A few words about our journey

A long time had been passed since this editorial board had taken the charge of this journal. The first issue which was published by us was a combined issue of number 1 and 2 in the year, 2003. Since then in each year two issues had been published regularly. The number of members of this journal are also increasing which increase our responsibility. Many advice and criticism came from our advisory board and well wishers. We had tried to modify the journal accordingly. Thus vancouver pattern of reference had been incorporated. Many internationally reputed scientists had contributed here in response to our request. These were and are being published as “invited article.” We are grateful to them. We are also thankful to the reviewers, who, despite of their busy schedule are giving valuable suggestions for the improvement of the articles. The contents of all the articles published since 2003 are given in this issue. If anybody is interested in any particular article can contact us for reprint. We are also thankful to the sponsorers for their constant support. We know that the members of this journal are our real asset. Without their contribution we could not reach where we are today. We hope that all help will be continued in the coming days also, so that we can improve this journal. The cover page is changed from this issue to give the journal a new look. Please let us know whether you like it or not. Readers are also requested to express their views and share their experiences with us. If we have sufficient communications, we can introduce a new column, “Letters to the editors”. The editorial board acknowledge all the cooperations it is having to make its journey smooth.
Early diagnosis of tuberculosis is essential to fight against the global threat of the disease. The well established conventional methods are time consuming and occasionally inconclusive. Molecular methods on the other hand are rapid, sensitive and specific. But as these methods are expensive, their beneficial effects are far from the reach of people of developing countries like India. The molecular methods to diagnose tuberculosis is discussed in details in this article.

Introduction

Despite the discovery of Mycobacterium tuberculosis, more than a hundred years ago, tuberculosis remains as a major killer disease worldwide. Presently about a third of world population is infected with Mycobacterium tuberculosis and related pathogens. About 10 million new cases each year with 3 million deaths account for the maximum number of mortality due to a single pathogen in the would. (1)

This corresponds to 52,000 deaths per week or above 7000 deaths per day and accounts for 1000 new cases per hour, each day. (2) In India, each year, about 2 million patients develop active disease and upto half a million die. (3) More over emergence of Multi Drug Resistant strains and their sinister association with HIV/AIDS has posts of serious threat to the tuberculosis control programmes in different countries. (4) MDR is associated with high mortality rate of 52 to 80% and spans a relatively short time (4-16 weeks) from diagnosis to death. (5)

Conventional Diagnostic Tools

In the face of this global threat of tuberculosis, highly sensitive and specific tools for accurate diagnosis of the disease is the need of the time for early initiation of proper treatment. For pulmonary tuberculosis, demonstration of Acid Fast Bacilli by conventional Ziehl Neelson stain or Auramin Rhodamine Fluorescent stain still remains as the gold standard. (6) But in Extra Pulmonary Tuberculosis (EPTB) like Tubercular Meningitis, pleural effusion or ascitis. AFB staining is positive in less than 10% of patients with pleural effusion in most reports, whereas pleural fluid culture is positive in 12-70% of cases. (7) As it takes 6 weeks to obtain results of conventional culture on solid LJ slants or liquid Middlebrook’s broth with nutritional supplements, treatment decision have to be made before the laboratory results are available. Culture based techniques also require viable organisms and this may pose a problem in treated cases. (8) In a life threatening condition like Tubercular meningitis, demonstration of AFB and culture is seldom helpful. In a review by Molavi and Lefrock, cerebrospinal fluid smears were positive in only 10-40% of cases, while culture isolations was positive in 45-90% cases. (9) In another study by Kennedy and Fallon, 37% smear & 52% culture positivity were found. (10) It is to mentioned here that for smear positivity at least $10^4$ Organism / ml is required.
This is not to underestimate the role of modern non-radiometric and radiometric cultural identifications which yield a rapid and more accurate diagnosis. The methods are as follows:

**Non Radiometric Systems**

(A) **Micro Colony Detection in solid media**\(^{(1)}\) Middle Brook 7H11 Agar: Results may be available in 7-10 days. Less expensive but less sensitive & labour intensive.

(B) **Septic Check AFB Method**\(^{(12)}\): Requires 3 weeks of incubation. Species identification of different Mycobacteria is possible.

(C) **MG/T 960 mycobacteria detection**\(^{(13)}\): Time taken for detection: 10-16 days. It is a fluorescence based automated continuous growth monitoring system. It is rapid than conventional culture & accurate but expensive.

(D) **MB/BacT System**\(^{(14)}\): Also an automated and computerised system depending on detection of CO\(_2\) released by grown mycobacteria. Average time taken for the detection is 12 to 20 days.

