 hours = 15-40%

Table-XII : *UIC in different thyroid functional status of patients with multinodular goitre and controls.*

	Urinary iodine concentration. (mg/dl) (Mean± SD) Patient	Control (n-60)	P value
Euthyroid (n=45)	10.35± 1.45		0.003[1]
Hypothyroid (n=7)	11.19± 2.46	11.35±76	0.8776[2]
Hyperthyroid (n=14)	10.19 ± 2.53		0.1015[2]
F	0.7606		
P	0.4716		
LSD (at P <0.05 level)	1.2970		

Analysis by one-way ANOVA (along column)

[1]Statistically significant by Student's unpaired 't' test

[2] Analysis by Mann-Whitney U test

Table-XIII : *Distribution of the patients by the median UIC of controls (11.64mg/dl).*

UIC(mg/dl)	No. of patients	Percentage
< 11.64	50	75.76
≥ 11.64	16	24.24

Table-XIV : *Distribution of patients with different thyroid functional status by the median UIC of controls (11.64mg/dl).*

Functional status	Urinary iodine concentration (mg/dl)	
	<11.64 µg/dl No (%)	≥11.64 µg/dl No. (%)
Euthyroid (n=45)	36 (80.00)	9 (20.00)
Hypothyroid (n=7)	4 (57.14)	3 (42.86)
Hyperthyroid (n=14)	10 (71.43)	4 (28.57)

Table-XV : *Correlation of different parameters in the patients.*

Determinants of r	r value	P value
UIC		
Vs age	-0.1179	0.346
Vs duration of goitre	-0.1390	0.266
Vs T3	-0.1654	0.185
Vs T4	-0.1371	0.272
Vs T3/T4 ratio	-0.0052	0.967
Vs TSH	0.0852	0.496
Vs RAIU- 2 hours	-0.3502	0.004*
Vs RAIU - 24 hours	-0.4476	0.000*
Vs FNAC	0.796	0.149
Vs thyroid functional status	0.1293	0.301

Analysis by Spearman correlation-cofficient

*Significant correlation

Discussion

The present study was designed to find out the iodine status in patients with multinodular goitre.

Sixty-six patients were recruited from the thyroid clinic, IPGM&R, Dhaka, and at the same time 60 healthy subjects were taken as controls during the period of January, 1996 to December,1997.

Bangladesh is a zone of endemic goitre.[14] In several studies,[3-5,11] iodine deficiency was considered to be a prime factor for the development of endemic goitre and its consequences, like simple and toxic multinodular goitres. During iodine deficiency, adaptive changes occur in the thyroid gland where the principal regulator is TSH, which by prolonged stimulation causes goitre formation.16 In endemic goitre, serum T4 decreases, but serum T3 increases and TSH is normal or increased.[16]

Along with deficiency of iodine, environmental goitrogens also contribute to endemic goitre formation.[17] Subthreshold iodine intake is associated with increased TSH, goitre,decreased T_4 and increased T_3/T_4 ratio.On the other hand,excessive iodine intake can also cause increased TSH and decreased T4, goitrous hypothyroidism.[18] These findings are also observed in endemic goitre.[19] From these observations, it is apparent that serum T_3, T_4, TSH levels do not always reflect the actual iodine status in an iodine deficient individual.

In the iodine deficiency state, RAIU is increased with a slow turnover. But this test can be affected regular intake of iodine containing salts, foods, drugs, etc.[20] Approximately 90 percent of ingested iodine is usually excreted in the urine, unless there are defective intestinal absorbtion and impairment of renal function.[15] Therefore,measurement of urinary excretion of iodine from the casual sample has been taken as a principal parameter to observe iodine status both in patients and healthy controls for comparison.

The National IDD survey in Bangladesh (1993) revealed total goitre prevalence of 47.1 percent,[14] but no study has been carried out in this country on general population regarding the prevalence and aetiology of different types of goitre. A retrospective study conducted in the thyroid clinic, IPGM&R, revealed the spectrum of thyroid disorders, of which simple multinodular goitre and toxic multinodular goitre comprised 8.4 and 3.1 percent, respectively, and were considered to be iodine deficiency related disorders.[6] The present study was undertaken to find out the iodine status in the multinodular goitrous patients in comparison with healthy controls.

Among the studied patients, 68.18 percent were euthyroid, 21.21 percent were hyperthyroid and the rest 10.61 percent were hypothyroid, which corroborates with those of other studies.[6,21]

Age of the patients in this series ranged from 15 to 70 years, the majority being in the fourth decade of

life. The age incidence of multinodular goitre in this series also conforms to those found in other studies.[6,22] Most of the patients with euthyroid multinodular goitre were in the age group of 30-39 years and that of toxic multinodular goitre were in between 40-49 years. These age incidences tally with study of Alam et al,[6] in which, peak age incidence of simple multinodular goitre was in the fourth decade and that of toxic multinodular goitre was in the fifth decade. Gradual shift of the peak age incidence to the higher age groups in these thyroid disorders is a testimony to the concept of progression of iodine deficiency related simple diffuse goitre and nontoxic solitary nodule through multinodular goitre, eventually to toxic multinodular goitre.[8-10]

In this series, 25.7 percent of patients were found in the age group of 50-70 years.But in other studies, in which solitary thyroid nodule and multinodular goitres were not considered separately, most of the nodular goitres were observed in elderly subjects.[23] In severe endemic, multinodular goitre is reported to present at earlier decades.[17] This may explain the occurrence of the condition at relatively early parts of life in this series.

In the present study, the mean (±SD) UIC of the patients in the age group of 30-39 years was significantly lower than that of the controls. The mean (±SD) UIC were also less than those of controls in other age groups, but the differences were not statistically significant. May be the fourth decade is the most vulnerable part of life to the development of multinodular goitre. It may explain the peak incidence of multinodular goitre in this age group of patients in the studies conducted in our population.[6, 21,22]

Thyroid disorders are more common in female. This is also true for nodular goitre. The female to male ratio in the present series was 7.25:1. Alam et al.[6] observed female to male ratio of 5.30:1 and in the study of Mansur,[21] it was 5.25:1. Higher female to male ratio in this study may be due to variation in selection groups and small number of population studied. The female preponderance was also observed in the subgroups, i.e. euthyroid multinodular goitre, multinodular hypothyroid and toxic multinodular goitre. The mean (±SD) UIC in female patients was lower than that of male but the difference was not significant statistically and the same observation was made in case of the female and male controls. No significant difference of Mean (± SD) UIC was observed in male patients and male controls, though Mean (±SD) UIC was found lower in male patients than that of male controls. However, Mean (±SD) UIC of female patients was lower than that of female controls with significant difference.It can be deduced that females are most likely to be affected, which conforms to the higher prevalence of multinodular goitre in female in different studies.[6,21,22]

The Mean (± SD) duration of goitre among the subgroups was longest in cases of multinodular hyperthyroid and shortest in hypothyroid patients. Prolonged duration of multinodular hyperthyroid correlates with the concept of natural history of toxic multinodular goitre which developed in longstanding simple multinodular goitre in older age group.[8-10] There was significant difference of Mean (± SD) duration of goitre among the patients with different thyroid functional status. But no significant correlation was observed in duration of goitre with UIC.

In the present study, mean serum T_3, T_4, and TSH were within normal limits but mean T_3/T_4 ratio was raised in patients with euthyroid multinodular goitre.

In iodine deficient goitre, serum T_3 is maintained at normal or elevated levels despite a lowering of serum T_4. This situation can best be explained by increased thyroidal secretion of T_3 relative to T_4 in these goitrous subjects. The mechanism of preferential T_3 secretion by the iodine-deficient thyroid remains incompletely understood but is probably due to the combination of increased thyrotrophic stimulation and hypoiodinated thyroglobulin.[24] Furthermore, recently synthesized thyroglobulin molecules which contain greater quantities of T_3 than T_4 are particularly sensitive to TSH and undergo proteolysis earlier than old thyroglobulin which gets progressively more iodinated with a higher T_4 content.[25] On the other hand,when a patient of iodine deficient goitre is supplied with increased dietary iodine, initially there is rapid increase of serum T_3 associated with rapid decrease of serum TSH. This is followed by gradual increase of serum T_4 and subsequent decrease of serum T_3.[26] In the same way, increasing use of iodized salts by the patients after the development of goitre might have changed the serum T_3, T_4 and TSH levels. This is the most likely reason why serum T_3, T_4 and TSH levels were found normal in the present series in euthyroid multinodular goitre.

In iodine deficiency state, serum TSH is expected to be high,[19] though some authors found no change in TSH concentration in iodine deficiency goitre.[27,28] TSH concentration may become normal in longstanding goitre when it reaches a stable level.[27,28] So, normal serum TSH in euthyroid multinodular goitre could be explained by most longstanding euthyroid multinodular goitre state in a substantial proportion of cases in the present study.

An alteration of serum T_3 / T_4 ratio in favour of T_3 has been reported by many authors.[19,27] Some authors reported a fall in serum T_4 only with no change in T_3.[19,28,29] Others observed similar changes occurring in experimental iodine deficient rats and have documented an increase in the ratio of immunoassayable T_3 and T_4.[30,31] Thus, raised serum T_3/T_4 ratio in euthyroid multinodular goitre in the present series conforms to above observations.

The observed thyroid hormonal status in multinodular hypothyroid and toxic multinodular goitre were compatible with the functional status of the individual disease.Statistically, no significant correlation was found in serum T_3 , T_4 and TSH with urinary iodine concentration.

The RAIU of the patients in an iodine deficient area is usually increased due both to increased specific activity of iodine tracer as well as the stimulation of the thyroid gland by TSH. It is important in nontoxic goitre – high uptake supports iodine deficiency, antibody negative Hashimoto's disease, where low uptake is seen in silent thyroiditis, subacute thyroiditis, excess iodine intake and Hashimoto's thyroiditis.32 In the euthyroid patients, Mean (± SD) RAIU in 2 hours and 24 hours were 5.84 (±1.43) and 15.07 (±6.43) percent, respectfully. These normal mean values may be due to the fact that patients were taking iodized salt after the development of goitre which had influence over the RAIU.

In toxic multinodular goitre, two patterns of RAIU with scan can be distinguished, some patients show increased RAIU with irregular patchy distribution and in others,distinct hot nodules occur with marked localized RAIU and no uptake between the hot nodules.[2] Administration of supplemental iodine to subjects with endemic iodine deficient goitre can result in the overproduction of thyroid hormones. This response termed Jodbasedow phenomenon in which RAIU is low. The Mean (± SD) RAIU in 2 hours and 24 hours were 13.50 (± 13.06) and [24.29] (±17.45) percent, respectively. These pictures might be due to the effects of iodized salt on RAIU, as because patients were taking iodized salt. However, there was significant negative correlation of RAIU in 2 and 24 hours with UIC.

The Mean (± SD) UIC in the patients and controls were 10.41 (±1.83) and 11.35 (± 1.76) µg/dl, respectively, and the difference was statistically significant. In 75.76 percent cases, UIC was below 11.64 µg/dl (median urinary iodine level of the controls).But none had urinary iodine concentration below 5 µg/dl. The median value of UIC of the patients and controls were 10.02 and 11.64µg/dl, respectively. It appears that the patients with multinodular goitre had been suffering from iodine deficiency in comparison with control subjects.

The only epidemiological criteria available for assessing the severity of IDD is based on median urinary iodine levels, in which median urinary iodine level >10.0 µg/dl, was considered as normal.[15]

It is established that UIC depends on the intake of iodine.[15] Salt iodization programme has been started in Bangladesh since 1988. Since then, the people have been taking iodized salt more or less regularly. In addition, the patients particularly became conscious regarding the intake of iodized salt after the development of goitre.Thus it is not unexpected to find median urinary iodine levels of both the patients and control subjects above the critical level. Despite the conscious intake of iodized salt, the patients had lower median urinary iodine level in comparison with controls and Mean (± SD) UIC of the patients was significantly lower than that of controls.From these observations, it appears that if the patient had not been suffering from iodine deficiency,there should not be any significant difference of urinary iodine concentrations between the patients and control subjects.

Therefore, it can be deduced that the patients with multinodular goitre had been suffering from iodine deficiency for long time and have been taking iodized salt after the development of goitre, with consequent rise in UIC, but not up to the level of control values.

A study done in 1993, the median urinary iodine level was found 7.75 µg/dl among the population of Bangladesh irrespective of presence or absence of goitre .[16] In 1994, a study amongst the students of Dhaka University showed median UIC 5.03 and 9.64 µg/dl in the resident and non-resident students, respectively.[33] Another study in 1995, amongst the school children of Dhaka University campus showed median value of UIC 10.1 µg/dl irrespective of the presence or absence of goitre.[34] The above observations reflect the iodine deficiency states in different groups of population in different periods with gradual overcoming of iodine deficiency states due to salt iodization programme. This concept agree with the view that iodine deficiency existed among the patients with multinodular goitre, the urinary iodine level subsequently increased due to intake of iodized salt after the development of goitre .

In conclusion it reveals that the patients with multinodular goitre in this series had been suffering from iodine deficiency and despite the intake of iodized

salt after the development of goitre , majority of them were still in a relatively iodine deficiency state.

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EFFECT OF IPRIFLAVONE ON PAIN DUE TO RECENT OSTEOPOROTIC VERTEBRAL CRUSH FRACTURE AND ON RADIAL BONE MINERAL DENSITY

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Summary

Ipriflavone conserves bone mass in postmenopausal osteoporosis. Salmon calcitonin and alendronate, two other anti-resorptive drugs, are found to have analgesic effect in osteoporotic acute vertebral fracture. This study was undertaken to find out if ipriflavone also possessed such effect. Opportunity was taken, at the same time, to study the effect of one-year ipriflavone therapy on the bone mineral density of patients with recent vertebral fractures. Thirty-two women with recent osteoporotic vertebral fractures were randomly assigned to ipriflavone or placebo groups. Ipriflavone was given at the dose of 200 mg thrice daily. NSAIDs were given ad lib. *Calcium carbonate 1 g daily was given to all subjects. Intensity of pain at rest, on movement and on pressure, pain rating on a 10-point visual analog scale (VAS), degree of mobility impairment and supplementary analgesic use at the end of three months were used for assessment of the analgesic effect. Distal radial BMD was measured for assessment of the effect of the drug on bone mineral density. Serum calcium, phosphate, 24-hour urinary calcium & phosphate and calcium/creatinine ratio were measured to study its effect on calcium metabolism. Fourteen in the ipriflavone and 12 in the placebo groups completed the trial. At the end of three months, all pain variables decreased significantly in both groups. Intensity of pain at rest & at pressure and supplementary analgesic use were significantly lower in the ipriflavone compared to the placebo groups. BMD of the distal radius increased from 0.313±0.035 to 0.325±0.034 g/cm^2 (not significant) after one year of treatment with ipriflavone. Serum calcium level increased from 8.98±0.30 to 9.36 ± 0.39 mg/dl (p=0.01). 24-hour urinary phosphate dropped from 508.14±162.46 mg to 455.28 ± 93.43 (p=0.01). In the placebo group, BMD decreased from 0.309±0.032 to 0.301±0.032 g/cm^2 (not significant). 24-hours urinary phosphate decreased from 607.66±244.97 to 544.41±198.75 mg (p=0.02). Compared to the placebo group, the BMD was significantly higher (p=0.02) and 24-hour urinary calcium excretion significantly lower (p=0.03) in the ipriflavone group at the end of 12 months.*

Other parameters of calcium metabolism did not change significantly. The study shows that ipriflavone exerts an analgesic adjuvant property in acute osteoporotic vertebral fracture. Its long-term use is associated with a favorable calcium homeostasis and conservation of bone mass.

Introduction

Osteoporosis is defined as a systemic disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in fragility and susceptibility to fracture[1]. Bone mineral density (BMD) measurements form the basis for a more practical and operational definition of osteoporosis with better clinical utility[2]. By current definitions, osteoporosis is defined as BMD >2.5 standard deviation (SD) below the young adult mean value [3]. The aim of treatment of osteoporosis is to maintain and to restore the bone mass density,[2] so as to prevent fractures and immobility in late life. Physical exercise, adequate calcium intake and avoidance of smoking and alcohol are important general measures for treatment and prevention of osteoporosis. Recent meta-analyses have shown the efficacy of estrogens, bisphosphonates and calcitonin in increasing the BMD and in reducing the incidence of fractures [4,5]. The adverse effects of long term therapy with estrogen and other agents provides strong rationale for development of alternatives to estrogen for the treatment of osteoporosis [6].

Ipriflavone is a synthetic isoflavone derivative. The efficacy of ipriflavone in preventing loss of bone mass and in increasing bone mineral content in patients with osteoporosis has been demonstrated in many randomized controlled trials [7-16]. In most of these studies, patients with recent fractures were excluded. Salmon calcitonin is found to have analgesic effect in acute osteoporotic vertebral fracture [17-19]. In one study, ipriflavone was found to possess the ability to

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decrease the acute bone pain caused by vertebral fracture of osteoporotic origin [20]. The present study was undertaken with a view to evaluating the analgesic effect of ipriflavone in a 3-month period of treatment and also to see its effect on BMD in a one-year period of treatment in postmenopausal osteoporotic women with vertebral fracture.

Materials and Methods

This open, randomized, prospective, controlled clinical trial was conducted in the department of Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Patients presenting with recent (within preceding two weeks) vertebral fracture due to postmenopausal osteoporosis in the rheumatology clinic between June and November 1998 were included in the study. Departments of orthopedic surgery, gynecology and some private practitioners in these fields were requested to send such patients to the rheumatology clinic during this period.

All patients were assessed clinically. Body mass index (BMI) was calculated. Following investigations were carried out routinely: complete blood counts including ESR, fasting blood glucose, alkaline phosphatase, ALT, serum calcium, serum inorganic phosphate, serum creatinine, routine urinalysis, 24 hours urinary calcium & phosphate, calcium/creatinine ratio, X-ray of thoracic and of lumbar parts of the vertebral column in antero-posterior and lateral views and BMD measurement. Densitometry of the distal radius of the non-dominant arm was done with the help of single x-ray absorptiometry. Women with severe back pain with onset within two weeks, age ³55 but £75 years, BMI <30 kg/m^2, with osteoporosis with vertebral fracture(s) confirmed by X-ray and BMD value of distal radius of non dominant arm lower than -2.5 SD (T-score) were included in the study. Those with peptic ulcer disease, serious co-morbidity, or known hypersensitivity to ipriflavone, with neurodeficit arising out of the vertebral collapse, with substantial scoliosis, moderate to severe osteophytosis or spinal fusion (determined by antero-posterior dorsal and or lumber spine X-ray), with secondary osteoporosis, were excluded from the study. Informed consent was obtained before entry into the trial.

The subjects were randomly assigned to either ipriflavone or placebo groups. Ipriflavone was given at the dose of 200 mg thrice daily after meals. Dummies of identical shape, size and color containing cellulose were given to the subjects in the placebo group. Non-steroidal anti-inflammatory drugs (NSAIDs) were given *ad lib*. Omeprazole 20 mg was given daily. Calcium carbonate 500 mg twice daily was given to all subjects. All subjects were seen by a physiatrist for appropriate physical treatment.

The subjects were instructed to keep an account of number of tablets used during the whole period of treatment. All patients were followed up monthly for a period of three months and then at the end of the study- after one year. In addition to this routine follow up, the subjects were advised to visit any time if they found any difficulty during the whole course of study. Pain variables and the safety of the drugs were assessed monthly for a period of three months. The following variables were used for assessment of pain: intensity of pain at rest, on movement and on pressure, pain rating on a 10-point visual analog scale (VAS), degree of mobility impairment and supplementary analgesic use. The intensity of pain was graded as follows: 0 = no pain, 1 = mild pain, 2 = moderate pain which interferes with function and needs control, 3 = severe pain present most of the time of the day demanding almost constant attention, 4 = totally incapacitated.[21] For assessment of drug safety, history of any new symptom was taken and following laboratory tests were performed: routine urinalysis, complete blood counts, Hb%, ESR, blood glucose, alkaline phosphatase, ALT, serum creatinine. For assessment of the effect of ipriflavone on bone mass, distal radial BMD was measured at the end of one year. Serum calcium, inorganic phosphate, 24-hour urinary calcium & phosphate and calcium/creatinine ratio were measured at one year to assess the effect of the drug on calcium metabolism.

Statistical analysis

Data were collected in a structured questionnaire. They were entered into the computer and analyses were done using SPSS PC 10+. Comparison of baseline characteristics of two groups of patients was tested by Mann-Whitney U test. The significance of the differences between pre and post treatment values of the continuous variables in the same group (intra-group) was estimated by Wilcoxon signed rank test. The significance of the differences between ipriflavone and placebo groups (inter-group) was evaluated by Mann-Whitney U test.

Results

A total of 32 female patients were included in the trial. Seventeen were assigned to the ipriflavone and 15 to the placebo groups. One patient in the ipriflavone group abandoned the study due to adverse reactions. Two subjects in the ipriflavone and three subjects in the placebo groups discontinued the study due to loss of interest or personal reasons. Eventually,

fourteen subjects belonged to the ipriflavone and 12 to the placebo groups. Data of these 26 valid completers were finally used in all subsequent comparative analyses. Baseline values of the relevant variables of these 26 subjects are shown in table 1. These values did not vary significantly between the ipriflavone and placebo groups (table 2).

At the end of three months, all outcome variables, viz., intensity of pain at rest, on movement and on pressure, VAS, degree of mobility impairment and supplementary analgesic use, showed significant improvement in both groups (tables 3 and 4). Intensity of pain at rest & at pressure and supplementary analgesic use were significantly lower in the ipriflavone group compared to the placebo group at the end of three months (table 5). The differences between the two groups in other pain variables were insignificant.

BMD of the distal radius increased from 0.313±0.035 to 0.325±0.034 g/cm^{2}after one year of treatment with ipriflavone, although this difference was not significant. Among the parameters of bone metabolism, serum calcium level was significantly (p=0.01) higher whereas 24-hour urinary phosphate level was significantly (p=0.01) lower in the ipriflavone group. Serum inorganic phosphate, serum alkaline phosphatase, 24 hours urinary calcium, and calcium/creatinine ratio in urine were lower (not significant) after one year of treatment with ipriflavone (table 6).

In the placebo group, BMD decreased from 0.309±0.032 to 0.301±0.032 g/cm^2 after one year of treatment. This decrease was not significant. Twenty-four hour urinary calcium, calcium/creatinine ratio increased (not significant) but serum alkaline phosphatase decreased insignificantly. Twenty-four hour urinary phosphate excretion decreased significantly in this group (table 7). Compared to the placebo group, the BMD was significantly higher (p=0.02) and 24-hour urinary calcium excretion significantly lower (p=0.03) in the ipriflavone group at the end of 12 months (table 8).

Six patients in the ipriflavone and five in the placebo groups developed adverse effects. All of them developed gastrointestinal symptoms. Anorexia, constipation, abdominal discomfort, nausea were the common adverse effects, and were present in both groups, suggesting a possible effect of calcium supplement and NSAIDs at least in some of them. Headache, vertigo, insomnia were also experienced by both group of patients (table 9). One patient treated with ipriflavone was withdrawn from the study because of dizziness and abdominal discomfort. No serious or life threatening events occurred during the study. In both the groups, there were some insignificant alterations in ALT, serum creatinine and in absolute lymphocyte or absolute granulocyte count during the one-year treatment (table 8). Two subjects in the placebo group, and none in the ipriflavone group, developed new fractures in course of the study.

Table-1: *Baseline characteristics of patients.*

Parameters	Patients (n-26)
Age (year) 62.12± 4.01	
Body mass index (Kg/m^2)	22.31 ± 3.46
Bone mineral density (BMD) g/cm^2	0.311 ± 0.033
Intensity of pain At rest	
At movement	
At pressure	1.30 ± 0.47
	2.61 ± 0.49
	2.38 ± 0.49
VAS	7.26 ± 0.87
Degree of mobility impairment	2.61 ± 0.50
Supplementary analgesic use	1.88 ± 0.32
Hb% (gm%) 11.04 ± 04	
ESR (mm) 34.50 ± 4.30	
TC (K/µL) 8.15 ± 1.52	
Alkaline phosphatase (U/L)	207.30 ± 38.77
Serum creatinine (mg/dl)	0.92 ± 0.12
Serum calcium (mg/dl)	9.00 ± 0.28
Serum inorganic phosphate (mg/dl)	4.09 ± 0.53
24 hours urinary calcium mg/24h	181.23 ± 41.47
24 hours urinary phosphate (mg/24h)	554.07 ± 206.61
Calcium/creatinine ratio in urine (mg/mg)	0.19 ± 0.04

Table 2: *Comparison of baseline characteristics of two groups of patients.*

Parameters		Ipriflavone (n-14)	Placebo (n=12)	P
Age (year)		61.21 ± 4.30	63.17 ± 3.54	NS
Body mass index (Kg/m^2)		22.47 ± 3.49	22.13 ± 3.56	NS
Bone mineral density (BMD) (g/cm^2)		0.313 ± 0.035	0.309 ± 0.032	NS
Intensity of pain	At rest	1.35 ± 0.49	2.57 ± 0.51	
	At movement	2.57 ± 0.51	2.66 ± 0.49	
	At pressure	2.50 ± 0.51	2.55 ± 0.45	NS
VAS		7.21 ± .80	7.33 ± 0.98	NS
Degree of mobility impairment		2.57 ± 0.51	2.66 ± 0.49	NS
Supplementary analgesic use		1.85 ± .36	1.91 ± 0.28	NS
Hb% (gm%)		10.87 ± 1.87	11.24 ± 0.87	NS
ESR (mm)		32.64 ±15.11	35.90 ± 14.01	NS
TC (K/µL)		8.08 ± 1.01	8.23 ± 1.33	NS
ALT (U/L)		22.17 ± 6.03	19.89 ± 5.62	NS
Alkaline phosphatase (U/L)		209.55 ± 34.71	202.70 ± 29.72	NS
Serum creatinine (mg/dl)		0.95 ± 0.09	0.89 ± 0.13	NS
Serum calcium (mg/dl)		8.98 ± 0.30	9.03 ± 0.26	NS
Serum inorganic phosphate (mg/dl)		4.15 ± 0.67	4.01 ± 0.32	NS
24 hours urinary calcium (mg/24h)		177.57 ± 39.41	185.50 ± 45.12	NS
24 hours urinary phosphate (mg/24h)		508.14 ± 162.46	607.66 ± 244.97	NS
Calcium/creatinine ratio in urine (mg/mg)		0.19 ± 0.03	0.19 ± 0.04	NS

Table-3: *Treatment response in the ipriflavone group after 3 months*

Parameters		Ipriflavone	(n=14)	P-value
		Month- 0	Month-3	
Intensity of pain	At rest	1.35 ± 0.49	0.71 ± 0.26	0.001
	At movement	2.57 ± 0.51	0.85 ± 0.36	0.001
	At pressure	2.50 ± 0.51	0.71 ± 0.46	0.001
VAS		7.21 ± 0.80	1.21 ± 1.25	0.001
Degree of mobility impairment		2.57 ± 0.51	0.85 ± 0.53	0.001
Supplementary analgesic use		1.85 ± 0.36	0.71 ± 0.26	0.001
Alkaline phosphatase (U/L)		209.55 ± 34.71	192.85±44.73	NS

Table 4. *Treatment response in the placebo group after 3 months (n=12).*

Parameters		Month- 0	Month-3	P
Intensity of pain	At rest	2.57 ± 0.51	0.58 ± 0.51	0.005
	At movement	2.66 ± 0.49	1.08 ± 0.51	0.001
	At pressure	2.25 ± 0.45	1.00 ± 0.00	0.001
VAS		7.33 ± 0.98	1.41 ± 0.79	0.002
Degree of mobility impairment		2.66 ± 0.49	1.16 ± 0.57	0.002
Supplementary analgesic use		1.91 ± 0.28	0.75 ± 0.45	0.002
Alkaline phosphatase (U/L)		202.70 ± 29.72	193.95 ± 24.32	NS

Table 5. *Comparison of the pain variables after 3 months in the ipriflavone and placebo groups.*

Parameters		Ipriflavone(n=14)	Placebo	
		P value	*(n=12)*	
Intensity of pain	At rest	0.71 ± 0.26	0.58 ± 0.51	0.009
	At movement	0.85 ± 0.36	1.08 ± 0.51	NS
	At pressure	0.71 ± 0.46	1.00 ± 0.00	0.03
VAS		1.21 ± 1.25	1.41 ± 0.79	NS
Degree of mobility impairment		0.85 ± 0.53	1.16 ± 0.57	NS
Supplementary analgesic use		0.71 ± 0.26	0.75 ± 0.45	0.01

Table 6. *Treatment response in the ipriflavone group after 12 months (n=14)*

Parameters	Month 0	Month 12	P
Bone mineral density (BMD) (g/cm^2)	0.313 ± 0.035	0.325 ± 0.034	NS
Alkaline phosphatase (U/L)	209.55 ± 34.71	184.75 ± 24.02	NS
Serum calcium (mg/dl)	8.98 ± 0.30	9.36 ± 0.39	0.01
Serum inorganic phosphate (mg/dl)	4.15 ± 0.67	3.83 ± 0.62	NS
24-hour urinary calcium mg/24h	177.57 ± 39.41	167.57 ± 27.69	NS
24-hour urinary phosphate (mg/24h)	508.14 ± 162.46	455.28 ± 93.43	0.01
Calcium/creatinine ratio in urine (mg/mg)	0.19 ± 0.03	0.18 ± 0.05	NS

Table 7. *Treatment response in placebo group after 12 months (n=12).*

Parameters	Month 0	Month 12	P
Bone mineral density (BMD) (g/cm^2)	0.309 ± 0.032	0.301 ± 0.035	NS
Alkaline phosphatase (U/L)	202.70 ± 29.72	183.54 ± 17.77	NS
Serum calcium (mg/dl)	9.03 ± 0.26	9.30 ± 0.32	NS
Serum inorganic phosphate (mg/dl)	4.01 ± 0.32	4.11 ± 0.64	NS
24-hour urinary calcium mg/24h	185.50 ± 45.12	193.83 ± 52.49	NS
24-hours urinary phosphate (mg/24h)	607.66 ± 244.97	544.41±198.75	0.02
Calcium/creatinine ratio in urine (mg/mg)	0.19 ± 0.04	0.20 ± 0.04	NS

Table 8. *Comparison of the therapeutic response in the ipriflavone and placebo groups after 12 months.*

Parameters	Ipriflavone (n=14)	Placebo (n=12)	P value
Bone mineral density (BMD) (g/cm^2)	0.325 ± 3.42	0.301 ± 3.25	0.02
Absolute lymphocyte count (K/µL)	2.58 ± 0.75	3.16 0.64	NS
Absolute granulocyte count (K/µL)	4.91 ± 0.88	5.09 ± 0.87	NS
ALT (U/L)	22.78 ± 4.13	19.33 ± 4.03	NS
Alkaline phosphatase (U/L)	184.75± 24.02	183.54± 17.77	NS
Serum calcium (mg/dl)	9.36 ± 0.39	9.30 ± 0.32	NS
Serum inorganic phosphate (mg/dl)	3.83 ± 0.62	4.11 ± 0.64	NS
24 hours urinary calcium mg/24h	167.57 ± 27.69	193.83± 52.49	0.03
24 hours urinary phosphate (mg/24h)	455.28 ± 93.43	544.41±198.75	NS
Calcium/creatinine ratio in urine (mg/gm)	0.18 ± 0.05	0.20 ± 0.04	NS

Table 9 : *Adverse effects in both groups.*

Symptom	Ipriflavone	Placebo
Gastrointestinal disturbances	6	5
Headache	2	1
Vertigo	1	1
Insomnia	1	1

Discussion

The mechanism of action of ipriflavone is not exactly known. It has been demonstrated that it suppresses bone resorption, inhibits differentiation of osteoclasts and reduces parathyroid hormone induced bone resorption [22-24]. It is reported to potentiate the effect of estrogens on bone metabolism [25-26], although the drug itself is devoid of intrinsic estrogenic activity [27]. This has rendered the drug suitable in situations where estrogenic effects are not desired, or, where vigilance for adverse estrogenic effects is difficult to organize.

In the present series, there was insignificant increase and decrease of the BMD in the ipriflavone and placebo groups respectively. In most of the randomized controlled trials with ipriflavone, BMD had increased significantly in the ipriflavone and decreased in the placebo groups [7-8, 12-13, 28-29]. In some others, it increased insignificantly, as in the present study, or remained static [10-11, 15-16]. In a few of these studies, the BMD was measured at distal radius [8, 10-11, 29]. The change in BMD was insignificant in the studies of Adami *et al*[10] and Gennari *et al* [11]. In the study of Valente *et al*, vertebral BMD increased significantly, whereas the BMD at distal radius increased insignificantly [8]. These findings may hint at a possibility that the ipriflavone induced increase in BMD was less pronounced at distal radius than in the axial skeleton. Since continued bone loss is the characteristic feature of patients with osteoporosis, conservation of the bone mass may be an acceptable goal of treatment. Moreover, inclusion only of patients with recent fractures limited the sample size. The gain in bone mass in the ipriflavone group might have been rendered significant if the sample size were larger. The increase in serum calcium and the decreases in 24-hour urinary phosphate and calcium excretion may indicate a favorable calcium homeostasis in the ipriflavone group. The decrease in 24-hour urinary phosphate in the placebo group might have been caused by concomitant calcium supplementation. Because of the lack of laboratory facilities, we could not measure serum bone Gla-protein, calcitonin, parathyroid hormone, osteocalcin and urinary hydroxyproline. Although, the present clinical trial was not designed to study the effect on fracture rate, the development of new fractures in two patients in the placebo group may also indicate a positive effect of ipriflavone on bone health.

The more important part of our study was the evaluation of the analgesic effect of ipriflavone in acute pain of osteoporotic vertebral fracture. Alendronate, another antiresorptive drug, is reported to reduce the number of days of bed disability and days of limited activity caused by back pain in postmenopausal women with existing vertebral fractures [30]. Salmon calcitonin is found to possess analgesic effect in acute pain due to recent osteoporotic vertebral crush fractures [17-19]. The effect of ipriflavone in acute or chronic pain in post-menopausal women with osteoporotic vertebral fracture is less well studied. Scali *et al* reported on analgesic effect of ipriflavone in osteoporotic vertebral pain [20]. Agnusdei *et al* also reported significant improvement in pain and motility in patients treated with ipriflavone [31]. In the present study, as expected from the natural course of any fracture pain, all pain variables decreased significantly in both the groups. As patients in both groups were allowed to use NSAIDs *ad lib*, supplementary analgesic use remained the most important variable to assess the analgesic effect of the drug. The intensity of pain at rest and on pressure, and the supplementary analgesic use were significantly lower in the ipriflavone than in the placebo group.

Finally, it may be concluded from this limited study that ipriflavone is a safe anti-resorptive drug for post-menopausal women with osteoporotic vertebral fracture. Long-term use of this agent conserves the bone mass. In acute pain of vertebral fracture, it works as an analgesic adjuvant, thereby limiting the consumption of NSAIDs.

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ESTABLISHMENT AND VALIDATION OF MINI-DOSE (1μCi) ^{14}C-UREA BREATH TEST FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECITON

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Summary

Helicobacter pylori is associated with a number of common pathologies including peptic ulcer, gastric cancer and MALT lymphoma. Eradication of Helicobacter pylori alters the natural history of these conditions. But, no unique regimen is developed so far for the universal use. So, diagnosis of this is important not only for treatment but also for documentation of eradication. It can be diagnosed by invasive and non-invasive methods. Breath test is a noninvasive method and can be done by using isotope carbons. ^{14}C- Urea is one of the isotopes, that can be used for breath test. The Urea Breath Test is an easy, simple, non-invasive global test for the diagnosis of H. pylori infection. As it associated with radioactivity, the dose (3 μCi of the ^{14}C is needed to be reduced to an acceptable level. With this aim, this study was undertaken to establish the test with a lower level of radioactivity and to validate this test with other standard tests for H. pylori infection. Initially the test was performed with 1 μCi dose of ^{14}C- Urea on 7 known positive and 9 known negative patients. A cut of value was calculated and this value was then validated against standard tests-Histology and CLO on 98 patients of unknown H.pylori status. ^{14}C- Urea -breath test was positive in 44 patients. Histology and CLO were positive in 43 and 39 patients respectively. Against 45 H.pylori positive (based on positively of either or both and histology) positive ^{14}C- Urea breath test were judged; 42 were true positive, 0 was true negative, 2 were false positive and 3 were false negative. So, the sensitivity and specificity of' this test were 93.33% and 96.23% respectively.