**Radiometric System**

**BACTEC 460 TB method**\(^{(15)}\): Based on mycobacterial growth in 14\(_c\)-labelled palmitic acid in 7H12 media. \(^{14}\)CO\(_2\) released by mycobacterial growth is measured by BACTEC System instrument & reported in terms of Growth Index (GI). Average time of reporting: 7-14 days.

Apart from the modern culture based methods, there are techniques based on LAM (Lipo arabino mannan) and other antigen detections, demonstration of antibodies and bacteriophage base studies like FAST plaque TB. Many of the tests are expensive and limited to research works but not for widespread use for clinical diagnosis in a large scale.

One test must be mentioned to complete the list which is demonstration of IFN gamma by activated T Cells of tuberculosis patient by Quanti FERON—TB Gold (QFN-Gold) test (celletic Limited, St. Klida, Australia and T-SPOT-TB (Oxford Immunotec, Oxford UK). These are internationally accepted commercial kits to identify release of IFN-gamma or detection of T-lymphocytes themselves, respectively.

**Molecular Biological Tools**

The different conventional diagnostic methods mentioned so far are well established and inexpensive. But they are time consuming, labour intensive and provide inconclusive results on many occasions. As already mentioned, the demonstration of AFB is less sensitive and conventional cultures are time consuming, so much so that they seldom contribute to the timely treatment of the patient in clinical practice. The modern culture based methods are more sensitive but still require several weeks time for the reporting.

The introduction of molecular methods has revolutionised the identification of mycobacteria in the following ways—

1. Highly sensitive and specific
2. They have sufficiently reduced the time of reporting. Can be reported even on same day.
3. Can detect much lower number of bacteria (upto the extent of 10 bacteria / ml).
4. Can be used in species identification (genotyping)
5. Can be used to identify Rifampicin, INH and other drug resistant strains.
6. Fingerprinting can be used as a powerful epidemiological tool.

The different molecular biological tools used in diagnosis of tuberculosis can be broadly classified into the following categories:—

**A. Nucleic Acid Amplification** (PCR Based Methods) and amplification with reverse hybridisation :

The basic technique is amplification of a highly conserved gene present in the mycobacterial genome i.e. IS 6110 or Hsp 65 gene. They are available in different forms with some modifications:
Molecular Diagnosis of Tuberculosis

(a) Ordinary and multiplex PCR
(b) Transcription Mediated Amplification (TMA)
(c) Nucleic Acid Amplification (NAA)
(d) Ligase Chain Reaction
(e) Strand Displacement Amplification (SDA)
(f) Nucleic Acid Sequence Based Amplification (NASBA)
(g) Branched DNA (b-DNA)

B. DNA probe based assay
DNA probe technology for identification of fastidious organisms is still one of the most successful molecular diagnostic method (e.g. Accuprobe). Different probes are available for identifying mycobacteria belonging to M. tuberculosis complex & M. avium complex. They can be used on culture grown either on solid and liquid media. A sonicator for cell lysis and luminometer for final reading is required.

C. DNA Microarray
DNA microarrays are used for rapid examination of large number of DNA sequences by a single hybridization step. There is simultaneous identification of mycobacterial species and identification of mutation to rpoB gene (responsible for Rifampicin resistance). This is also called ‘Genechip’ method. Though restricted to research laboratories till date, this technology is likely to invade the diagnostic laboratories soon.

Discussion of some important molecular biological tools in details
PCR Based Methods (without or with hybridisation) : The polymerase chain reaction allows sequences of DNA present in only a few copies of mycobacteria to be amplified in vitro such that the amount of amplified DNA can be visualised and identified. If appropriate sequences specific for M. tuberculosis are selected, 10^1 to 10^3 organism / ml can be easily identified. It is rapid (result can be obtained in 24 hours) sensitive an specific. (17) It permits direct detection of M. tuberculosis complex. (18-19)
In case of tubercular meningitis and other EPTB cases, PCR based identification is much better than conventional methods including Adenosine Deaminase estimation. (20)

The most common target used in PCR is IS 6110. This sequence is specific for M. tuberculosis complex and is present upto 20 times in the genome thus offering multiple targets of amplification. PCR detection of IS 6110 in sputum (in pulmonary TB) when compared to culture has a sensitivity, specificity and positive predictability of 83.5%, 99% & 94.2% respectively.
In case of tubercular pleural effusion, it yields a sensitivity of 42-100% and specificity of 85-100%. (21) Using improved PCR techniques, the contribution to diagnosis of EPTB cases have greatly increased. (22) 10% of all cases, may show absence of IS 6110 in the bacterial genome which was first demonstrated in the Vietnamese migrants in Australia. Thus a multiplex PCR involving 3 pair of primers may be of greater sensitivity & specificity.

(a) A pair of oligonucleotide primers for gene coding 65 KDa hsp protein.
(b) A pair of genus specific oligonucleotide primers based on nucleotide sequence of DNA J gene.
(c) A pair of oligonucleotide primers from IS 6110. (23)

This multiplex PCR is efficient in species identification of M tuberculosis complex and other MOTT (Mycobacteria other than tuberculosis) organisms even from clinical specimens. (24) (figure 1).

Other Modifications
Transcription mediated amplification (TMA) and nucleic acid amplification (NAA) : This approach identifies the presence of genetic information unique to M. tuberculosis complex directly from preprocessed clinical specimens. The NAA technique uses chemical rather than biological amplification to produce nucleic acid
so that within a few hours these tests distinguish between M. tuberculosis complex and NTM in an AFB positive specimen. Currently it is being used only for respiratory specimens (sputum, Bronchoalveolar lavage etc.)

A positive direct amplified test in connection with an AFB positive smear is highly predictive in TB disease. The M. tuberculosis direct test (MTD) and amplified mycobacterial direct test (AMDT) are highly sensitive (96%) and specific (100%) for M. tuberculosis that are smear positive for AFB. Occasional false negativity and false positivity may be due to fewer bacilli or contamination. Another disadvantage of this technology is that both viable and dead bacilli give positive result as DNA is amplified. Commercial kit for AMDT is available from Gen Probe (San Diego, CA, USA) as an isothermal transcriptase mediated amplification system.

**Principle of Amplified Mycobacterial Direct Test (AMDT)**

M. tuberculosis Complex-specific region of 16S Ribosomal RNA gene produces double-stranded ribosomal DNA, due to the combined action of reverse transcriptase & ribonuclease. In turn RNA polymerase catalyses the synthesis of multiple stretches of ribosomal RNA from the ribosomal DNA synthesized before. A new cycle starts when the newly produced ribosomal RNA undergoes further transcription by reverse transcriptase. The sensitivity of the method is increased by the presence of, in each bacterium, of a high number of 16S ribosomal RNA target molecules (about 2000) compared to only one copy of 16S ribosomal DNA. The detection of amplification products relies on hybridization with a specific, single strand DNA probe with chemiluminescent molecule (hybridization protection Assay). Other Commercially Available methods

**Amplicor M'TB test**

A 584 bp fragment of the 16S ribosomal RNA gene, comprising a species—specific region flanked by genus specific sequence, is amplified using biotinylated primers. In the mastermix, an adjunct to adenine, guanine, and cytosine and uracil is used. The later being used in place of thymine. As a result, the amplification product differs from target DNA due to presence of uracil. An enzyme uracil-N-glycosylase fragments DNA wherever uracil is present, but the uracil free target DNA is not damaged. Because of the genus specific nature of the annealing regions, 16S ribosomal DNA belonging to any mycobacterial species is amplified by the PCR. The use, in the revealing phase of magnetic beads located with M. tuberculosis complex specific probes allows the removal, by washing, of any other DNA. The detection of the specific amplification product is preformed by adding an avidin enzyme conjugate and a chromogenic substrate.

Apart from M. tuberculosis, this kit is available for M. avium-inter cellularae DNA also.

**BD probe tec ET**

This kit is based on isothermal strand displacement amplification (SDA) with DNA polymerase to produce multiple copies of IS 6110. This is a real time technology and the principle of strand replacement is extremely complex. Interested readers may refer to the original article.27, 28

**Ligase chain Reaction**

It is a variant of PCR, in which a pair of oligonucleotides are made to bind to one of the DNA target strands, so that they are adjacent to each other. A second pair of oligonucleotides is designed to hybrideze to the same regions on the complementary DNA. The action of DNA polymerase and ligase in the presence of nucleotides, results in the gap between the adjacent primers being filled with the appropriate nucleotides and ligation of the primers. The LCX® M. tuberculosis assay kit (Abbott) is mainly used for respiratory samples with a high overall specificity & sensitivity. But presently it is not in the market.
Genotypic methods
Following the extraordinary development of molecular methods, the identification of mycobacteria, previously based on phenotypic investigations, suddenly started to rely on genotypic methods as well. Though these are mostly practiced in research laboratories, some were used in diagnostic laboratory also and commercial kits are also available. Without going into detail, we just mention the name of methods

a. PCR restriction enzyme analysis (PRA)
Amplification of 441-bp fragment of the hsp 65 gene by PCR, followed by digestion to the amplified product with two restriction enzymes e.g. Bst EII and HaeIII according to the procedure first described by Telenti. The products of the digestion reaction are then separated and visualised by the agarose gel electrophoresis.