Introduction

Helicobacter pylori is a gram-negative microaerophilic curved flagellated organism which resides under the thick mucus layer of the antrum of the stomach and gastric metaplastic area of the duodenal bulb.[1] This infection is acquired mostly in childhood[3,4,5] and remains so if not eradicated by treatment. In adults, 40-50 percent of world population and around 80% of population of developing countries are infected with this organism.[2-6] Helicobacter pylori (H.pylori) is well-known for its association with a number of gastroduodenal pathologies e.g. Duodenal ulcer (85-100%), gastric ulcer (58-96%), gastric cancer (59-100%), mucosa associated lymphoid tissue (MALT) lymphoma (100%).[7-11] Eradication of H.Pylori cures its associated duodenal ulcer, gastric ulcer and MALT lymphoma.[12-16] Eradication in early stage of gastric adenocarcinoma prevents further progression[17]. Unfortunately, no unique treatment regimen is developed so far, for universal use for the eradication of H.pylori. Classical triple therapy with Colloidal Bismuth subcitrate (CBS), Metronidazole and Tetracycline or Amoxycilline is associated with side effects and poor compliance.[18,19] Metronidazole is seen to be resistance in many patients of developing counties.[20,21] Subsequently adopted regimen, composed of a proton-pump inhibitor (e.g. Omeprazole), Clarithromycin and Amoxycillin or Metronidazole was also variably effective in the eradication of H. Pylori.[22-24] H.pylori is the important but not the only factor for the mentioned gastroduodenal pathologies.[25] So, diagnosis of H.pylori infection in these clinical setting is not only for treatment but also for the documentation of eradication after treatment.

Diagnosis of H.pylori may be done either by direct demonstration of the organism through histology, culture and PCR or indirectly through rapid urease test (CLO), serology and urea breath test (UBT). Direct demonstration and (CLO) needs invasive procedure - endoscopy, serology, though highly sensitive (95%) and specific (95%)[26] is not suitable for all purposes because it remains positive for months to years even after eradication[27]. Urea breath test on the other hand is a non-invasive highly sensitive test for the diagnosis of H.pylori.[28]

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The strong enzyme, "urease" of H.pylori hydrolyses urea with the production of ammonia and carbondioxide (CO_2) which in the intestinal level is absorbed and is excreted in expiratory air.[29] Using isotope carbon (in urea) test meal, the amount recovered from the breath sample can be measured and thus urease activity may be assessed. Sensitivity and specificity of this test arc 96% and 100% respectively and positive result always indicates an active infection.[30] Two isotopes ^{13}C and ^{14}C urea may be used. ^{13}C involves time consuming, cumbersome procedure and requires costly machine, Mass Spectrometer, which is not available in many centres. On the other hand, breath test with ^{14}C - urea, may be done rapidly through easy and simple procedure and by relatively cheaper machine, Beta counter (Liquid Scintillating machine), which is available in most centres.[31] Only problem is with its radioactivity, the dose of which was successively reduced from initial higher level (10.8 μCi) to relatively lower (2 μCi) level.[32] This study was thus undertaken with the following aims and objectives.

Aims and Objectives

(1) To establish a urea breath test with a minimum dose of radio-activity of ^{14}C carbon.

(2) To evaluate the sensitivity and specificity of this urea breath test with other standard test for the diagnosis of Helicobacter pylori infection.

Patients and Methods

This was a prospective study done at the department of Gastroentesology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK from October '95 to July '96.

Patients attended the gastroenterology clinic of Hammersmith Hospital for various upper gastrointestinal complaints were primarily selected. Short history abut the complaints were taken. Exclusion criteria were, age below 18 and above 70 years. females in whom pregnancy could not be excluded, patients on or had antibiotics, including imidazoles, proton-pump inhibitors and colloidal bismuth subcitrate within 4 weeks of the study. Patients having reflux esophagitis and major debilitating diseases were also excluded. Detailed description of the procedures including exposure to radioactivity was explained to the patients. Informed written consents were taken from the patients. They also consented to be endoscoped and biopsied from the antrum and body of the stomach. The study was approved by the hospital ethics committee.

Modification of the standard ^{14}C urea breath test

Initial test with higher dose of ^{14}C-urea (10.8 μCi) was associated with a pre-test high calorie carbohydrate and fatty diet to delay the gastric emptying and thus maximizing the contact of urease with ^{14}C-urea test dose.[32,33] Mouth-wash with urea was also part of the procedure to eliminate the possible urease activity. Further reports on the procedure were without mouth wash and without pre-test meal, but the results were not changed.[34] In subsequent reports, the dose of radioactive isotope was gradually reduced without being changed sensitivity and specificity of the test. Following the publication to perform the test with 92 KBq dose of ^{14}C-urea, we soon came to realize that the difference between the counts in the breath-samples of H.pylori negative and positive patients were so great that we could reduce the amount of ^{14}C-urea to only 37 KBq (1 μCi), which is just below the amount used by others[35,36]. With this dose, we performed the test on histologically and microbiologically proven 7 H.pylori positive and 9 H.pylori negative patients. Results showed a highly statistical significant differences between two groups. A cut off value was set by adding 3 standard deviation (S/D) to the mean of 9 negative values arid this was validated against rapid urease test and histology of endoscopic biopsy samples of the stomach.

^{14}C-Urea breath test

After an overnight fast, a basal breath sample was collected from patients, attended the endoscopy unit of the department. Patients then removed false teeth (if present), cleansed their mouths with water and were given 37 KBq) (1 μCi) of ^{14}C - labeled urea dissolved in 25 ml of water which they drank through a straw with minimum contact with oral cavity. Patients were then asked to remain seated for half an hour and breath samples were taken at 10, 20 and 30 minutes by blowing through a disposable drinking straw into a liquid trap and then into a 20 ml vial containing 2 ml of methanol based 1 M Bensethonium hydroxide (hyamine) and a few amount of thymolphthalein, as pH indicator. Liquid trap prevent accidental ingestion of hyamine. 2 mmol carbonidioxide was trapped, which was indicated by change of deep blue color of the solution into a colorless fluid. Time taken for this was 1-3 minutes. Each 2 mmol of CO, collected at basal, 10, 20 and 30 minutes breath in hyamine were then transferred into the scintillation vials and by adding 1 ml of scintillatant (Hisafe-3) in each, radio activity was counted together with an activity standard containing 10% of the administered dose. Activity in each tube obtained as disintegration per minute (dpm). Results were expressed as follows :

$$CO_2, \text{ recovery} = \frac{\text{\% dose recovered}}{\text{mmol } C0^2 \text{ trapped}} \text{ X Body weight of the patient (Kg)}$$

Rapid Urease Test (CLO) and Histology of biopsy

All patients having performed ^{14}C-urea breath test were endoscoped on the same day and biopsies in duplicate were taken from the antrum, 5 cms proximal to the pylorus and body of the stomach. Biopsies from the antrum and body (one each) were used for rapid urease test. Two other biopsy specimens were used for histological examination. They were fixed in formaline, embedded in paraffin, and stained with modified Geimsa stains. Histopathologist examined the slides independently and reported according to his findings. H.pylori positive (HP+) patients had short, curved, rods seen adjacent to the mucosal cells in the stained sections.

Patients who showed either both or single test positive for H.pylori were considered as H.pylori positive (HP+) and those failed to show positively in either test were regarded as H.pylori negative (HP-) persons.

Statistical analysis of data

The result of ^{14}C-urea breath test of known HP+ and HP- patients were compiled and statistical analysis was done by the unpaired student's -t test to find the significant difference between these two groups.

Table-I : *Age distribution of observed patients (n=98)*

Age range (Years)	No. in each range	Average percentage	Mean age (Years)
21 - 30	13	13.2	
31 - 40	17	17.3 5	
41 - 50	25	25.51	47.89
51 - 60	21	21.43	
61 - 70	22	22.45	

Table-II : *Sex distribution of examined patients (n=98)*

Total No. of Patients	Male	Female	M : F
98	52 (53.06%)	46 (46.94%)	1.13

Table-III : *Values calculated) of ^{14}C-Urea breath test recovered in 7 known HP+ patients (n=7)*

Sl. No.	At 10 minutes		At 20 minutes		At 30 minutes	
	Each pts	Mean	Each pts	Mean	Each pts	Mean
1.	2.221		4.675		2.469	
2.	6.609		3.671		3.127	
3.	0.915	3.992	4.280	4.224	2.580	3.101
4.	5.094		2.959		1.922	
5.	4.553		4.862		5.759	
6.	4.890		4.907		4.293	
7.	3 .660		4.213		1.634	

Table-IV : *Values of ^{14}C-Urea breath test recovered in 9 known HP-patients (n=9)*

Sl. No.	^{14}C- at 10 min		^{14} C- at 20 min		^{14}C- at 30 min	
1.	0.505		0.494		0.487	
2.	0.502		0.491		0.491	
3.	0.500		0.492		0.4884.	
4.	0.499	0.5 ±	0.493	0.495 ±	0.490	0.49 ±
5.	0.500	0.02 SD	0.495	019 SD	0.492	0.018SD
6.	0.497		0.496		0.489	
7.	0.497		0.497		0.493	
8.	0.493		0.498		0.491	
9.	0.504		0.499		0.489	

Table-V : *Results of 3 tests done on all observed patients (n=98)*

Total No. of Patients	Urea breath test		Rapid urease test		Histology	
	+	-	+	-	+	-
98	44	54	43	55	41	57

Table-VI : *Helicobacter positive (HP+) and Helicobacter negative (HP-) on the basis of rapid crease test (CLO) and histology.*

Total No. of patients	Both rapid urease test (CLO) & Histology positive	Rapid urease test positive only	Histology positive only	Total positive	Total negative
98	39	4	2	45 (45.92%)	53 (54.08%)

Table-VII : *Comparison of ^{14}C-Urea breath test and positive HP patients*

Total No. HP+ patients	Total no. of Positive ^{14}C UBT	True Positive	True Negative	False positive	False Negative	Sensitivity	Specificity
45	44	42	0	2	3	93.33%	96.23%

Results

Primarily, we performed ^{14}C-Urea breath test with a dose of 1 μCi (37 KBq) on 16 patients, of which 7 were positive and 9 were negative for H.pylori, as diagnosed by histology and microbiology. Amount of radioactivity recovery at 10, 20 and 30 minute breath sample were measured. For the negative (HP-) subjects the mean values were 0.5 ± 0.02 SD, 0.495 ± 0.019 SD and 0.49 ± 0.018 SD respectively. Values for H.pylori positive (HP+) subjects were higher enough to differentiate from that of negative subjects.

Taking the average value of 0.55% (Mean ± 3SD) as cut-off between positive and negative, the ^{14}C-Urea breath test was validated against rapid urease test (CLO) and histology for H.pylori infection.

Altogether 98 patients were included in this study for validation of ^{14}C-Urea breath test with a dose of 1 μCi (37 KBQ) of ^{14}Carbon. The age range of these patients was from 22 to 70 years, with a mean of 47.89 years. Most (around 70%) of these subjects were above 40 years of age. Of 98, 52 (53.06%) were male and 46 (45.96%) were female, with a male : female was 1.13.

Forty four of 98 patients were found positive for ^{14}C-Urea breath test, while 54 were negative. Rapid urease test (CLO) was positive in 43 patients and negative in 55 patients. Histology for H.Pylori was positive in 41 patients, while negative in 57 patients.

In this study, examined subjects were considered H.pylori infected if both rapid urcase test and histology or any one of them was found positive. With these criteria, 45 (45.92%) of 98 subjects were H.pylori positive (HP+) and 53 (54.08%) were H.pylori negative (HP-).

When ^{14}C-Urea breath test was compared with these 45 positive patients, it was fund to be true positive (both group positive) in 44 cases, true negative (negative for UBT and other test) in 51 cases, false positive in 02 cases and false negative in 3 case. So, the calculated sensitivity and specificity were 93.33% and 96.23% respectively.

Discussion

Urea breath test (UBT) is the qualitative and to some extent the quantitative measure of the enzyme urease, of H.pylori. On ingestion of urea, it is hydrolyzed by this enzyme with the production of ammonia and carbondioxide.[37] In presence of H.pylori infection, the excretion of major amount of labelled Co_2 occurs within 2 hours. It could be logistic to trap the whole amount of expired air during this period and to measure the amount of labeled carbon recovered.[38] Practically it is cumbersome and almost impossible. By investigations, it was shown that, the amount of carbon excreted at different points of time within 30 minutes of ingestion of isotope-leveled urea are qualitative and quantitative.[39] This rises sharply within 10 minutes, if there is urease producing organism in the mouth and/or oesophagus, but declines rapidly after that if there is no H.pylori colonization in the stomach.[40] In presence of H.pylori,

it continuous to rise up to 20 minutes and then slowly declines over hours.[41] So it is the breath sample collected at 20 minute that can indicate the presence or absence of H.pylori infection in the stomach. In our preliminary study, to establish the ^{14}C-urea breath test, we collected the samples at 10, 20 and 30 minute and on calculation, it was found consistent with the result of other tests results. The value (mean ± 3 SD = 0.55), at 20 minute sample was chosen for validation against other standard tests (CLO and histology), because this sample avoids possible buccal urease activity and also avoid the exhaustion of the urease activity in low-grade infection at 30 minutes. Results of our study with a sensitivity and specificity of 93.33% and 96.23% respectively were compared with that of other studies of similar protocol. Other group used 400 KBq of ^{14}C- and found sensitivity as 100% and specificity is also around 100% by comparing with culture.[42]

Another group used 370 KBq (10 µCi) of ^{14}C- carbon and found sensitivity and specificity at 87% and 92% respectively by comparing the results with culture, histology and some with CLO[43].

Sensitivity and specificity of 94% and 89% respectively were found in another study, who used 185 KBq (5µCi) of ^{14}C- urea [40].

Two other groups used 110 KBq (2.97 µCi) and 92 KBq (2.48) respectively and sensitivity in both the cases were 100% with a variable specificity from 75 to 84%[44]. Results of our study have the similar sensitivity and specificity with others, despite lower dose of radioisotope used.

Mouthwash with urea before the start of the main procedure was included in the protocol of many investigators with the aims to exhaust the urease producing organisms in the buccal mucosa.[38,40,44.45] The nutrient meal before the test was also used by some to delay the gastric emptying and thereby prolonging the exposure time of labeled urea with the urease of H.Pylori.[39,42,43] A cold urea substrate was used by some other study groups with an intention to saturate all preformed urease and thus allowing hydrolysis of a uniform quantity of labeled urea over the study period.[38,39] These studies were around 90% sensitive and specific. Omission of these extra procedures simplified the test, keeping the accuracy intact.

^{14}C- has prolonged physical half life, but biological half life is short, because of rapid excretion of the isotope in breath and urine. Urinary bladder is the organ which gets maximum exposure to the radiation, that may be minimized to some extent by voiding every two hours. In a recent dosimetric study, the whole-body equivalent radiation dose from I µci (37 Kbq) ^{14}C-UBT has been estimated to be between 0.14 and 0.3 mrem (1.4 - 3 µSV) depending on sex and H.pylori status.[46] To put this into perspective, a dose of 4 µ SV (the "Worst" case) is less than 1 day's average natural background radiation, it is similar to the additional radiation received from cosmic rays during 1 µ jet flight. In terms of other radiological investigations, the dose from one ^{14}C-UBT is equivalent to roughly one-seventh of that from a chest X-ray (20 µSV), or one thousandth of that from a barium meal (3 SV). This margin of safety makes it acceptable to be used in the tests for continuous studies.

The advantages of the mini-dose ^{14}C-UBT, in addition, are the rapidity of the test result and low cost. ^{13}C-UBT analysis can be done by "mail order" but takes days to weeks time for result. In contrast, the results of our mini-dose test can be obtained within an hour, thus allowing management decisions to be made on urgent basis. The cost of consumables and analysis in the mini-dose test is only about one-fourth of that of the ^{13}C- UBT.

Conclusion

In conclusion, the mini-dose (1 µ Ci) ^{14}C- Urea breath test has proved to be a simple, rapid, cheap and reproducible test that detects H.pylori with high accuracy and minimal radiation exposure. It has very high degree of accuracy with a sensitivity and specificity of 93.33% and 96.23% respectively.

So my recommendations are -

This simple rapid and cheap mini-dose (1 µCi) ^{14}C-Urea breath test may be used for the diagnosis of active Helicobacter pylori infection.

This is specially important for

Epidemiological survey

Documentation of H.pylori eradication after treatment.

Long-term follow-up of H.pylori infection after eradication.

Though, minimum, it is associated with radiation exposure, So should not be used in children and pregnant women. Trial to perform urea breath test with further lesser dose of radio activity of ^{14}C- Urea may be done and its efficacy may be proved.

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USE OF COILS EMBOLIZATION IN THE TREATMENT OF CORONARY AND PERIPHERAL VASCULAR MALFORMATIONS

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Summary

Three cases of vascular malformations presented to us in National Institute of Cardiovascular Diseases (NICVD) in the month of September '2000, one of which was anomalous coronary artery connection and others were peripheral vascular malformations. All of them were markedly symptomatic. The patients were subjected to coronary and peripheral angiogram. Subsequently the vascular malformations were occluded by coil embolization Procedures were uneventful with good outcome.

Introduction

Embolization Coils used for arterial and venous vessel therapeutic embolization (occlusion) procedure. The embolization coils are made of stainless steel coil with attached synthetic fibers to promote maximum thrombogenicity. Embolization coils are delivered to tile target vessel using a soft straight wire guide through standard angiographic catheter[1]. Therapeutic coils embolization do not destroy healthy tissue.[2]

Coronary artery malformation are the most common hemodynamically significant congenital anomalies of the coronary artery system[3,4]. Direct connection between epicardial coronary artery with cardiac chambers or major vessels (Vena cava, coronary sinus, Pulmonary artery) is the most significant coronary artery anomally.[5]

Fistulas from right coronary artery (RCA) are more common than from left.[6] Complication of coronary arteriovenous malformation (AVMs) include myocardial ischemia, cardiac failure, infective endocarditis, atrial fibrillation.[3,5] So surgical repair either by ligation of the anomalous connection or direct closure of the fistula on cardiopulmonary by pass is recommended even in small children.[7] Recently, transcatheter embolization of fistulla either with detachable balloons of coils, has been reported in a small number of cases with an excellent success rate.[8-12] We have done a good number of successful coil embolization in the treatment of AVMs in NICVD Dhaka, in the month of September, 2000. Here we report the cases of therapeutic coil embolization

Materials and Methods

Transcatheter Coil Embolization in malformed Vessel between right coronary artery to pulmonary artery (PA) :

A 40 years old lady non-diabetic, non-smoker, normotensive was presented to us for evaluation of occasional palpitation & chest pain. Clinical examination findings were unremarkable except Holter monitoring showing ventricular extrasystole (VES) & ventricular Tachycardia (VT). She was subjected to coronary angiogram (CAG) on 4th September, 2000 and CAG showed a significant size abnormal communication from proximal RCA to rPA. Subsequently the abnormal communication was occluded by coil embolization

Selective angiogram of RCA was done through right femoral approach. The anomalous vessel was cannulated with 4F MP Catheter. A 3 cm x 4mm cook Embolization coil was deployed at desired location. After ten minutes, check angiogram showed complete occlusion of anomalous vessel with no antegrade flow. Procedure was uneventful . (Figure 1 & 2)

Fig.-1 : *From RCA to rPA anomalous connection before Coil Embolization*

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Fig.-2 *From RCA to RPA anomalous connection after Coil Embolization*

Coil Embolization in maxillary artery malformation on face :

A 21 years man presented with pulsatile swelling on right side of the face below the eye. The swelling was progressive and it was closing his eye lids. It was diagnosed as a case of vascular malformation at maxiillary artery territory.

Arteriovenous malformation (AVMs) in the territory of maxillary artery is difficult to treat. Coil embolization may be effective for its treatment like mandibular A-V malformation.[13]

The young man was subjected to peripheral angiogram and subsequent embolizatin of maxillary artery malformation. Selective angiogram of right External cardotid artery done through right femoral approach. Carotid artery angiogram showed hugely dilated malformation at maxillary artery territory. The feeding vessel was cannulated with 6F JR 3.5 Catheter. A 5 cm. X 5 mm. Cook embolization coil was deployed, After 10 minutes check angio showed complete occlusion of feeding vessel with no antegrade flow. Procedure was uneventful. (Fig. 3&4)

Fig.-3 : *Malformation in Maxillary artery before Coil Embolization*

Fig.-4 : *Malformation in Maxillary artery after Coil Embolization*

Coil Embolization for A-V malformation in left lower Leg :

A 10 years young girl presented to us with painful and pulsatile swelling at left lower leg. Swelling was progressive and was associated with sleep disturbance. She was diagnosed as a case of AVMS in left lower leg. The girl was subjected to peripheral angiogram and subsequent coil embolization of left lower leg A-V malformation. Selective angiogram of left superficial femoral artery was done through left femoral approach. Peripheral angiogram showed hugely dilated A-V malformation at left lower leg. The feeding vessels were cannulated with 5F JR 3.5 Catheter & 2 (3 cm x 4mm) Cook embolizing coils were deployed. After 10 minutes, check angio showed complete occlusion of feeding vessels with minimum flow to A-V malformation from collaterals. The procedure was uneventful. (Fig.-5 & 6).

Fig.-5 : *Lower leg AVM before Coil Embolization*

Fig.- 6 : *Lower leg AVMs after Coil Embolization*

Discussion

Surgical ligation of coronary, carotid and lower limb vessels malformations have been successful but accurately locating and closing malformed vessel connections without damaging the true vessels can be difficult. Selective angiography can clearly demonostrate the anatomy of malformed connections and the true vessels. So an intravascular approach to closure seems intuitively preferable to the surgical approach. As in these cases, illustrated that an Interventionalist can perform coil occlusion of a coronary-pulmonary artery malformation, A-V malformation in face & lower leg without the expense and risk of morbidity which are associated with surgery.

Transcatheter coil embolization in the treatment of coronary and peripheral vascular malformation is newer procedure in our country. It is cost effective, less traumatic but should be followed up for its any unwanted effect.

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CASE REPORTS

PROGRESSIVE SYSTEMIC SCLEROSIS WITH OESOPHAGEAL STRICTURE – A CASE REPORT

MT RAHMAN, MA RAHIM MIAH, PK ROY, SK SAHA

Introduction

Scleroderma or Progressive systemic sclerosis (PSS) is a multisystem and multistage disorder of connective tissue which varies widely in extent and severity. Oesophageal involvement is common and Achalasia – like syndrome may be the first manifestation of scleroderma.[1,2] Symptoms such as dysphagia and heart burn may occur with or without oesophagitis. However, oesophagitis occurs in about 40% of the patients.[2] Motility disorder and impaired peristalsis with delayed clearance of the refluxed acid is the most important factor for oesophageal mucosal damage and the resultant reflux oesophagitis in PSS[2,3] Endoscopy is the usual method for the diagnosis of reflux oesophagitis and stricture oesophagus.[1] Yet barium swallow oesphagogram is a suitable tool for screening of the patients with dysphagia due to motility disorder and stationary manometry of peristaltic activities is the gold standard investigation for the confirmation of oesophageal motility disorder.[4,5]

Case Note

A 25 years old unmarried female presented with 10 years history of dysphagia to both solid and liquid foods along with initial thinning followed by progressive thickening and hyperpigmentation of the skin associated with Raynaud's phenomenon. Dysphagia was associated with sticking of solid food at the lower oesophagus . During the period of last one year, dysphagia become more severe and she practiced self induced vomiting for the removal of the sticked food materials. The vomitus contained undigested food particles. She also complained of frequent nocturnal cough with respiratory difficulties for the last 5 years. She had epigastric pain, heart burn, anorexia, weight loss and deformities of the fingers for 6 months. No history of pulmonary TB or exposure to patient with pulmonary tuberculosis or ingestion of corrosives. Her mentrual cycle is very irregular occurring at an interval of six months to two years. In November 1998 she was admitted in the Medicine Unit of BSMMU and on the basis of clinical plus investigational data, was diagnosed as a case of progressive systemic sclerosis with bilateral interstitial lung disease. Accordingly she was treated by prednisolone, cyclophosphamide, sulbutamol and fluconazol. For the gastrointestinal symptoms she received H_2 –blocker, omeprazole, nifedipine, cisapride and sucralfate for a longer period without any significant clinical improvement. That is why she got readmitted in Gastrointestinal, Liver and Pancreatic Diseases unit of BSMMU for further treatment. On physical examination the patient was severely ill, grossly emaciated with a poor nutritional status, mild anaemia; expresionless facies with pinched nose having a parrot beak appearance; microstomia with exposed teeth, loss of nasolabial furrow and limited opening of the jaw. The skin of the whole body was thickened, teethered at places to the underlying structures with areas of hypo and hyperpigmentation. Hands showed gross wasting of all the muscles with a fixed flexion deformity of the right 2nd and 5th fingers with scarring at the finger tips. Abdominal examination did not show any physical sign other than generalized skin changes. Respiratory system revealed presence of crepitation bilaterally predominantly over the lower and mid zones. Other system did not revealed any abnormalities. Laboratory data showed haemoglobin – 60%, ESR-55 mm in first hour, TC of WBC -13 x 10^9/l with neutrophil leukocytosis and microcytic hypochromic anaemia in blood film. Fasting blood glucose was 4.5mmol/l, serum bilirubin 15.4mmol/l, serum ALT-32 u/l, serum ALP-201 u/l; serum total protein 11 gm/dl, Albumin 3 gm/dl and serum globulin 8gm/dl with a normal prothrombine time. Stool and urine microscopy were normal. X-ray chest PA view showed bilateral reticulonodular opacities predominantly in the mild & lower zones which are highly suggestive of bilateral interstitial lung disease. Oesophagogram and fluroscopy showed persistant long segment narrowing of the lower oesophagus with a proximal dilatation and aperistalsis suggestive of scleroderma associated stricture oesophagus with motility disorder (Fig.-1). X-Ray of the hands (Fig.-2) showed resorption of the terminal phalanges of the right second and fifth fingers with periarticular osteopenia. Endoscopy of upper GI tract revealed stricture of the oesophagus at 35th cm; the scope could not passed beyond the stricture.

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Fig.-1 : *Ba-swallow x-ray of the oesophagus showing oesophageal sticture.*

Fig.-2 : *X-ray of Hand showing resorption of terminal phalanges,*

She was seronegative for Rheumatoid factor, ANA and anti sclero-70. Her lung function tests were grossly abnormal (FEV_1-27%, FVC-26% and PEF-33%) which are suggestive of severe restrictive type of lung disease. Skin biopsy revealed thinning and hyperkeratosis of the dermis with increased collagen along atrophic adenexae, no subcutaneous tissue. All these are compatible with scleroderma.

Discussion

Scleroderma (PSS) is an uncommon multisystem collagen disease which involves the gastrointestinal tract in majority of the cases with a high frequency of oesophageal disorders (>50%)[6]. Skin lesions with Raynaud's phenomenon and dysphagia (CREST Syndrome) are the most common clinical pictures of PSS. Dysphajga, epigastric burning or retrosternal discomfort and regurgitation are the common symptoms suggestive of the oesophageal involvement[7]. According to Masi Rodman Criteria dysphagia is the presenting symptom in 82% of patients[6]. Peptic oesophagitis frequently occurs and may lead to narrowing and stricture of the lower oesophagus producing dysphagia. Major laboratory abnormalities suggestive of PSS are raised ESR, anaemia, hyperimmunoglobulinaemia mostly IgG (~50% cases), Rheumatoid factor (in 25%), ANA in >95% of case and anti-DNA. Anti-nuclear antibodies that have a high specificity for PSS is anti sclero-70 that could be detected in only about 20% of cases and are associated with diffuse cutaneous lesions, interstitial lung disease and other visceral involvement. Skin biopsy revealing accumulation of mucopolysacharides in the dermis and skeletal musle are important histologic pictures suggestive of PSS[7].

The reported patient presented with 10 years long history of progressive dysphgia to both solid and liquid food associated with classical skin lesions, Raynaud's phenomenon, sclerodactaly and typical scleroderma facies. Lastly she developed epigastric pain, heart burn and regurgitation of ingested food during the period of last one year. Although she practiced self induced vomiting, no history of upper GI bleeding and no evidence of oesophageal carcinoma. Barium swallow oesophagogram and endoscopic pictures are diagnostic of oesophageal sticture with hypomotility. All these are typical pictures of stricture oesophagus associated with PSS. Oesophageal manometry could not be done because of lack of facilities for the test. A low level of haemoglobin, raised ESR, hyperglobulinaemia are highly suggestive of PSS. But the patient is negative for RA test, ANF screening and anti sclero-70 which can be explained by the fact that test for RA factor usually found to be positive in only 25%, anti sclero-70 in only 20-30% of cases and ANA is more specific for SLE rather than PSS. Finally skin biopsy showing evidence of hyperkeratosis of the dermis with collagen tissue and atrophic adenexae are compatible with the diagnosis of PSS.

The diagnosis of PSS is not difficult in presence of Raynaud's phenomenon with typical skin lesions and visceral involvement. Abnormal skin texture provides

the definitive diagnostic criteria of PSS in >90% cases.[8] Considering the typical clinical features, laboratory data and histologic pictures the case was diagnosed as PSS. In view of 10 years long history of progressively worsening dysphagia plus radiological and endoscopic pictures the patient was categorized as PSS with benign stricture of the oesophages. Accordingly endoscopic dilatation of the oesophageal stricture was done by Eder Puestow dilators of 21-45 mm size. After dilatation endoscope easily passed into the stomach and seen upto the 2nd part of the duodenum but no lesion seen. Thereafter the patient showed a dramatic clinical improvement of dysphagia. Seven days later the patient was discharged with a H_2-blocker along with prednisolone and cyclophosphamide with an advice to take frequent small meals and sleep in a bed elevating the head end.

PSS is not a curable disease. However, treatment of the involved organ system can relief the symptoms and improve the quality of life. The doctor patient relationship is extremely important in the treatment.

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ASSESSMENT OF ANAEMIA IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Summary

In order to evaluate anaemia with Rheumatoid Arthritis (RA), a total of 45 cases based on American Rheumatolgical Association (ARA) diagnostic criteria were evaluated in a prospective observational study in Rheumatology unit of a Teaching Hospital. Patients haematological & marrow iron status was ascertained by semiquantitative estimation with Pearl's staining of marrow aspirate after grading 0 to 4. Marrow iron stores were found depleted (absent to decreased) in 21 (46%) and repleted (normal to increased) in 24(54%) of patients. On comparision of haematological & iron parameters between iron depleted & iron repleted groups, the serum ferritin levels in the iron depleted group were significantly lower (41.95 ± 17.66 vs 181.42 ± 148.78. p<0.00001). Positive correlation was found between serum ferrifin & marrow iron store. If serum ferritin level ≤70 ng/ml is taken as cut off level, it was a good predictor for iron deficiency anaemia with it's sensivity of 100% & specificity of 83%. The test had a positive predictive value of 84% & negative predictive value of 100% & accuracy of 92%. Total iron binding capacity (TIBC) was significantly higher in iron depleted group compared with iron repleted group (p<0.001). Negative correlation was found between TIBC & marrow iron store. If the TIBC level ≥350ug/dl is used as a cut off level to differentiate iron depleted from iron repleted condition, it's sensivity & specificity were 76% & 83% respectively & both positive & negative predictive values 80%. Serum iron was lower in both types of anaemia & the difference was not statistically significant (p=0.0736). Haemoglobin (Hb) was found significantly lower in iron repleted group (p = 0.0244) & ESR was found significantly higher in iron repleted gruoup (p= 0.0041).

Our study demonstrated that estimation of serum ferritin correlated well with body iron stores & is most reliable, least invasive as well as inexpensive means of estimation of iron status in anaemia with RA, which can also distinguished iron deficiency (iron depleted anaemia) from anaemia of chronic disorder (iron repleted anaemia). TIBC estimation can be used as supportive &/or alternative test for this purpose.

Introduction

Anaemia is one of the most common extrarticular manifestations of Rheumatoid arthritis (RA)[1]. The course of RA may be complicated by the development of anaemia. The most important causes are anaemia of chronic disorder (ACD) & iron deficiency anaemia (IDA)[2]. The differential diagnosis between these two iron deficiency anaemia is important because, potentially curable blood loss can be detected & therapeutic approach are completely different between two conditions but the detection may be extremely difficult unless bone marrow smears stained for iron are examined. The main reason for the difficulty is that the usual blood tests indicating iron deficiency, mean volume (MCV), mean corpuscular haemoglobin concentration (MCHC), serum iron are similarly affected in both types of anaemia. The magnitude of the problem is due to the fact that practically all patients with RA of a certain disease activity develop the ACD & upto 75% of the same patients also may have iron deficinency in anaemia with RA. However, marrow sampling can even in skilled hands be painful procedure for the patients, time consuming for the physician & expensive for the poor patients. Several authors have attempted to identify laboratory measures that predict marrow iron stores in patients with RA & thus avoid marrow sampling. [3-12]

Recently, serum ferritin has been considered the most suitable non invasive investigation for assessing iron status in patients with RA, which can distinguish

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IDA from ACD. Such studies, to the best of our knowledge have not been carried out in developing countries like Bangladesh where IDA is widespread.[4] The use of serum ferritin as determined by Radio Immunoassay (RIA) in patients with RA has received attention from many authors The method is not easily available in most of the under developed countries, where another modern immunological technique Immunometric Enzyme Immunoassay can be accessible & some other authors had used this technique to estimate serum ferritin. [13-15] An object of these studies has been to assess the relationship between haematological & iron parameters including serum ferritin with state of bonemarrow iron stores & to detect the level which could distinguish iron deficinecy from Aanaemia of chronic disorder in patient with anaemia of Rheumatoid arthritis.

Materials and Methods

This was a prospective study conducted at the Department of Internal Medicine & Rheumatological Clinic, Bangabahdnu Sheik Mujib Medical University (BSMMU) Dhaka during the period from October 1998 to June 2000. A total of 45 cases of RA of both sexes based on American Rheumatism Association (ARA) diagnostic criteria who were clinically moderate to severely anaemic were selected for the study. Patients receiving iron therapy in the past 4 weeks & on clinical conditions which could alter parameters included in the study were excluded from the study. Patients receiving drug therapy was not modified by any means.

All patients were first evaluated clinically, After obtaining informed consent, bone marrow examination & other investigations were done. The relevant findings & parameters were recorded in a pre designed data sheet. Investigations done were routine blood tests like Hb estimation by cyanmeth haemoglobin method, ESR by Westergren method, peripheral blood film (PBF) by Romanovsky's staining technique, bonemarrow study by Pearl's staining technique, serum ferritin by Elisa & serum iron & TIBC by International Committee for standardization in Hematology (ICSH) rcommended assay, marrow iron stores were assessed by grading semiquantitatively in to 0 to 4 gradings. Grade 0 & 1 were included in iron depleted (IDA) group & grade 2-4 were included in iron repleted (ACD) group by 2 experienced haematologists working independently & blinded to laboratory & clinical details. Observers agreed in 88% of samples. Where observers disagreed, if either not identified intracellular marrow iron, the patient was classified as IDA. Marrow smear which did not contain any particle was discarded as unsuitable for assessment. For the control a marrow iron known to contain & not to contain iron were always processed in the test samples. Quantitative assessment of serum ferritin was done in commercially available test kit (orgentee Diagnostika GmbH, Germany). Controls were provided with the kits & instructions supplied were meticulouusly followed during the laboratory investigations.

Data were analysed by using SPSS programme & comparison was done by using student 't' test. A cutoff value for some variables identified & then was selected the best discriminated value between IDA & ACD. Sensitivity, specificity & predictive values were calculated to find out the accuracy.

Results

A total of 45 patients were included in the study. Age of the patient ranged from 20-58 years (mean 41.4). Thirty Six (80%) were female & nine (20%) were male. The duration of illness ranged from 1 to 12 years (mean 5.13). Thirty one (69%) cases were Rheumatoid factor (RA) test positive & fourteen (31%) were negative. All the patient had active disease & used NSAID. Twenty eight (62.2%) had received DMARD & sixteen (35.5%) used steroid.

On bone marrow iron examination iron depletion was found in twently one (46%) & iron repletion in twenty four (54%) of cases. Iron depleted group were considered IDA & iron repleted group as ACD. Among the iron depleted patients, grade 0 was found in 13(29%) & grade 1 was present in 8(17%) of case. Among the iron repleted patients, grade 2 was present in 13(29%), grade 3 in 6 (13.5%) & grade 4 in 5(11.5%) of cases (Table 1).

Table 1 : *Iron status in Bone marrow in anaemia with RA (n = 45) :*

Group	Number of patient	Percentage
Iron depleted group (n = 21)		
Grade 0	13	29.00
Grade 1	8	17.00
Total	21	46.00
Iron repleted group (n=24)		
Grade 2	13	29.00
Grade 3	6	13.50
Grade 4	5	11.50
Total	24	54.00

Comparison of Hb, ESR, SF, SI & TIBC between two groups at various iron grade have been shown in the Table V-VI. In iron depleted group, Hb ranged from 4.2 to 10.2 g/dl (mean 7.45), where as in iron repleted group, Hb ranged from 5.8 to 11. g/dl (means 8.58). Hb level was significantly lower in iron deficiency anaemia than anaemia of chronic disorder (p=0.0244).