b. Use of DNA probes
Commercially available DNA probes (e.g. Accu Probe) is used for identification of mycobacteria from clinical specimens. The probes are single stranded DNA oligonucleotides, complimentary to a short, species sequence within a hyperviable region of the 16S ribosomal DNA. It is labeled with an acridinium ester, a chemiluminescent molecule, which gives light when properly excited. Once the mycobacterial cell has been lysed by sonication, the extract is mixed with the probe under stringent conditions, allowing their hybridization only in case they are 100% complementary.

The chemiluminescent marker, is protected in the double stranded hybrid. The addition of a hydrolysing agent makes the first undetectable without affecting the second. Any hybridization is accompanied by light emission, which is detected by a luminometer, thus signifying the identification of the test strain.

Line Probe Assay
Line probe assay is a very important technology not only for detection of mycobacterial DNA in clinical sample but also for detection of mutation in rpoB gene in rifampicin resistant case.
The line probe assay (Lipa) uses the reverse hybridization technology with differently specific DNA-probes immobilized in parallel lines on a paper strip. The target DNA, previously extracted by boiling, is PCR—amplified using biotinylated primers and finally incubated with the strip. The hybridization probe is revealed as a coloured band, developed following the addition of a streptavidin—labeled enzyme and a chromogenic substance. Three commercial kits are available i.e. INNA—LiPA MYCOBACTERIA (innogeneties, Belgium), Geno Type Mycobacterium (Hain, Germany) & Genotype MTBC (Hain, Germany) of which INNO—LiPA is most popular.

The line probes of INNO LiPA Mycobacteria are species-specific fragments of the internal transcribe spacer (ITS) region interposed between 16S and 23S ribosomal RNA genes. The system includes a genus Mycobacterium specific probe, 2 complex specific probes and 23 other probes suitable for identifying 18 species and several intra specific variants.
The method is very effective for characterization of rpoB mutations in rifampicin-resistant mycobacterium. It is also capable of direct detection of Multidrug resistant M. tuberculosis from clinical specimens. The sensitivity and specificity varies from 78.37% to 100% in different studies.

The line probes of Geno Type Mycobectorum are fragments of the 23S ribosomal RNA gene mostly showed by more than one specimen. The newly developed Geno Type MTBC is a reverse hybridization system developed for identification of the species belonging to the M. tuberculosis complex which cannot be differentiated by analysis of any of the most frequently investigated conserved regions (i.e. 16S ribosomal DNA, ITS, 23S ribosomal DNA).

DNA microarray
DNA microarrays are small, solid supports,
typically glass, filler or silicon wafer, upon which DNA molecules of known sequences are deposited on synthesized in a predetermined partial order so that they can be made available as probes in a high-throughput, parallel manner. There are three major applications of DNA microarray technology.

(a) Identifying the sequence (gene/gene mutation)
(b) Determining the expression level (abundance) of genes of one sample.
(c) Comparing gene transcriptions in two or more different cell types.

This method is not routinely used for diagnosis of tuberculosis. But it is a very useful tool in clinical trial of new drugs, invention of vaccines, patients, follow up, disease and strain identification, treatment selection and observation of therapy efficacy. For the most cost is a limiting factor, but the objective of the specialists in biotechnology is to reduce the production cost in order to make the advanced technology routinely accessible.[32]

DNA Sequencing & Finger printing
These are not required for diagnostic purpose but to identify mutation, different strain and for epidemiological studies. The whole scheme of laboratory diagnosis of infection is presented in figure 2.

Conclusion
The application of molecular biology has opened up an entirely new horizon in the arena of diagnosis of tuberculosis. But inspite of the rapidity, sensitivity and specificity, the molecular biological tools are not cost effective in a country like India, till today. So it remains as a supportive instrument to AFB microscopy and culture and not as an alternative to them. With advancement of science, the boon of molecular biology is likely to come down within the reach of common people for its greater and better use.