All the values were below 11 g/dl which indicated that all patients were actually anaemic. In all cases ESR was hgiher than normal. In IDA, it ranged from 30 mm in 1st hour, which was the lowest in the series to 140 mm in 1st hr. (mean 74.81), where as in iron repleted group, it ranged from 50 to 150, which was the highest value in the series (mean 102.67). ESR value was significantly higher in ACD than IDA (p = 0.0041).

Serum Ferritin ranged from 11 to 70 ng/ml (mean 41.95) in IDA & all the values were below the level of 70 ng/ml & one value was <15 ng/ml, which otherwise differentiated IDA in absense of inflamatory disorder. In ACD, it ranged from 58 to 462 ng/ml (mean 181.42). SF was significantly lower in IDA than ACD (p<0.0001). The range of serum iron was from 12 to 62 ug/dl (mean 34.76) in IDA & 18 to 221 ug/dl (mean 54.63) in ACD. In both the group most of the values were lower than normal & it did not show any significant difference between the two groups (p 0.=0736). TIBC ranged from 252 to 492. µg/dl (mean 385.00) in IDA & 211 to 404 (mean 372.75) in ACD. Most of the values were found higher than the normal in IDA in comparison to ACD where most of the values were lower than normal. TIBC was significantly higher in IDA than ACD (p<0.001).

Table II : *Clinical features in relation to anaemia in the iron depleted & iron repleted group (n= 45):*

Features	Iron depleted group (n= 21)		Iron repleted group (n= 24)	
	No.	%	No.	%
Weakness	21	100	24	100
Breathlessness	12	57.00	8	33
Palpitation	10	48.00	4	17
Dizziness	4	19.00	1	4
Pallor :				
Moderate	15	71.00	21	87.00
Severe	5	29.00	3	12.00
Smoth Tongue	9	43.00	2	8.00
Nail changes	4	19.00	0	0

Table III : *Findings on Peripheral blood film in the Iron depleted & Iron repleted group (n=45)*

Features	Iron depleted group (n=21)		Iron repleted group (n=24)	
	No	%	No	%
Normochromic-Normocytic	6	13.00	15	34.00
Hypochromic with or without Microcytic	12	27.00	2	4.00
Non specific/Anisochromic-Anisocytic	3	6.00	7	16.00
Total	21	46.00	24	54.00

Table IV : *Erythropoetic changes on routine bone marrow examination in the iron depleted & iron repleted group (n= 45)*

Features	Iron depleted group (n=21)		Iron repleted group (n=24)	
	No.	%	No.	%
Hyper cellular marrow	19	90.00	15	63.00
Normal cellutarity	2	10.00	9	37.00
Total	21	100.00	24	100.00

Table V : *Comparison of patients with iron deficiency & anaemia of chronic disorder :*

Iron depleted (n=21)							Iron Repleted (n=24)						
Case No.	BMI gm dl	Hb mm in 1st hr.	ESR ng/ml	SF ug dl	SI ug dl	TIBC	Case No.	BMI gm/dl	HB mm in 1st hour	ESR ng/ml	SF ug/dl	SI ug/dl	TIBC
2	0	4.5	70	22	43	399	1	2	8.8	60	462	221	228
4	0	7.5	72	35	18	492	3	2	11.0	78	99	68	267
7	0	7.2	130	30	25	400	5	2	9.0	140	230	22	225
14	0	4.2	100	11	20	440	6	2	6.4	127	58	20	259
14	0	8.5	45	33	15	340	11	2	8.2	130	87	48	222
15	0	10.2	42	27	32	392	12	2	10.4	140	60	34	292
21	0	7.2	555	51	30	412	20	2	7.9	100	75	48	280
22	0	9.2	90	36	22	450	24	2	7.8	102	56	52	256
27	0	7.0	60	48	28	408	31	2	9.5	65	86	43	252
29	0	6.0	199	18	27	370	33	2	7.0	105	59	62	250
30	0	8.4	45	47	48	366	34	2	11.0	70	99	68	260
32	0	5.6	62	29	18	402	37	2	10.5	50	91	38	392
45	0	6.4	45	20	12	425	43	2	6.5	80	109	18	404
9	1	10.0	90	49	22	350	8	3	8.0	110	220	43	299
10	1	9.6	30	63	14	360	17	3	5.8	145	302	41	211
16	1	8.4	140	70	62	348	36	3	9.8	65	177	120	260
19	1	8.7	120	70	48	252	38	3	8.0	125	105	80	240
23	1	7.5	50	64	35	270	40	3	10.7	125	135	50	230
25	1	8.6	75	57	32	405	44	3	9.0	132	109	44	252
35	1	5.6	60	45	30	402	18	4	8.0	150	413	30	240
39	1	6.2	90	56	14	402	26	4	8.0	115	180	40	240
							28	4	10.0	60	216	60	382
							41	4	7.8	100	292	46	260
							42	4	6.8	90	643	20	245

Table VI : *Comparison of Haematological & Iron parameters between iron depleted & iron repleted :*

Parameters	Iron depleted group (n= 45) Grade 0-1	Iron repleted group (n= 24) Grade 2-4	p value
Hb (gm/dl)	7.45 ± 1.71	8.58 ± 1.53	0.0244*
ESR (mm in 1st hr)	74.81 ± 30.57	102.67 ± 30.82	0.0041*
SF (ng/ml)	41.95 ± 17.66	181.42 ± 148.78	<0.0001*
SI*ug/dl)	34.76 ± 29.32	54.83 ± 41.98	0.0736ns
TIBC (ug/dl)	385.00 ± 54.79	272.75 ± 57.94	<0.001*

Hb= Haemoglobin. ESR = Erythrocytic Sedimentation Rate. SF= Serum Ferritin. SI = Serum Iron, TIBC – Total Iron binding capacity.
NS = Not significant, * = Significant ($p<0.05$)
Group analysis was done using Unpaired Student 't' test iron depleted vs. Iron repleted.

Table VII : *The sensitivity, specifity, predictive values & accuracy of serum ferritin, TIBC & PBF at various levels :*

Tests	Cut off value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Serum	≤ 60	81	83	81	83	82
Ferritin	≤ 70	100	83	84	100	92
(ng/ml)	≤ 75	100	79	81	100	90
TIBC (ug/dl)	≥ 350	76	83	80	80	80
PBF	Hypochromic with or without microcytic Anaemia	57	92	85	71	75

Sensitivity : Referred to proportion of IDA who are marrow iron grade 0 & 1.
Specificity : Referred to proportion of ACD who are marrow iron grade 2-4.
Positive predictive value : Referred to proportion of patients with marrow iron grade 0 & 1 who are IDA.
Negative Predictive value : Referred to proportion patients with marrow iron grade 2-4 who are ACD.
Accuracy : Referred to sensitivity plus specificity divided by two.

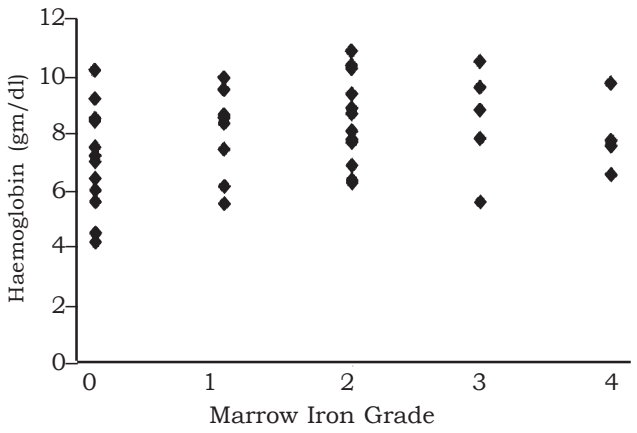

Fig. 1. *Relationship between bone marrow iron grade and haemogiobin.*

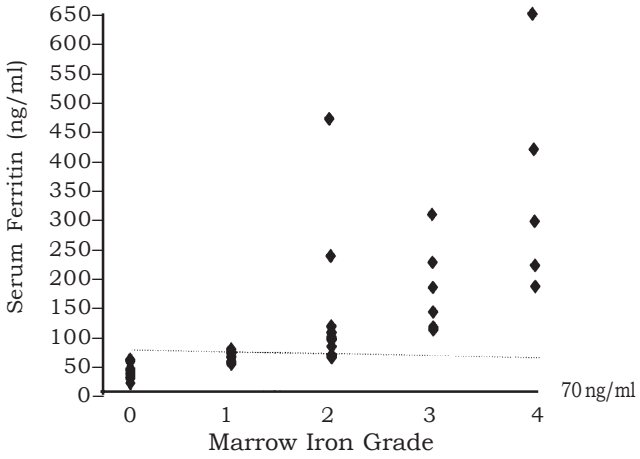

Fig. 3 : *Relationship between bone marrow iron grade and serum ferritin.*

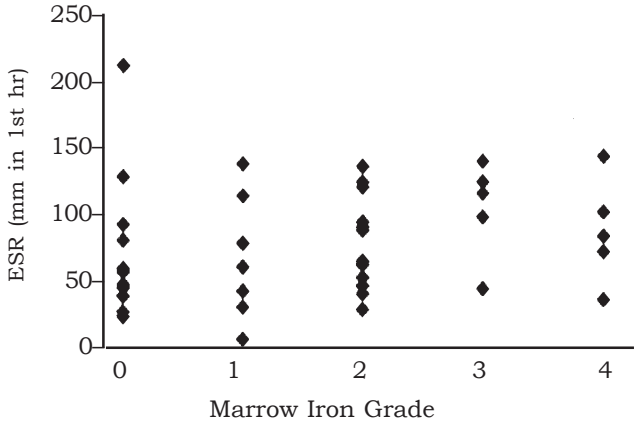

Fig. 2 : *Relationship between bone marrow iron grade and erythrocyte sedimentation rate (ESR).*

Fig. 4 : Relationship between bone marrow iron grade and serum iron.

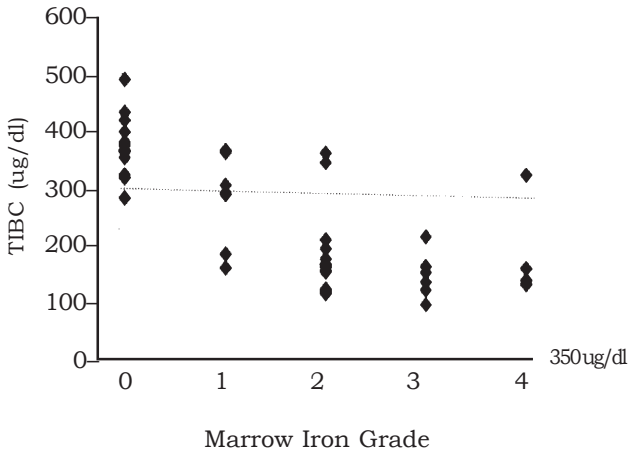

Fig. 5 : *Relationship between bone marrow iron grade and total iron binding capacity (TIBC).*

Discussion

The significance & differentiation between the ACD & IDA which are commonest causes in patients with RA has recently been reviewed. Differentiation between two types is important for the management of Aanaemia. In IDA, iron should be given where as in ACD, it is contraindicated. The course of RA may be complicated by development of anaemia & in the long term, death related to RA are more common in patients who are anaemic at the time of diagnosis[5]. Recently, serum ferritin has been considered the most suitable noninvasive investigation for assessing iron status in patients with RA. However, there has been considerable debate in the literature regarding the usefulness of serum ferritin as a measure of body iron stores in the presence of chronic inflammation.[4,10] It is agreed that in patients without inflamatory disease, serum ferritin < 15 ng/ml indicates the presence of iron deficiency.[12] This vlaue is inappropriate for patients with RA as the inflamatory process increases the level of serum ferritin. Therefore an elevated level of serum ferritin should be considered to indicate iron deficiency in these patients. Several workers had reported with various outcomes. The result may be influenced by various factors like socioeconomic status, anorexia & lack of intake, food habits, parasitic infestations, use of NSAID & unawareness of prolonged GI blood loss etc.[2,4,10] Considering all these points, this study was done.

In this dtudy, out of total 45 anaemic patients with RA, 21(46%) had IDA & 24(54%) had ACD. The result was consistent with Mulherin et al in which, they reported out of their 45 anaemic patients with RA, 18(47%) had IDA & 21(53%) had ACD[3]. Droube et al studied 28 patients 14(50%) had IDA & remaining 50% had ACD[5]. Where as Shroff et al had reported 19(56.7%) IDA & 13(43.3%) ACD in their series of 30 cases.[4]

Comparison of haematological & iron parameters between two groups, serum ferritin level showed positive correlation with marrow iron. Serum ferritin value was lower in IDA than in ACD, the difference was highly significant ($p<0.001$). The result was consistent with the findings of Shroff et al, Dieghton et al, Nielsen et al, Bentely et al & Walter et al. On comparision of serum ferritin level at various level, SF level of >70 ng/ml could be considered as a cutoff point to reliably indicate iron deficiency with much higher sensitivity (100%), specificity (83%), positive predictive value (84%), negative predictive value (100%) & accuracy (92%) (Table VII). This cutoff value is five times higher than that in patients of uncomplicated iron deficiency anaemia. Consistent with this result, elevated levels of serum ferritin in patients of RA with iron deficiency was shown by Nielsen et al & bentley et al.[16,17] Doube et al reported < 60 ng/ml with the sensitivity of 86%, specificity of 88% & predictive value of 83%.[18] Vreugdenhil et al had mentioned < 50 ng/ml with 79% Sensitivity & 100% specificity for iron deficiency[19] Multherin et al had adviced as a precise level of serum ferritin that distinguishes iron deficiency from ACD varied between studies, but was 40-60 ng/ml[3]. Whereas Porter et al had mentioned if <75 ng/ml was taken as cutoff value, had sensitivity of 92% & predicitive value of 86%.[2]

TIBC was found significantly higher in IDA in comparison with ACD ($p<0.001$) (Table VI). The result was consistent with the findings of Doube et at & Bentley et al[15,17]. This study had shown Negative corelation with Bone marrow iron store. If TIBC value >350 ug/dl was taken as cut off value to differentiate IDA from ACD, which showed sensitivity & specificity of 76.2% & 83.33% respectively & positive & negative predictive values of 80% (Table VII). Though the sensitivity, predictive values were consistent with serum ferritin value when taken <60 ng/ml, sensitivity decreased to 76% from 80%. It indicated that serum ferritin level <70 ng/ml or even <60 ng/ml is better for differentiating IDA from ACD. TIBC >350 ug/dl could be used as a supporive &/or alternative test to differentiate IDA from ACD in anaemia with RA. Previous workers did not show much interest in TIBC to differentiate these two conditions through it correlate with marrow iron grading, probably it was due to the fact that SF represents well with total iron storage of body & it remains in balance between body iron storage & serum ferritin[3-4].

Most of the serum iron value were at lower level in both the groups & the difference was not statistically significant (p = 0.0736) (Table VI). It did not correlate with marrow iron gradings which coincided well with the findings of Shroff et al Mulherin et al & Nielsen et al[3,4,16].

ESR were found raised in both the groups but was higher (102.67 ± 30.82) in ACD than IDA (74.81 ± 30.57). The difference was statistically significant (P = 0.0041) (Table Vii). Whereas, Hb level ranged from 4.5 to 11.0 g/dl in IDA, it was between 4.5 to 10.2 g/dl (7.45 ± 1.71) & in ACD group, between 5.8 to 11.0 g/dl (8.58 ± 1.53). The difference between two was significant (P<0.05) (Table VI). Similar findings were reported by Porter et al & Mulherin et al[2,3]. In PBF, hypochromic with or without microcytic blood picture was common in IDA, where as normochromic normocytic type was common in ACD nonspecfic findings were found in both the types, but was slightly higher in ACD. When the hypochromia with or without microcytic blood picture is taken for differentiating iron deficiency from ACD in RA, it's senstivity & specificity were found 57% & 92% respectively. Positive predictive value was 85%, negative predictive value 71% & accuracy was found 75% (Table VIII). To consider this test , the sensitivity was found much lower. In this study, 4% of cases of ACD, showed hypochromic or without microcytic anaemia. Among them, one case had Hb 8.2 g/dl, ESR-130 mm in 1st hour, SF 87 ng/ml, Sl-48 ug/dl & TIBC-222 ug/dl. The another patient had Hb-9.5 g/dl, ESR-65 mm in 1st hr, SF-86 ng/ml, SI-43 ug/dl & TIBC- 252 ug/dl. It indicated that both patients were moderately anaemic with SF level >70 ng/ml. bone marrow iron grading were 2 in both the patients. It clearly indicated that both the patient had ACD. It was probably due to the fact that ACD may behave functionally as iron deficiency though iron is sufficiently stored in macrophages, these are not released to Erythroblasts to utilize & bone marrow may behave functionally as iron deficiency. On routine bone marrow examination, changes in bone marrow erythopoesis was studied, in which hypercellular marrow was found in both types of anaemia with RA but it was common in IDA.

In this study, there were 36(80%) female & (20%) male the ratio of 4:1. This ratio can be compared favorably with sex ratio of 4:1 in series of Smith et al [20]. Age ranged from 20-58 years (mean 41.4). In the series of Smith et al it was 19 to 73 years (mean 55.3)[20]. The difference was noticed as probably due to the fact that very few number of elderly patients attended the Rheumatological Clinic or did not give consent for bone marrow examination in our centre. The duration of illness in our series was 1 to 12 years (mean 5.13) which was close to the range of 5 to 10 years in the series of Shroff et al[4]. RA test was found positive in 31(69%) & negative in 14(31%) of patients which actually coincided well with overall positivity of RA test in patients with RA. All the patients were suffering from active disease for which they attended the clinic due to pain, swelling, morning stifiness of joints. We did not score activity of disease because it was out of scope of our study. All patient received nonsteroided anti-inflemmatory drugs (NSAID), 28(62.2%) received disease modifying antirheumatic drugs (DMARD) & 16(35.5%) received steriod during the course of their illness.

We conclude from this study that iron deficiency anaemia is cause of anemia in a considerable proportion of anaemia with RA. The cause of which should be searched for & explained. Estimation of serum ferritin correlates well with body iron status & is the most reliable, least invasive as well as less expensive means of estimation of iron status in anaemia with RA. A cut off value <70 ng/ml can be used to identify iron deficiency naemia from anaemia of chronic disorder with high sensitivity, specificity, predictive value & accuracy in anaemic patients with RA. This test can be considered as a routine basis in patient with anaemia with RA. It is justifiable to advice iron therapy for those patients who have serum ferritin <70 ng/ml & iron therapy should be forbidden for them who have higher than this level. Estimation of TIBC & cut off level of >350 ug/dl can be used to differentiate iron deficiency as a supportive &/or alternative test to serum ferritin. Serum iron level does not help to distinguish these two conditions. RBC hypochromia is a marker of iron deficiency with high specificity but its sensitivity is low & it may fail to identify iron deficiency in some patients.

Acknowledgement

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OUTCOME OF MANAGEMENT OF HYPOTHYROIDISM : EVALUATION OF DIAGNOSIS AND THYROXINE DOSAGE

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Summary

Thirty eight (Female-28, Male-10) cases of hypothyroidism, previously diagnosed and on thyroixne replacement therapy, registered to the Thyroid Clinic of Chittagong Medical College Hospital, Chittagong were studied to see the outcome. The previous diagnosis was evaluated to the basis of thyroid function tests performed at the initial diagnosis. The success of treatment before and after registration was evaluated on the basis of clinical features and triidothyronine (T_3) thyroxine (T_4) and thyroid stimulating hormone (TSH) assays and dose of thyroxine registration and at six months of continued thyroxine therapy. At the initial diagnosis in many cases of suspected hypothyroidism radio-active iodine uptake and thyroid scan was used as thyroid function tests. Before registration to Thyroid Clinic, diagnosed patients of hypothyroidism were treated for a mean duration of 6.8±1.1 months. But all the patients were still remaining hypothyroid clinically and biochemically (T_3-1.09±0.36 nmol/l, T_4 56±30 nmol/l, TSH-18.93±7.67 miu/l) because of inadequate doses of thyroxine (69±21µg/day). After registration, with adequate doses of thyroxine (176±7.30µg/day) treatment, all patients became euthyroid clinically and biochemically (T_3-1.79±0.51 nmol/l, T_4-131±31 nmol/l, TSH 2.90±2.27miu/l) at six months of therapy. The treatment of hypothyroidism is specific and effective. But the adequate doses of thyroxine should be used to achieve and maintain euthroidism.

Introduction :

The development of new sensitive immuno-radiometric assay (IRMA) to measure serum thyroid stimulating hormone (TSH) has been established as a valuable tool in the diagnosis and management of thyroid diseases. Expected normal range for TSH is 0.5-5.0 miu/l. The older insensitive TSH radioimmuno-assays could only measure concentrations as low as 0.5 miu/l. With the new sensitive IRMA, TSH concentrations as low as 0.001 miu/l can be detected, and the new assays can reliably distinguish between normal and suppressed TSH concentrations.[1,2] The American Thyroid Association (ATA) recommends that a decrease in serum free thyroxine (T_4) estimate and a raised level of serum TSH confirm the diagnosis of hypothyroidism caused by thyroid gland failure.[3] In the current cost-conscious era, significant issues focus on the cost effectiveness and medical outcomes. The appropriate laboratory evaluation is critical to establish diagnosis and in the management of hypothyroidism in the most cost-effective way. The most valuable test is a sensitive measurement of TSH level. A TSH assay should always be used as the primary test to establish the diagnosis of hypothyroidism.[4,5,6] The clinical diagnosis of primary hypothyroidism is difficult as many of the symptoms and signs of the disease are non- specific. One the diagnosis is made, the replacment with thyroxine is curative. At present, the mean replacement dosage of levothyroxine is thought to be 125 µg/day or 1.6 µg/kg/day, although the appropriate dosage will vary in individual patients.[7,8,9] The half-lift of levothyroxine is about 7 days, so it needs to be given orally once daily.[9] The purpose of our study was to evaluate the method of diagnosis, treatment and the outcome of treatment in previously diagnosed cases of hypothyroidism on thyroxine replacement therapy.

Materials and methods

Fifty-four previously diagnosed cases of hypothyroidism on thyroxine replacement therapy were registered to 'Thyroid Clinic Chittagong Medical College Hospital, Chittagong from Dec, 1993 to Dec, 1997. Of them, 38 patients (female 28, male 10) were included in the analysis of the present study.

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All patients were on thyroxine replacement for at leart 5 months but were biochemically hypothyroid at the time of registration.

Patients found en thyroid biochemically, non compliant to taking may and possible follow-up or unable to perform hormone assay were excluded from the study.

At registration to 'Thyroid Clinic' the previous diagnosis of hypothyroidism was evaluated on the basis of thyroid function tests (TFTs) at the time of initial diagnosis when available. After registration the patients was evaluated both clinically and biochemically by hormone assays. Serum T_3, T_4 and TSH were assayed for all patients. All the patients were individually assessed for the doses compliance of thyroxine therapy initially and at each follow-up visit. The thyroxine replacement therapy was continued with increasing doss according to the need of the individual patient. The patients were followed at one-month interval for clinical evaluation. At 3 months of follow-up, serum TSH was assayed for every patients. At 6 months of therapy (the end point of this study) serum T_3, T_4 and TSH were measured for all patients in addition to clinical evaluation Serum TSH assay was done using IRMA methods. Serum T_3 and T_4 assays were done using RIA method. For the purpose of this study the commonly accepted normal range of 0.5-5 miu/l for TSH, 62-165 nmol for T_4 and 0.7-2.8 nmol/l for T_3 was used. Thyroxine sodium (oroxine® 50 μg tab, Welcome) was used for treatment of hypothyroidism. The clinical and biochemical thyroid status of patients at the of time registration was compared to that of patient at the end of follow-up therapy. The total daily dose of thyroxine used to achieve euthyroid before registration was compared to that used after registration. Written consent was taken from the subject after information them about the nature and purpose of the study.

Results

The age distributions of 38 (Female-28, Male-10; Female : Male = 2.8:1) patients are shown in Table-I. The mean (±SD) age of female and male patients were 40.68 (±16.28) years respectively. On evaluation of previous diagnosis of hypothyroidism it was found that hormone assays were done for 27 patients out of 38. For 17 patients serum T_3, T_4 and TSH; for 5 patients serum T_3 and T_4; for 2 patients serum T_3 and TSH; for 1 patient serum T_3; for 1 patient serum T_4; and for only 1 patient TSH alone were measured. The mean serum T_3, (n=25), T_4 (n=23) and TSH (n=20) levels were measured 1.00 ± 0.56 nmol l 36±19 nmol/L and 26.45±12.35 miu/l respectively . For 21 patients RAIU tests and for 12 patients thyroid scan was done for diagnosis. Five patients were diagnosed on the basis of low RAIU test and thyroid scan. Six patients were diagnosed only on clinical assessment without any function tests. On evaluation of thyroid status at registration it was found that all patients were remaining still hypothyroid clinically and biochemically (T_3=1.13±10.41 μmol/l; T_4=55±8 nmol/l and TSH=18.08+7.00 miu/l) in spite of 6.8±1.1 months of thyroxine therapy. The mean doses of thyroxine used before registration was 69±21 μg daily. After registration to our clinic the mean doses of thyroxine used to achieve euthyroid was 176±30 μg daily (Table. II). At 3 months of follow-up therapy. 23 (60%) patients were found clinically and biochemically euthyroid. Serum TSH level of euthyroid patients was measured 2.61±1.72 miu/l. At the end of 6 months therapy all patients were found euthyroid clinically and biochemically (T_3=1.86±0.49 nmol/l, T_4=128±30 nmol/l and TSH=2.69±2.12miu/l. The clinical status of patients with 6.8±1 months of thyroxine therapy at the time of registration and at the end of the study with 16 months of thyroxine therapy after registration to thyroid clinic are compared in Table-III and Table-IV respectively. The hormonal status of patients at initial diagnosis, at the time of registration with 6.8±1.1 months of thyroxine therapy and at the end of the study with 6 months of thyroxine therapy after registration are shown in Fig-1. Nearly in all cases thyroxine was used in multiple daily doses before registration. After registration we used thyroxine in a single daily dose.

Table-I : *Age distribution of patients*

Age group	No. of patients	Percentage
0-10	1	2.68
11-20	5	13.16
21-30	2	5.26
31-40	10	26.32
41-50	9	23.68
51-60	7	18.42
60-	4	10.52
Total	38	100

Table-II : *The doses of thyroxine used before and after registraiton to achieve euthyroid*

Thyroxine Sodium mu/day (tab/day)	No. of patients before registration	No. of patyents After registration
25 (.5 tab)	03	
75 (1 tab)	10	
75 (1.5 tab)	17	
100 (2 tab)	08	
125 (2.5 tab)		03
150 (3.5 tab)		14
175 (3.5 tab)		02
200 (5 tab)		17
225 (4.5 tab)		01
250 (5 tab)		01
Total patients	38	38
Doses of thyroxine used µg/day), Mean ± SD)	69 ± 21	176 ± 30

Table-III : *Clinical status (Symptoms) of patients with 6.8 ± 1.1 months of thyroxine therapy before registration and with 6 months of therapy after registration to thyroid clinic*

Peristing symptoms	With 6.8 ± 1.1 months of Thyroxine therapy before registration (% of patients)	With 6 months of thyroxine therapy after registration (% of patients)
Fatigue/weakness	84	10
Cold intolearance	76	05
Swelling of face/leg/body	76	0
Constipation	73	29
Palpitation	68	23
Aches and pain in muscle	60	0
Weight gain	55	0
Increased sleep	42	10
Hoarse voice	34	0
Disturb memory	23	08
Menorrhagia	23	8
Swelling in front of neck	23	18
Pain in neck	13	08
Difficulties in swallowing	08	08
Heat intolerance	13	21

Table-IV : *Clinical status (Physical signs) of patients with 6.8±1.1 months of thyroxine therapy before registration and with 6 months of therapy after registration to thyroid clinic*

Persisting signs	With 6.8 ± 1.1 months of thyroxine therapy before registration (% of patients)	With 6 months of thyroxine therapy after registration (% of patients)
Delayed relaxation of ankle jerks	86	05
Thick/rough/dat/cold skin	86	10
Edema/puffy face	45	10
Hoarse voice	42	0
Delayed relaxation of biceps jerks	34	0
Goiter	32	26
Depression	16	05
Deafness	08	0
Bradycardia (pulse < than 60)	10	0
Pleural effusion	05	0

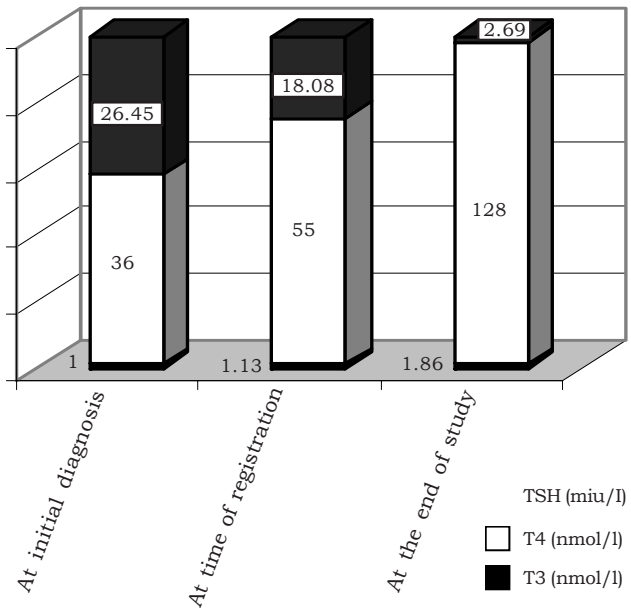

Fig.-1 : *Hormonal status of patients at initial diagnosis T_3 = 1.00±0.56 µmol/l, T_4 = 36 ±19 µmol/l, TSH = 26.45 ±12.35 µ/l), with 6 months thyroxine therapy at the time of registration (T_3=1.13±0.41 µmol/ 1, T_4=55+28 µmol/ 1, TSH = 18.08 ± 7.00 µ/l) and at the end of study (T_3=1.86±0.49 µmol/l, T_4 = 128±30 µ mol/l, TSH = 2.69 ±2.12 µ/l). Hormonal levels are expressed in mean±SD. Only mean levels are shown in figure.*

Discussion

Over the past decade it has been generally accepted that the serum TSH represents the best biochemical marker of thyroid function when measured using an adequately sensitive assay[10]. Considerable debate centers on the role of other TFTs in supplementing TSH and on the recommendations as to which patients should be tested. The health Insurance Commission in cooperation with the Australian Medicare Program implemented a policy in 1994 that denies payment for TFTs other than an assay for TSH except in limited cases.[11]

The Royal College of Physicians of London has recently recommended that, to confirm the diagnosis of hypothyroidism or hyperthyroidism, concentration of TSH and total or free thyroxine (direct or indirect) must he measured.[12] The National Academy of Clinical Biochemistry recommended serum TSH measurement using a precise and sensitive method as the initial step in the diagnosis of hypothyroidism. Measurement of T_4, FT_4 or FT_3 should not be used as the initial step. Routine measurement of thyroid antibodies in primary hypothyroidism is not recommended unless there is a specific clinical reason.[13]

In the present study the cause of hypothyroidism was the primary thyroid failure in all cases. It has been found that for the initial diagnosis the physicians measured serum T_3 for 25 patients and T_4, for 23 patients. Serum T_3 level in hypothyroidism is variable and often remains within the normal range as occurred in the present study (T_3=1 00+0.56 mmol/ l at initial diagnosis), it's measurement is not recommended for the diagnosis of hypothyrodism.[9,13,14] Measurement of T_4 or FT_4, should not be used as the initial step, but may be indicated in selected cases of suspected hypothyroidism with elevated TSH levels.[11,15] In this study we found that radioactive iodine uptake (RAIU) tests and thyroid scan was done in 21 and 12 patients respectively for initial diagnosis, and 5 patients were diagnosed as on the basis of low RAIU only. The RAIU test provides a useful assessment of thyroid function, in general the higher the iodine uptake the more active the gland. However, in case of patients with hypothyroidism such as Hashimoto's disease the % uptake may be low, normal or high depending on the steps affected in thyroid hormone synthesis. This is therefore of no value in establishing the diagnosis of hypothyroidism Thyroid scan is also of no value in establishing the diagnosis of hypothyroidism and should not be recommended [16].

The role of other thyroid hormone measurements to supplement an assay for TSH in monitoring replacement therapy is controversial. The general consensus is that the sensitive TSH assays represent the "gold standard" for evaluating thyroid hormone replacement levels in patients with an intact hypothalamo-pituitary thyroid (HPT) axis.[7,11,13,17] The replacement dose should be adjusted to keep the TSH in the normal range, neither suppressed nor elevated.[18]

Generally a period of 4-6 weeks is required for TSH levels to return to normal after a hypothyroid patient is treated with thyroxine replacement.[10] The patients fees better within 2-3 weeks of treatment, symptoms and signs of hypothyroid disappear usually within 3-6 months. In the present study it has been observed that with 6.8 months of replacement therapy with thyroxine before registration to our clinic all patients were still remaining hypothyroid clinically (Table III & Table IV) and biochemically (T_3=1.13±10.41µmol/L T_4=55±8 nmol/l, TSH=18.08±7.00 miu/l) at the time of registration. This was because of inadequate dose of thyroxine used in replacement therapy. A mean dose of 69 µg thyroxine daily was used, in contrast to usual replacement dose of 125mg/day or 1.6 Ig/kg/ day.[7,9] After registration to our clinic we used a mean dose of 176 mg/day (Table II). After 3 months of therapy 60% patients were found euthyroid (TSH=2.61±1.72 miu/l) At the end of the study with 6

months of thyroxine therapy all patients were found euthyroid clinically (Table III & Table IV) and biochemically (TSH = 2.691±2.12 miu/l. T_4=1281±30 nmol/l). In most of the can thyroxine was used in multiple daffy doses before registration to our clinic. After registration we used thyroxine as a single daily dose. As the half-life is long it should always be used in a single dose.[9] Even once or twice weekly dose of thyroxine has been found satisfactory in patients with poor drug compliance.[19]

In many cases of suspected hypothyroidism thyroid function tests had been done for initial diagnosis were not rational. The treatment of hypothyroidism is specific and effective. But the adequate doses of thyroxine should be used to achieve and maintain euthyroidism.

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TREATMENT OF ADULT CHRONIC REFRACTORY ITP WITH AZATHIOPRINE

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Summary

Acute idiopathic thrombocytopenic purpura often undergoes spontaneous remission especially in the young. In patients with chronic conditions complete remission is about 50% of adults and 10% of children, neither corticosteroids nor splenectomy is satisfactory. Long-term steroid therapy may cause many undesirable side effects. The immunosuppressive drugs azathioprine (Imuran) was used in long term management of chronic ITP who were resistant to steroid but refused splenectomy as an alternate madality of treatment. Out of 13 patients, 5 patients (23%) had compete remission, 3 patients (23%) had partial remission, 2 patients (15%), showed minimal responses and 3 patients (23%) failed to respond. The median time to response with azathioprine was 4 months. Short term prednisolone was given concomitantly with azathioprine initially. With the exception of transient and reversible leukopenia, no significant toxic effect was observed even after 2 years of therapy. In the management of chronic idiopathic thrommobocytopenic purpura, refractory to steroid and when splenectomy is contraindicated or to be avoided due to any reasons, therapy with azathioprine as an alternative modality of treatment can be considered.

Introduction

Idiopathic thrombocytopenic purpura occurs in all ages in acute and chronic forms. Children mainly have the acute forms, which usually follows a recent viral illness, occurs equally in both sexes and generally resolves within six months. Chronic idiopathic thrombocytopenic purpura occurs more often in adults, often has an insidious onset and shows female preponderance. Both forms are now thought to be due to an anti-platetet antiobody usually of the IgG class which coats autologous platelet and leads to their phagocytosis and destruction by the reticuloendothelial system. In most patients, the spleen is the major site of the production of this antiobody and destruction of platelets.[1]

Criteria for chronic refractory ITP are : 1. Prior treatment with glucocorticoids and/or splenectomy. 2. Age of 10 years or older, 3. No concurrent illness that could cause thrombocytopenia, 4. Platelet counts of <50,000/ml (< 50 x 10^9/L) and 5. Duration of ITP of more than 3 months.[2]

Adult chronic refractory Immune thrombocytopenic purpura (ITP) are not rare. With some extrapolation the frequency can be estimated to be approximately 10 new patients per one million population per year.[2] About 14,000 to 16,000 new cases occur each year in the United States. Initial classical treatment with corticosteroids and splenetomy results in normal or "Safe" platelet counts in more than 70% of patients. Treatment of patients refractory to these two modalities of treatment is difficult.[3] The best treatment of patients with ITP who are refractory to or have contra-indications for splenectomy and corticosteroids remains uncertain. Many difficult modalities for these refractory patients have been published with reports of success. These include trial with imuno-suppresant drugs tike azathioprine, cyclophosphamide, vinca alkoloids, danazole, intravenous immunoglobulins (IVIG) and high doze dexamethasone etc. Among these, azathioprine was the first immunosuppressive durg reported to be effective in patients refrectory to glucocorticoids and splenectomy. In comparison to other immunosuppressant drugs mentioned above, azathioprine has less side effects, well tolerability and relatively high incidence of response to therapy. This drug has been trialed in refractory ITP when splenectomy has failed or contraindicated.[4-13]

The aim of this study was to-reevaluate the role of azathioprine in the treatment of adult chronic ITP who were resistant to prednisolone alone but not interested for splenectomy or splenectomy was contraindicated.