References
7. Agarwal AN, Gupta D, Jindal SK. Diagnosis of Tuberculosis Pleural effusion. Indian J Chest Dist 1999; 1 : 89 - 100


**Figure 1**: Multiplex PCR amplification of 165bp, 365bp and 541bp regions of the clinical specimens and control strains of *M. tuberculosis* and Non tubercular mycobacteria (MOTT) on 2% agarose gel. Lane 1: pGEM Molecular Marker; Lane 2-3: Patients’ samples (Pleural Fluid); Lane 4: H37RV (control strain) of *M. tuberculosis*; Lane 7-9: Other Atypical mycobacteria; Lane 10-12: Sputum sample of patients; Lane 13: Positive Control; Lane 14: Negative Control

**Figure 2**: Laboratory Diagnosis of Infections

- Detection of antibodies (e.g., ELISA)
- Detection of nucleic acids (e.g., DNA hybridization)
- Oligonucleotide probe
- Detection of nucleic acids (e.g., DNA hybridization)
- Pathogenic organism
- DNA
- RNA
- DNA/RNA
- in vitro nucleic acid amplification (e.g., PCR, NASBA)
- Automated DNA sequencing
- Microscopy
- Culture
- Biochemical characteristics
LEVELS OF TUMOR NECROSIS FACTOR ALPHA IN PATIENTS OF GUILLAIN BARRÉ SYNDROME BEING TREATED WITH INTRAVENOUS IMMUNOGLOBULINS

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Abstract
A total of 28 patients of GBS were taken to study the levels of Tumor Necrosis Factor \( \alpha \) (TNF-\( \alpha \)) before and after intravenous immunoglobulins (IVIg) in patients of Guillain Barre Syndrome (GBS). Out of these, 20 patients were treated with IVIg while 8 were not given IVIg. Serum TNF-\( \alpha \) levels were measured before and after IVIg to investigate the role of TNF-\( \alpha \) as a prognostic marker of successful treatment. Mean TNF-\( \alpha \) levels in 20 patients declined from 34.01 pg/ml \( \pm \) 32.91 (pre-treatment) to 12.61 pg/ml \( \pm \) 7.53 after IVIg therapy \( (p=0.006) \). This correlated well with neurological recovery which suggests the role of TNF-\( \alpha \) as a prognostic marker of GBS patients treated with IVIg. Raised TNF-\( \alpha \) levels before treatment indicate their role in the pathogenesis of GBS. Fall in TNF-\( \alpha \) levels with IVIg and its correlation with neurological recovery suggests the protective role of IVIg and TNF-\( \alpha \) as a prognostic marker in GBS patients.

Introduction
Guillain Barre Syndrome (GBS) is an immune mediated, acute, frequently severe demyelinating polyradiculopathy with wide range of clinical symptoms and outcome. Typical illness counts of symmetrical ascending flaccid paralysis of legs, arms and sometimes respiratory muscles and bulbar muscles with areflexia, parasthesias followed by spontaneous gradual recovery over several weeks to months.\(^1\) It can also lead to persistent fatigue (67% patients), persistent disability (5-10% patients) and even death (4-15% patients).\(^2\)
Pathologically GBS patients have an acute multifocal lymphocytic infiltration in their peripheral nerves and spinal roots causing primary demyelination.\(^3\) GBS is thought to be due to an autoimmune response triggered by a preceding infection commonly by campylobacter jejuni.

Management of GBS includes various types of immunomodulatory therapies. Plasmapheresis has shown to be an effective management line.\(^4\) Steroids are proven to be of no use. Recently it has been shown that intravenous immunoglobulins (IVIg) is as effective as plasmapheresis.\(^5\) Because of ease of administration and equal efficacy, it is now recommended as the standard treatment of GBS.\(^6\)
Benefits of IVIg has been a watershed event for GBS. This is because of the fact that cellular interaction mediated through release of cytokines (TNF-\( \alpha \), Interleukin-1) play a role in the pathogenesis of GBS.\(^7\) TNF-\( \alpha \) is regarded as one of the immune factors that can induce demyelination.\(^8\) Treatment with IVIg modify the course of the disease but precise mechanism remains unknown. IVIg suppresses antibody dependant cellular toxicity, decreases natural killer cell function, inhibits autoantibodies...
production, neutralizes circulating pathological antibodies and interferes with complement activation\(^9\) therefore reducing TNF-\(\mu\) levels in patients of GBS.\(^{10}\) To provide evidence based role of IVIg for the management of GBS indicating role of TNF-\(\mu\) as a prognostic marker of successful treatment, the present study was undertaken to study the levels of TNF-\(\mu\) before and after IVIg therapy.

**Materials and Methods**

A total of 28 patients who fulfilled the criteria of GBS\(^{11}\) admitted in our hospital between January 2004 to March 2005 were included for the study. All the 28 patients were in progressive stages of minor weakness, disability grade IV or V and disease duration was less than 2 weeks. CSF examination showed increase in protein levels in 53.6\% (15 patients) only. Detailed history, CSF examination, nerve conduction studies and routine investigations were done in all patients. Inclusion criteria for IVIg therapy was: duration of symptoms less than 2 weeks; patients unable to walk greater than 5 meters unsupported.