Materials and methods

13 adult chronic ITP patients of both sexes were registered for this study. All the patients were resistant to prednisolone alone. All patients were opted for 2nd line immune supressant drugs before splenectomy or refused splenectomy or had contraindication to splenectomy. At this stage of this treatment mean age of these patients was 37 years (range = 17 to 55 years), 6 of them were males and 7 of them females. They were registered either in BSMMU or in Green View Clinic, Dhaka, Bangladesh.

Treatment protocol

All patients were treated with Azathioprine (Imuran) 100-150mg daily P.O. and Tab. Prednisolone 0.5mg/

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kg body wt. along with Omeprazole 20mg PO BD initially for a short period. These patients were followed up every monthly by complete blood count and random blood sugar. Azathioprine was continued for 4 months irrespective of responses. Then the dose was gradually reduced by 25 mg in every occasion for the complete responders and completely tappered off by 3 months if the platelet count did not fall below normal limits. For patients whose platelet count fell down to below normal limit on reduction of the dose of azathioprine, the dose was increased by 25 mg till platelet count came to normal. This dose was continued for 2 months & then gradually tappered off accordingly. Likewise, the dose of prednisolone was reduced by 5mg/week and gradually tapered off. For the partial responders, this dose of azathioprine was reduced by 25mg and maintained at minimal dose with which the platelet count remained stable within normal ranges. The dose of prednisolone was reduced by 5mg/week and gradually tapered off. Splenectomy was done for 1 patient and infusion of vincristine was given (1.4 mg/kg) slowly over for 4 patients for minimal responders or non-responders. Prednisolone had to be discontinued for 3 patients who showed impaired glucose tolerance.

Evaluation of response :

All the available patients were followed up monthly by CBC and blood sugar and observed clinically for any side effects.

Responses were classified accordingly to the standard criteria of post therapy platelet count as follows :

CR = Complete Response; if the platelet count = > 150 x 10^9/L

Partial response (PR) if platelet count = 50-150 x 10^9/L

Minimal response (MR) = Platlet count increased from baseline upto 50 x 10^9/L

Any response (AR) = CR + PR + MR

No response (NR) = No significant change of platelet count[4,14].

Results

Among 13 patients, 10 patients showed responses, 5 showed complete response (CR), 3 partial response (PR), 2 Minimal response (MR) and 3 complete failure.

All the 5 complete responders showed response upto 1 year, 4 of which showed persistent response 6 months after discontinuation of treatment. 3 of the complete responders showed persistent response at the end of 1 year of stopping treatment. 3 patients showed partial response, of which 1 relapsed after 6 months despite treatment and lost follow up.

2 patients showed minimal response. The opted for other modalities of treatment. They were given vincristine. Among the 3 nonresponders, 1 died of severe internal bleeding. 2 opted for vincristine infusion. 4 patients were (2 non-responders, 2 minimal responders) given vincristne infusion. 2 of them showed response, 1 complete response, 1 partial response, 1 minimal transient response and 1 showed no response. The last patients opted for splenectomy and was improved.

Table-I : *Profile ITP patients : demographic, platelet count, therapy and response.*

Case No.	Sex	Age years	Platelet Count Initial X10^9/L	Platelet Count Corrent X10^9/L	Results	Azathloprine daily doses in mg/day	Duration of Azathloprine therapy	Duration CR or PR month	Previous therapy month	Subsequent therapy	Current therapy
1	M	22	<30	300	CR	150	20	18	Prednisolone		None
2	F	55	40	225	CR	150	4	20	Prednisolone		None
3	F	34	40	120	PR	100	24	19	Prednisolone		Azathioprine
4	M	39	<30	Lost follow up	Failure	150	5		Prednisolone	Vincristine with splenectomy	Lost follow-up
5	F	41	<31	250	MR	150	6		Prednisolone	Vincristine	None
6	M	52	45	250	CR	150	20	10	Prednisolone		None
7	F	43	<30	Died	Failure	150	3		Prednisolone	Died	Died
8	M	26	<30	Lost follow up	MR	150	8		Prednisolone	Vincristine	Lost follow-up
9	F	48	<30	175	CR	150	13	12	Prednisolone		Azathioprine
10	F	17	40	130	PR	100	20		Prednisolone		Azathioprine
11	M	50	<30	350	CR	150	8	10	Prednisolone		Azathioprine
12	M	19	<30	lost follow up	Pr	150	12	6	Prednisolone		Azathioprine
13	F	30	40	lost follow up	failure	100	7		Prednisolone	Vincristine	Azathioprine

CR = complete response
PR = Partial response
MR = Minimal response

Table –II : *Response of ITP patients*

Description	Frequency / duration
CR	5/13 (39%)
PR	3/13 (23%)
MR	2/13 (15%)
Overall response (CR + PR + MR)	10/13 (15%)
No response	3 /13 (77%)
Median time for response	4 months
Median duration of treatment	20 months

One responded completely. The other one inatially showed partial response than become resistant and lost followup :

Table-III : *Adverse effects observed among the steroid patients*

Types of toxicity	No. of patients
Gastro – intestianl (nausea and vomiting)	3
Granulocytopenia (<4000 x 10^9/L)	3
Anemia (<10mg Hb/dl)	1
Infection	1
Fever	1
Myalgia / arthralgia	1

Discussion

Refractory patients (who comprise 25% to 30% of patients with ITP) are defined as those in whom treatment with standard dose of corticosteroids and splenectomy failed and who requried further therapy because of safe platelet counts or clinical bleeding.[3] Though the treatment (for those patients refractory to corticosteroid and splenectomy or for whom splenectomy is contraindicated or refused include immunosuppression with azathioprine, vinca alkaloids, cyclophozphamide, 6-MP, danzaole, etc.[3-5,7,8,15] We treated patients with chronic ITP who are refrectory to corticosteroid alone but refused splenectomy. These patients were relactant to operative procedure and opted for other modalities of treatment.

The reason for choosing azathioprine was to avoid toxicities of other drugs like vincristine, cyclophosphamide, danazole etc and also as the azaathioprine is cheaper than these drugs. Our results are consistent with some other studied carried out previously.[4,15,16] The median time for response was about 4 months, which correlates with the report of Guiquandom I et al.[4] We gave corticosteroids initially with azathioprine for short period of time initially to enhance the effecacy of azathioprine and stabilize initial platelet count to a safe level as the effect of azathioprine appears[4,12]. The complete response rate of azathioprine in earliar reports were 45%, 57%, 50%, 29% and 47%.[4,6,15-17].

In our study we observed 39% complete response rate, 26% partial response, 15% minimal response and 23% failure. 23% patients showed continued response after discontinuation of therapy. We observed minimal side effects of azathioprine as shown in table-II, transient neutropenia in 3 patients and GI problem in 3 patients were the only significant side effects in our series.

It is evident from our results and earliar reports[4,15] that the dose of azathioprine needed to obtain a therapeutic effect in patient with ITP dose not influence cellular depression of bone marrow or induce harmful degree of immurosuppression in patient treated. We did not observe significant incidence of infection in patient treated with azathioprine. It was demonstrated previousy[18] that although the primary and secondary immune responses may be inhibited by the azathioprine therapy levels of immunoglobalin presumably having the usual spectrum of antiviral and antibacterial activities are mentioned.[18] The effect of azathioprine or the platelt count may not become apparent for weeks, an observation has been supported by the other investigators[15,16]. It seem advisable to begin treatment cocomitantly with prednisolone, which should have a more rapid effect. As the platelet reach normal levels, the prednisolone is reduced gradually and discontinued. If this platelets then remain normal, the dose of azathioprine is gredually reduced and finally discontinued.[15,16] It is evident that our observation and previous results show azathioprine has to be maintained at least 3 for 4 months to maintain a remission. The minimum period of azathioprine reduired three months as reported previous report[15] but it was 4 months in our observation and in the study of Quiquandom I et al.[4]

Azathioprine interferes with the synthesis of DNA or RNA or both. To understand the beneficial effect of azathioprine in chronic ITP, the pathogenic mechanisms involved this disease should be discussed in the light of clonal selectors theory of Burnet.[6,19,20] It is postutea that there is a rise of immunologically competent cells from the “forbidden clones”. That are producing antibodies against patients own platelets. Azathioprine is thought to act by permanent suppression or destruction of these anomalous cells. It could also be postulated that the amount of antigen present was no longer capable of stimulating maxmum antibody production since it has been shown that the minimum dose of angigen required for an insult.[17-18] Azathioprine may have had its effect by preventing the formation of a sufficient large “pool” of immunologically competent cells capable of giving a maximum antibody response.[6]

In summary, we feel that corticosteroid should be the initial treatment in adult cases of ITP and if there is no favourable response, splenectomy is the treatment of choice However, where splenectomy is refused or instead conservative treatment and other modalities of treatment is descried azathioprine may be tried as it is less toxic in comparison to other drugs and have acceptable efficacy.

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FAMILY GENETIC STUDY OF CHILDHOOD AUTISM

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Summary :

There is considerable evidence from family and twin studies that aetiology of autism is predominantly genetic. However, the definition of the phenotype is questionable and it is suggested that the genetic liability for autism may be expressed in nonautistic relatives in a phenotype that is milder but qualitatively similar to the defining features of autism. This study was aimed to evaluate the rate of childhood autism and autism like features among the first degree relatives of austistic probands. Sixty five consecutive cases of childhood autism attended in Child Mental Health Outpatient Clinic of Bangabandhu Sheikh Mujib Medical University, Dhaka, from July 1998 to June 2000, satisfying clinical diagnosis of ICD-10 were included for this study. Same number of patients were selected as controls from children outpatient services of the University. A structured family history interview was used to assess the autism or autistic like features in first degree relatives of the probands of the both groups.

Though family history of childhood autism is insignificant, a considerable rate of social and communication impairments and restrictive & repetitive activities and interests were found in the first degree families of the autistic probands which are significantly higher than that of the families of the controls. Further studies are needed to establish the strengths of these autism phenotypic behavioural features and these should be included in the genetic analyses of childhood autism.

Introduction

Autism is a rare but severe developmental disorder characterized by lack of social relatedness, poor communication skills and absence of imaginative activity coupled with repetitive stereotypic behaviour and with early onset. Aetiology and pathogenesis of autism can view as a behavioural syndrome with various neurobiological causes with predominantly of genetic aetiology. It was estimated that about 47% of the autistic individuals had chromosome abnormalities and about half of whom showed the fragile X.[1] Another study showed the rate of fragile X in autism was about 2.5%.[2] The risk of recurrence of autism in families (i.e. the frequency of autism in subsequently born siblings) is estimated 6-8% or up to 200 times the risk in the general population.[3] Twin studies of geographically defined population[4-6] detected pairwise concordance rate ranged from 37-91 % between MZ twins and 0% in DZ twins, producing an average heritability estimate over 90%.

Although the importance of genetic factors in autism has been firmly established, the definition of the phenotype is questionable. A number of family and twin studies have suggested that a behavioural phenotype that is qualitatively similar to but more broadly defined in relatives of autistic individuals than in the general population. August et al[7] reported familial aggregation of cognitive impairment in the siblings of autistic probands. Wolff et al[8] interviewed the parents of autistic children and the parents of nonautistic mentally retarded comparison subjects and found that the parents of the autistic children were more often judged to lack emotional responsiveness and empathy, show impaired rapport with the examiner and have histories of oversensitivity to experience, special interest patterns, and oddities of social communication. Piven et al[9] detected significantly higher rates of social deficits in the parents of autistic children than in the parents of children with Down syndrome, using a semi structured personality interview. Bolton et al[10] examined features of autism in the family histories of the first-degree relatives of autistic and Down syndrome probands using semistructured family history interview. The results indicated that the relatives of the autistic probands had significantly higher rates of communication and social deficits and stereotyped behaviours than the relatives of the Down syndrome probands. Piven et al[11] reported similar findings among the relatives in families with multiple incidence autism in comparison with relatives of Down syndrome probands by using same family history method. All of these studies suggested that the genetic liability for autism may be expressed in nonautistic relatives in a phenotype that is milder but qualitatively similar to defining features of autism. Present study was designed to evaluate the rate of childhood autism and autism like features among the first degree relatives of autistic probands.

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Materials and methods

Selection of Autism Families

The study was carried out in the Child Mental Health Outpatient Clinic of the Department of Psychiatry, Bangabandhu Sheikh Mujib Medical University in Dhaka. The present sample consists of first degree relatives of the families of a consecutive series of 65 new cases of childhood autism between July 1998 and June 2000. Childhood autism was assigned according to clinical diagnosis of ICD -10.[12] These autism families included 56 male and 9 female autistic probands. The boy and girl ratio was 6.2:1. Their age ranged between 3 to 13 years with a mean of 5.80(± 2.65) years. Urban rural distribution were 48 and 17 cases respectively. Thirty three cases were predominantly of middle income group followed by 21 and 11 cases of high and low income group respectively.

Selection of Comparison Families

The first degree relatives of same number of families of nonautic cases with some medical problems having no clinically identifiable developmental disorder were selected from children outpatient department of the University for control comparison group in this study. They were randomly recruited matching with age group, sex and socioeconomic condition of the autistic probands.

Assessment of autism and autism like features

Available first degree family members (parents and siblings) of autistic and control subjects were interviewed for assessing the autism. For each, thorough clinical assessment was undertaken using semistructured case assessment sheet. The diagnosis was phenomenologically based according to ICD-10 criteria for childhood autism.[12]

Then, the family were interviewed with a structured family history interview to assess the presence of a range of autistic like features that hypothesized to possibly be generally related to autism. This instrument including operational definition within it was prepared on the basis of family history interview applied by Bolton et al[10] with some minor modifications and exclusion of the items not directly related to autism. It included communication skills, scholastic skills, social behaviour and adult and childhood functioning. Features were rated as absent (rating = 0), mild or probably present (rating = 1) or severe or definitely present (rating =2). The items were grouped into three principal features that parallel the defining features of autism: social impairment, communication impairment and restrictive, repetitive and stereotyped behaviour.

The parents in each family interviewed about themselves and about the siblings of the probands. Information about the first degree family members who were not available also collected from either parents which further strengthened by the reliable informants to avoid extensive involvement due to limited resources.

As the relatives within same family for the autism and autism like features were not statistically independent, the family was treated as the unit of analysis. The comparison was made between autistic families and control families. Statistical analyses involved two tailed t- tests and x^2 tests with Yates' correction.

Results

Sixty-five fathers, 65 mothers and 115 siblings from the first degree relatives of the 65 autism probands and 65 mothers, 65 fathers and 182 siblings from the first degree relatives of the 65 control probands were included in this study. Table-I shows that only one parents and 2 siblings were found to be autistic in autism group. In the control group, no incidence of autism was reported. Another autism father met the criteria for autism on the basis of current behaviour, but no informant was available to detect the presence of autism in childhood and thereby not considered as a case of autism.

First degree relatives of the autism and control probands were compared with total scores of autism like features from Family Diagnostic Interview by means of two tailed t-tests. There was a general excess of scores in autism families. Particularly, the autism fathers and siblings showed higher rates of social impairments, communication impairments and repetitive and stereotyped behaviour than did control fathers and siblings. However, the difference of scores between two groups was too low to reach the level of significance (Table-II).

Analysis of the autism like features among the first degree relatives of the autism and control probands revealed that 11 (16.92%) fathers, 2(3.08%) mothers and 26(22.61 %) siblings had one or more autism like features in the autism group. In the control group 1(1.54%) fathers, no mothers and 5(2.86%) siblings had had autistic like features. Overall, the difference was significant at 5% level. Table -III shows the comparison of the frequency of these features between two groups. More than one feature were recorded among the relatives. The significance of difference between two groups was tested by x^2 using Yates' correction when appropriate for each features. This analysis indicated that overall increased

frequency of autism like features in the all types of first degree relatives of autistic probands was paralleled by increased frequency of individual features. Significantly higher rates of social and communication impairments but not repetitive and stereotyped behaviours were detected in the autism fathers whereas due to very small numbers for meaningful comparison, no difference was found in the autism mothers and controls. The significantly higher rates of social impairment, communication impairment, and repetitive and stereotyped behavoiour were detected in the siblings of the autism group. Statistical comparison of the rates of individual item from the family history interview between the first degree relatives of the autism and control probands was not undertaken because of low rates of occurence to reach significance.

Table-1: *Comparison of existence of autism in the first degree relatives of the families of autism and control probands*

Type of relatives	Autism group		Control group		X^2 sig.
	N	%	N	%	
Fathers	1	1.54	0	0.00	NS
Mothers	0	0.00	0	0.00	NS
Siblings	2	1.74	0	0.00	NS

Table-II : *Comparison between first degree relatives of autism and control probands with total scores of autism like features on Family Diagnostic Interview**

Type of relative	Autism group	Control group	t-test
Fathers	0.9 (±11.71)	0.0 (±00.00)	0.47, NS
Mothers	0.1 (±16.72)	0.0 (±00.00)	0.04, NS
Siblings	1.0 (±09.51)	0.0 (±18.86)	0.66, NS

* Data are expressed as X = SD

Table-III : *Comparison of frequency of autistic features in the first degree relatives of the autism and control probands*

Austistic features	Autism group		Control group		X^2 sig.
	N	%	N	%	
Fathers					
Social impairment	8	12.30	0	0.00	<0.05
Communication impairment	6	9.23	1	1.54	<0.02
Repetitive & stereotyped behaviour	1	1.54	0	0.00	NS
Mothers					
Social impairment	1	1.54	0	0.00	NS
Communication impairment	1	1.54	0	0.00	NS
Repetitive & stereotyped behaviour	1	1.54	0	0.00	NS
Siblings					
Social impairment	18	15.65	1	0.55	<0.01
Communication impairment	13	11.30	3	1.65	<0.01
Repetitive & stereotyped behaviour	7	6.09	1	0.55	<0.05

Discussion

In this study, incidence of childhood autism in the first degree family members of autism probands was found only 4.6% in total which was 0% in the similar family members of the control probands. The difference was insignificant and statistically not analyzable but indicating the risk of occurence of autism among family members. It was estimated that risk of recurrence of autism in families is 6-8.3%.[3] However, the findings of the large scale genetic studies on autism can only evaluate the findings of the present study.

Though quantitative analyses revealed high rate of autism like features among the first degree family members of autistic probands as of control probands, the result was not significant because of low rate of scores. This is partly due to mild form of features existing among the relatives, lack of information from multiple sources, inability to conduct extensive and direct interview and assessment of all the relatives.

In this study, it was revealed that the first degree relatives of autistic probands had familial aggregation of behaviours that was milder than but qualitatively similar to the defining features of autism. This finding broadly simulates and replicates the findings of other studies.[4,5,7,9-11] Within this study, the findings for the fathers and siblings are particularly significant which suggests that genetic liability for autism are possibly expressed through father shared by the siblings. Several twin studies[4-6] support this view partially at least for the siblings, again it needs intensive family genetic study on mode of genetic transmission of the autism phenotype.

To our knowledge, the present study is the first to examine genetic liability for autism among the relatives of the autism probands in Bangladesh. An additional strength of this study is the control comparison sample of children who were nonautistic, having no developmental disorder and more nearer to the general children population. However, there are several limitations in this study. Due to little or no scope of investigations, it was not possible to exclude the aetiologically related co-occurring conditions including fragile X screenings and formal IQ testing was not undertaken due to lack of resources. Further, all the family members were not directly interviewed though information were obtained from reliable informants due to limited resources. All of these factors could create the biasness of the results.

The result of this study suggests the existence of genetically related features of autism which demand to be included in the genetic analyses of autism and indicate the need for further detailed genetic linkage studies.

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ASSOCIATION NEWS

A press briefing was organised by the association due to dengue fever epidemic for awareness of the people through print media. Prof. M Hasan, presided and questions were answered by Prof. MN Alam. A CME programme was also held on Dengue fever on 31st July, 2000. Prof. M Tahir, Past president of the association and Pro Vice-Chancellor of Bangabandhu Sheikh Mujib Medical University chaired the seminar. A large number specialists and practitioners attended the seminar. On 13th December, a CME was organised in DMC gallery on Bronchial asthma which was chaired by Prof. Ferdous Ara J Janan, Professor of Medicine, Dhaka Medical College. "Tuberculosis : at the dawn of 21st Century" was the topic of another CME, held in Bangabandhu Sheikh Mujib Medical University, which was chaired by Prof. Harun-ur-Rashid, Chairman, Department of Nephrology, BSMMU on 17th, December 2000.

12th Annual Convention & Scientific Session will be held on 31st March - 1st April 2001. The inaugural ceremony will be held at Dhaka Sheraton Hotel. Former Chief Justice Muhammad Habibur Rahman will be the Chief Guest.

VALIDITY OF PEAK EXPIRATORY FLOW MEASUREMENT IN ASSESSING REVERSIBILITY OF AIRWAY OBSTRUCTION

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Summary

Spirometric measurement of FEV_1 (Forced Expiratory Volume in one second) for "Reversibility Test" is widely used in the USA for every patient suspected of having bronchial asthma, at least for initial assessment. But in a poor country like Bangladesh, spirometry can not be afforded for every patient suspected of having bronchial asthma at least in general practice due to limited resources. Most of the patients with bronchial asthma are looked after by general practitioners. In this setting a simple, cheap and reliable method is needed to assess the reversibility of airflow obstruction in patients suspected of having bronchial asthma.

A prospective case control study was carried out over 90 patients with bronchial asthma and 30 normal healthy controls in the asthma centre, Institute of Diseases of Chest & Hospital (IDCH). This study was carried out during a period of two years from January 1997 to December 1998 for determination of validity of peak expiratory flow measurement in assessing reversibility of airflow obstruction. All the 120 subjects (90+30) completed the study. In the control group (Group-A), M : F ratio was 1:31, age range was from 15 to 68 and of 5 different occupation (service, business housewife, student and worker). In patient group (Group-B), M : F ratio was 1. 2 5 :1, age range was from 15 to 70 and of 5 different occupation (service, business, housewife, student and worker). There was no statistically significant difference between the two groups regarding age, sex and occupation. In group-A, the bronchodilator drug could not produce any significant improvement in FEV_1 and PEFR (Peak Expiratory flow rate) but in group- B, there was highly significant improvement in FEV_1 and PEFR after bronchodilator. The improvement in FEV_1 was compared with improvement in PEFR. There was a positive correlation between improvement in PEFR and improvement FEV_1 ($r = 0.700433$, $P<0.001$). From the study, it has been shown that reversibility of airflow obstruction in patients with asthma can be assessed by means of simple PEFR measurement and can give comparable results to those obtained by FEV_1. The criterion of an absolute increase in PEFR of > 60 L/min avoids having to make any calculations and appears to be an excellent way of predicting reversibility.

Introduction

Bronchial asthma affects approximately 5% of the population of USA and Europe'. In Bangladesh, bronchial asthma is a common disease but we do not know the actual percentage of Bangladeshi population suffering from bronchial asthma. But it appears that its prevalence in Bangladesh is not very less than any reported figures in the Western countries, like USA and Europe. Bronchial asthma represents one of the major health problems both in terms of human suffering and in cost to society. The diagnosis of bronchial asthma depends on (i) history (ii) physical finding (iii) Pulmonary Function tests (PFT) (iv) some other tests. Now, regarding PFT in bronchial asthma, obstructive defects are found which are reversible or at least partially reversible.

Spirometric measurement (FEV_1 etc) before and after the patient inhale a short acting bronchodilator that is the "Reversibility Test" should be undertaken for patients in whom the diagnosis of asthma is being considered.[2,3] This helps to determine whether there is airflow obstruction and whether it is reversible over the short term.

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Now, we know that PEF within and between subjects correlates well with FEV_1[5]. Also, a number of other studies proved that PEFR correlates with FEV_1.[4,6] Although the use of the peak flow meter is widely advocated, we are not aware of the data of the validity of peak flow measurement in assessing reversibility of airflow obstruction in patients with asthma and there is no practical criterion for assessing the reversibility of airflow obstruction in general practice. This study is designed for the purpose.

Patients and Methods

This prospective case control study was carried out in Asthma centre, Institute of Diseases of Chest and Hospital (IDCH), Mohakhali, Dhaka, during the period January 1997 to December, 1998.

A total number of 120 subjects were studied for "Reversibility Test". Of which, 90 subjects were patients with Bronchial Asthma without any criteria of exclusion attending asthma centre, IDCH. Thirty (30) subjects were taken as healthy control who were doctors, nurses and employees of IDCH as well as attendant of the patients. All the 90 patients with bronchial asthma were treated previously by general practitioner in different parts of the country.

The diagnosis of bronchial asthma was based on history suggestive of bronchial asthma such as - recurrent difficulty in breathing; recurrent cough, worse particularly at night or in early morning; recurrent wheeze; recurrent chest tightness.

Physical findings suggestive of bronchial asthma such as- breath sound, vesicular with prolonged expiration with bilateral expiratory rhonchi. Pulmonary Function test: Obstructive defects at least partially reversible by bronchodilator drug with or without, Sputum eosinophils.

Inclusion Criteria:

For cases- patients of both sexes having bronchial asthma diagnosed on the basis of above mentioned criteria; non-smoker; age 15 years and above upto 70 years; respiratory symptoms for greater than 1 year; only clinically clean cases without complications.

For control group- normal healthy persons of both sexes; non smoker; age from 15 years to 70 years.

Exclusion Criteria

Patients having - COPD; Patients of bronchial asthma with other concomitant disease such as active TB, Bronchiectasis, Pneumonia, Pneumothorax Pulmonary fibrosis, DM, uncontrolled hypertension CCF, corpulmonale, recent M.I. (within 8 wks). CVD, Systemic and local sepsis, renal failure, any malignancy and filariasis etc.; Asthma patients with pregnancy; Severe acute Asthma; Cardiac asthma.

A standard proforma with questionnaire was designed and filled up to identify patients with bronchial asthma. The patients were identified according to the predetermined criteria as well as inclusion and exclusion criteria. A thorough physical examination was done and following investigations were done.

TC, DC, Hb% and ESR; CXR PA view; Spirometry; Sputum for Eosinophils; ECG-if age is more than 40 yrs.; CFT for filariasis if needed.

All the data were analysed by the help of SPSS software program (version 6.0). Comparison. between the groups was done by student's 't' Test and 'r' test. P values <0.05 were considered statistically significant.

Aims and Objectives

The aims and objects of the present study are :

1. To find out a simple, cheap and easily accessible way for "Reversibility test" which will help in the diagnosis of Bronchial asthma.
2. To see the relationship between FEV_1, and PEFR.
3. To prove the validity of PEFR measurement in assessing reversibility of airflow obstruction.

Observation and Results

A total no. of 90 patients with bronchial asthma who attended the asthma centre, IDCH, during the period from January 1997 to December 1998, having no criteria of exclusion, were included in this study. All the 90 patients completed the study and were grouped as group-B. 30 normal healthy subjects were taken as control and were grouped as group-A. All the control subjects were in good health without any complains.

Pulmonary function before and after bronchodilator are shown in Table-I. In group-A, before bronchodilator, FEV_1 ranged from 1.94 to 3.92 liters with mean 2.84 liters and after bronchodilator, FEV_1 ranged from 1.94-3.92 liters with mean 2.85 liters. P value was not significant ($P>0.05$; $t = 1.8$). That is there was no significant change in normal healthy controls. Similar was the case with PEFR after bronchodilator in normal healthy controls. Before bronchodilator, PEFR ranged from 320-660 L/min with mean 485.33 L/min and after bronchodilator PEFR ranged from 320-670 L/min. That is the change was not statistically significant ($P>0.05$, $t= 1.8$). This is both FEV_1, and PEFR did not change significantly by bronchoidilator drug in normal healthy controls.

Consequently there is no question of reversibility of airflow obstruction in normal healthy controls.

But in group-B before bronchodilator FEV_1 ranged from 0.61 to 3.32 liters with mean 1.57 liters and after bronchodilator, FEV_1 ranged from 0.76 to 3.64 liters with mean 1.88 liters. P value was highly. significant P<0.001, t=18.87. That is there was significant change in FEV_1 after bronchodilator in asthmatic patients. Similar was with PEFR after bronchodilator in asthmatic patients. Before bronchodilator, PEER ranged from 100 to 650 L/min with mean 292.67 L/min and after bronchodilator, PEFR ranged from 160 to 680 L/min with mean 357.78 L/min. That is the change was highly significant (P<0.001, t= 26.98).

This is both FEV_1 and PEFR did change significantly by bronchodilator drug in asthmatic patients.

Reversibility : Sixty of the Ninety patients (group-B) with airflow obstruction (66.67%) showed a reversibility of 9% or more of predicated FEV_1. Seventy four of the ninety patients (group-B) with airflow obstruction (82.22%) showed a reversibility of 200 ml or more in FEV_1 as absolute value (Table II)

There was correlation between improvement in PEFR (as absolute value) and in FEV_1 (as percentage of predicated value), P<0.001, r= 0.70043. There was also correlation between improvement in PEFR (as absolute value) and in FEV_1 (as absolute value), P<0.001, r = 0.59, (Table-II).

Table-I : *FEV_1 and PEFR before and after bronchodilator (BD) in Group-A (n=30) and Group-B (n=90)*

	Group-A			Group-B		
	Before BD Mean (Range)	After BD Mean (Range)	P value	Before BD Mean (Range)	After BD Mean (Range)	P value
FEV_1 (Liter)	2.84 (1.94-3.92)	2.85 (1.94-3.92)	N.S. (P>.05 (t-1.8)	1.57 (.61-3.32	1.88 (.78-3.64)	S (P<0.001) (t-18.87)
PEFR (L/min)	485.33 (320.660)	486.33 (320-670)	N.S. (p>.05 (t=1.8)	292.67 (100-650)	357.78 (160-680)	S (P<0.001) (t=26.98)

Table-II : *Increase in $FEV_1 \geq 9\%$ or <9% of Predicated value and $\geq$ 200 ml or < 200 ml as absolute value in Group – B (n=90)*

	> 9% of predicated Value		> 9% of predicated Value		Total	
	No.	%	No.	%	No.	%
Increase in FEV_1	60	66.67%	30	33.33	90	100
	> 200 ml as absolute value		< 200 ml as absolute value		Total	
	No.	%	No.	%	No.	%
Increase in FEV_1	74	82.22	16	17.78	90	100

Table-III : *Correlation between increase in PEFR (as absolute vlaue) and increase in FEV_1 (AS% of predicted value), and between increase in PEFR (As absolute value) and increase in FEV_1 (as absolute) in Group-N (n=90)*

Increase in FEV_1 as % of predicoted value Mean with Range (%)	Increase in PEFR as absolute value Mean with Range (L/min)	R	P
10.87 (1.47-30.43)	65.11 (20-120)	0.70043 3	<0.001
Increase in FEV1 as Absolute value Mean with Range (ml)	Increase in PEFR as absolute value. Mean with Range (L/min)	R	P
317.33 (50-840)	65.11 (20-120)	0.599	<0.001

Discussion

Spirometric measurement (e.g. FEV_1) for assessing reversibility of airflow obstruction is, not widely used in Bangladesh because spirometric measurement is expensive and most patients in Bangladesh are poor and they can not afford test by spirometer. Again spirometer is available in only few limited centres in Bangladesh. For the above reasons, we need an alternative method to this spirometric measurement. This alternative method should be simple, cheap, reliable and all general practitioner should have access to it. Present study was under taken to establish that peak Expiratory Flow measurement is valid in assessing reversibility of airway obstruction. In this study 30 healthy controls and 90 patients of Bronchial asthma were included. The controls (Group-A) were of different ages ranging from 15 to 68 yrs. with mean 34.5 yrs, and M:F ratio was 1:3:1. The patients (Group-B) were of different ages ranging from 15 to 70 yrs with mean 37.84 yrs. and the M:F ratio was 1.25:1 . In previous study made by Dekker FW et at[7], the age range of patients was from 40 to 84 yrs. with mean 61.9 yrs.

Table-I shows Pulmonary function before and after bronchodilator. In Group-A i.e. in normal healthy control subjects, there is no significant change in FEV_1 after bronchodilator (P>0.05, t= .1.8) . Similar is the case with PEFR after bronchodilator in normal healthy control subjects (P>0.05, t=1.8) . That is both FEV_1 and PEFR do not change significantly by bronchodilator drug in normal healthy controls. Consequently, there is no question of reversibility of airflow obstruction in normal healthy controls. But in Group-B, there is significant difference in FEV_1 after bronchodilator (P<0.001, t=18.87) . Similar is the case with PEFR after brochodilator (P<0.001, t= 26.98) . That is, there is highly significant change in both FEV_1 and PEFR by bronchodilaor drug in asthmatic patients.

In this study 60 patients out of 90 patients i.e. 66.67% of patients have reversible airflow obstruction depending on the criterion of 9 % or more increase in FEV_1 as a percentage of predicoted value[10] and 74 patients out of 90 patients i.e. 82.22% of patients have reversible airflow obstruction depending on the criterion 200 ml or more increase in FEV_1 as absolute value[8].

In previous study by Dekker FW et al[7], these were 42.5% and 53.4% respectively. The difference is due to the fact that both COPD and asthmatic patients were included in their study and in this study, only asthmatic patients are included.

In this study positive correlation between increase in PEFR (as absolute value) and increase in FEV_1 (as % of predicoted value) where P<0.001, r=0.700433. And it also shows positive correlation between increase in PEFR (as absolute value) and increase in FEV_1 (as absolute value) where P<0.001, r=599.

In previous study by Dekker FW et al[7], the correlation between the change in PEFR and change in FEV_1 was 0.67 (r=0.67, P<0.001). That is both in this study and previous study by Dekker FW et al[7], there is positive correlation between change in PEFR and change in FEV_1.

The result of this study depends on the measure of reversibility that have been used. Various criteria for reversibility have been proposed. It has been found that increase in FEV_1 expressed as a percentage of

predicoted value or as absolute increase in FEV_1 are better ways of expressing reversibility of airflow obstruction than increase in FEV_1 expressed as a percentage of baseline FEV_1 .[9,10,11,12]

The moderate correlation between improvement in PEFR and FEV_1 is not unexpected (in this study correlation coefficient r=0.700433, P>0.001 and in previous study by Dekker FW et al,[7] r= 0.67,P<0.001). Although these two indices of pulmonary function are determined by somewhat different physiological mechanism[13], the relation between single measurement of PEFR and FEV_1 is good[5]. The result of this study suggest that the bronchodilating effect of a a adrenergic agent can be documented by either variable i.e. FEV_1. or PERFR. Dekker FW et al[7], also found that the bronchodilating effect of a β adrenergic agent could be documented by either variable FEV_1 or PEFR. They concluded that absolute changes in PEFR could be used as a simple technique to diagnoses reversible airflow obstruction in patients from general practice.

Failure of the FEV_1 or PEFR to respond to a bronchodilator on a single occasion, does not imply that the patient has irreversible airflow obstruction. Some patients who do not show reversible airflow obstruction as judged by a peak flow measurement may have reversible airflow obstruction which would have been detected by the FEV_1 or at a follow up visit after appropriate treatment by either PEFR or FEV_1. For some patients, a 2 to 3 weeks trial of oral corticosteriods therapy may be required to demonstrate reversibility.[14]

The finding of this study has clinical implications for general practice. Previous criteria for reversibility of airflow obstruction have mainly concerned FEV_1. Result of this study has shown that the reversibility of airflow obstruction in patients with asthma can be assessed by means of a simple PEFR measurement and give comparable result to those obtained by FEV_1. The criterion of absolute increase in PEFR of 60 L/ min avoids having to make any calculations and appears to be an excellent way of predicting reversibility.

Conclusions

From the above study it can be concluded that the bronchodilating effect of a β adrenergic agent can be documented by either variable, FEV_1 or PEFR. Peak expiratory flow i.e. PEFR is valid in assessing reversibility of airflow obstruction in general practice.

Absolute increase in PEFR $\geq$60 L/min can be used as a simple technique to diagnose reversible airflow obstruction in patients with bronchial asthma or in patients with suspected bronchial asthma who are 15 years in age or above in general practice.

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