Among all the patients 20 were given IVIg and 8 patients were not given IVIg. Serum TNF-\(\mu\) levels were measured at admission, after IVIg or day 5 in whom no IVIg was given. Patients were given intravenous immunoglobulins at a dose of 400 mg/kg body weight for five consecutive days within first 2 weeks of illness. Clinical outcome with IVIg therapy was assessed using a widely adopted clinical disability scale in treatment trials.\(^{12}\)

**TNF-\(\mu\) assay.** TNF-\(\mu\) levels were measured in serum using TNF-\(\mu\) immunoassay kits based on the principle of sandwich ELISA (using polyclonal coating anti- TNF-\(\mu\) antibodies). TNF-\(\mu\) present in the sample binds to antibodies absorbed on to microwells. A biotin conjugated monoclonal TNF-\(\mu\) antibody is added & binds to TNF-\(\mu\) captured by the first antibody. After incubation, unbound biotin conjugated TNF-\(\mu\) is removed by washing, streptavidin-HRP is added which binds to biotin conjugated TNF-\(\mu\). After incubation unbound streptavidin-HRP is removed, substrate added, reaction stopped by addition of acid & colored complex is measured at 450nm. A standard was run on each plate using recombinant human TNF-\(\mu\) standard in serial dilution (1000 to 0 pg/ml). A standard curve was prepared from seven standard solutions & TNF-\(\mu\) concentration determined.

**Statistical analysis.** Data collected in respect to various variables was analysed using statistical package SPSS VERSION 11.5. Mean and S.D. were computed. Difference in mean was seen by applying paired student t-test. Results were classed as significant if p<0.05 and highly significant if p<0.01.

**Results**

TNF-\(\mu\) levels were measured in all the 28 patients. 85.7\% patients (n=24) had raised TNF-\(\mu\) levels at admission with a mean of 32.69±26.76 (Table-1). The normal TNF-\(\mu\) in serum is less than 8 pg/ml. 20 patients who were given IVIg, had mean TNF-\(\mu\) levels 34.01 pg/ml±32.91 before IVIg. The levels declined to a mean of 12.61 pg/ml±7.53 after IVIg (p<0.01) (Table-2). P value was 0.006 which is significant indicating effect of IVIg on fall in serum TNF-\(\mu\) levels. In 8 patients who were not given IVIg mean TNF-\(\mu\) did not change from the day of admission to day 5 (Table-3), (15.92pg/ml±7.76 vs 22.91pg/ml±17.41), (p=0.230). The mean values of TNF-\(\mu\) at admission and at day 5 were found to be similar (P value=0.230). It indicates that there was no significant change in TNF-\(\mu\) levels at admission and on day five.

Out of 20 patients who were given IVIg, 16 patients showed clinical improvement (fall of 1 or >1 disability score) at four weeks following IVIg therapy. Among these 16 patients, 14 had raised TNF-\(\mu\) before IVIg therapy and later on showed fall in serum TNF-\(\mu\) levels after IVIg.
Discussion

The serum TNF-\(\mu\) levels were found to be raised in patients of GBS in early stages of disease, indicating its role in the pathogenesis of the disease.

On the basis of microscopic identification of lymphocyte and macrophage infiltrations, an immune disorder was proposed to be responsible for demyelination observed in Guillain Bare Syndrome.\(^{(13)}\) A lymphocytic infiltration precedes the influx of macrophages, the cells believed to cause demyelination. Several proinflammatory cytokines such as TNF-\(\mu\) and gamma interferon are elevated in serum of Guillain Bare Syndrome patients as reported by previous studies.\(^{(7,14)}\) A study reported TNF-\(\mu\) as a primary mediator of inflammation known to induce demyelination of nervous system.\(^{(15)}\) TNF-\(\mu\) has also been reported to be capable of inducing selective and specific damage to myelin in vitro.\(^{(16)}\)

The benefits of IVIg however were first reported in GBS.\(^{(17)}\) It has also been shown by previous studies that circulating levels of TNF-\(\mu\) decreased after treatment with intravenous immunoglobulin therapy but remained high in untreated patients.\(^{(18)}\)

In a study out of 36 patients of GBS with motor weakness, 26 patients (72.2%) showed elevated serum TNF-\(\mu\) levels prior to IVIg therapy.\(^{(9)}\) Following course of IVIg, a progressive decrease in the serum TNF-\(\mu\) concentration was observed. This is comparable to our study where among a total of 28 patients of GBS, 24 patients (85.7%) showed increased levels of TNF-\(\mu\) at admission. The mean TNF-\(\mu\) levels were found to be 32.69 pg/ml \(\pm\) 29.76 when compared to normal of less than 8 pg/ml (standard).

In our study, among the patients who were given IVIg (n=20), the raised TNF-\(\mu\) levels (mean 34.01 pg/ml \(\pm\) 32.91) were seen in 16 patients. Following therapy, there was a fall (\(p=0.006\)) in serum TNF-\(\mu\) levels to a mean of 12.61 pg/ml \(\pm\) 7.53 in 92.8%. This clearly indicates that IVIg administration has a significant effect on circulating TNF-\(\mu\) levels. (Also clinical improvement was compared by using the same clinical disability scale used in our study and similar results were obtained). Thus the results of our study were compared with previous study and show beneficial effect of IVIg on treatment of GBS and TNF-\(\mu\) levels. Among 4 patients who did not show neurological improvements, one patient was that who died of ventilator related respiratory complication. The other three patients who did not improve neurologically were those who were in grade 5 and showed slow neurological recovery. In our study, 8 patients who were not given IVIg, mean TNF-\(\mu\) at admission 15.92 pg/ml \(\pm\) 7.76 rose to 22.91 pg/ml 17.41 at day 5 (\(p=0.130\)). This means that patients who were not given IVIg therapy showed no change in TNF-\(\mu\) levels at admission and day 5. This trend was also observed by previous study.\(^{(17)}\)

In another study elevated serum TNF-\(\mu\) levels in 22 patients showed a steady decline at the end of IVIg therapy.\(^{(18)}\) At discharge from the hospital, there was a positive correlation between neurological recovery and decline in TNF-\(\mu\) concentrations, which is again comparable to our study. A prior study showed neurological recovery in 16 patients following IVIg therapy.\(^{(19)}\) As IVIg infusion does not require central venous access and does not reduce blood volume, it is generally preferred over plasma exchange in treatment of GBS in children and elderly.\(^{(20)}\)

Thus IVIg reduces the serum TNF-\(\mu\) concentrations in GBS patients having elevated levels prior to IVIg and may play a protective role by inhibiting the demyelinating effect of TNF-\(\mu\) in the peripheral nerves of patients with Guillain Bare Syndrome. Hence, at the end, our study and previous studies conclude that serum TNF-\(\mu\) levels decline with IVIg therapy which correlates with neurological recovery in patients of GBS.
Table 1: Serum TNF-μ level at admission

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (n=28)</th>
<th>Percentage (100%)</th>
<th>Mean (pg/ml)</th>
<th>S.D.</th>
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<tr>
<td>TNF-μ level ≥ 8 pg/ml</td>
<td>24</td>
<td>85.7%</td>
<td>32.69</td>
<td>26.76</td>
</tr>
<tr>
<td>TNF-μ level &lt; 8 pg/ml</td>
<td>4</td>
<td>14.3%</td>
<td>5.75</td>
<td>1.48</td>
</tr>
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Table 2: Comparison of serum TNF-μ value before and after IVIG therapy

<table>
<thead>
<tr>
<th>Time of Serum collection</th>
<th>Mean TNF-μ Level (pg/ml)</th>
<th>No. of Patients (n)</th>
<th>S.D.</th>
<th>t Value</th>
<th>df</th>
<th>P Value</th>
<th>95% confidence interval of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before IVIG</td>
<td>34.01</td>
<td>20</td>
<td>32.91</td>
<td>3.074</td>
<td>19</td>
<td>0.006</td>
<td>6.82 35.97</td>
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<td>After IVIG</td>
<td>12.61</td>
<td>20</td>
<td>7.53</td>
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Table 3: Comparison of serum TNF-μ value at admission and day 5 in patients to whom no IVIG therapy given

<table>
<thead>
<tr>
<th>Time of Serum collection</th>
<th>Mean TNF-μ Level (pg/ml)</th>
<th>No. of Patients (n)</th>
<th>S.D.</th>
<th>t Value</th>
<th>df</th>
<th>P Value</th>
<th>95% confidence interval of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Admission</td>
<td>15.92</td>
<td>8</td>
<td>7.76</td>
<td>1.314</td>
<td>7</td>
<td>0.230</td>
<td>19.56 5.59</td>
</tr>
<tr>
<td>Day 5</td>
<td>22.91</td>
<td>8</td>
<td>17.41</td>
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</table>
References

URIC ACID STATUS IN SUBCLINICAL AND OVERT HYPOTHYROIDISM

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⁵Department of Pathology, CNMC, Kolkata

Abstract
Uric acid metabolism in untreated sub clinical and overt hypothyroidism was studied in a hospital based case control study. Serum uric acid, Serum uric acid / Serum creatinine ratio and urinary uric acid / urinary creatinine ratio were found to be insignificantly altered (p > 0.05) in both sub clinical and overt hypothyroid subjects. At the same time there is no significant difference (p > 0.05) of BMI (Body Mass Index) amongst controls and two subgroups (sub clinical and overt) of hypothyroid subjects. It was concluded that insignificant change of uric acid metabolism among sub clinical and overt hypothyroid subjects in our study may be related to the insignificant changes in BMI of these two groups. Hypothyroidism often results in obesity, which together with hyperuricemia are characteristic of Metabolic Syndrome.

Introduction
Uric acid is the end product of purine base, nucleoside and nucleotide metabolism. The kidneys excrete it. This includes glomerular filtration, reabsorption in the early proximal tubule, secretion into proximal tubule and post secretory proximal tubular reabsorption. Increased serum uric acid level or hyperuricemia can result from an increased production, reduced renal excretion or a combination of both. With the onset of renal insufficiency, there is a fall in urinary uric acid with a corresponding rise in serum uric acid level. It has been documented that hyperuricemia has been associated with increased cardiovascular morbidity and mortality.¹ Long term effects of hyperuricemia include salt sensitive form of hypertension, insulin resistance and endothelial dysfunction.² In fact hyperuricemia may be a part of metabolic syndrome seen in diabetic patients.³ Serum uric acid contributes up to sixty percent of antioxidant activity in healthy subjects. However high concentration of uric acid acts as pro-oxidant.⁴ Thus hyperuricemia is a risk factor for oxidative stress associated disorders. Allopurinol, the drug used to treat hyperuricemia is shown to improve endothelial dysfunction.⁵ A group of researchers have shown that in patients suffering from pregnancy induced hypertension, serum uric acid concentration increases in the antenatal period.⁶ They showed a high degree of positive correlation between serum uric acid level and severity of preeclampsia with respect to hypertension and proteinuria. Increased incidence of perinatal mortality and mortality was shown to be associated with hyperuricemia in the study. Thyroid hormones influence the metabolic rate of the body.

In hyperthyroidism the metabolic rate of the body
increases, while in hypothyroidism metabolic rate decreases. In hyperthyroidism increased serum uric acid has been found.\(^7\) However, regarding serum uric acid level in hypothyroidism, literature is full of contradictory findings. Sato et al\(^8\) reported hypouricemia in hypothyroid subjects. Whereas Yokogoshi et al\(^9\) showed hyperuricemia in subjects of hypothyroidism with myopathy. Raber et al\(^10\) showed no relationship between serum uric acid level and \(T_4/TSH\) ratio in hypothyroid (both sub clinical and overt) subjects. This prompted us to assess the uric acid status in both sub clinical and overt hypothyroid subjects.

**Materials and Methods**

The study was carried out on 93 subjects (31 sub clinical hypothyroid, 32 overt hypothyroid, 30 euthyroid subjects) in the Department of Biochemistry of IPGME & SSKM Hospital for a period of eight months. The subjects were drug naïve for thyroid hormone preparation. Patients' previous uric acid status was enquired and only those patients were selected with a previous normal serum uric acid values. Valid informed consent was obtained from all subjects.

**Biochemical Measurements**

Serum TSH and \(T_4\) were estimates in the department of Biochemistry, IPGME & SSKM hospital by ELISA (Biotex Lab Inc, Houston Texas). Diagnosis of Primary overt hypothyroidism and sub clinical hypothyroidism was done according to a previously published study.\(^11\) Raised Serum TSH (>6\(\mu\)IU/ml) and low serum \(T_4\) (4\(\mu\)g/dl) constitutes overt hypothyroidism whereas raised serum TSH (>6\(\mu\)IU/ml) and normal serum IU (4 – 11.4 \(\mu\)g/dl) with few or no apparent clinical features, constituted sub clinical hypothyroidism. Serum creatinine and urinary creatinine (after suitable dilutions were estimated by alkaline picrate method using Jaffe's reaction, originally developed by Bonsues and Taussky (1945).

Serum uric acid and urinary uric acid (after suitable dilution) were estimated by coupled uricase peroxidase method.

Statistical analysis between cases and controls were done by one way ANOVA. \(P\) value < 0.05 was taken to be statistically significant.

**Results**

Demographic profile and thyroid profile of euthyroid controls, sub-clinical hypothyroid and overt hypothyroid cases are shown in table 1. Serum uric acid, serum uric acid/creatinine ratio and urinary uric acid / creatinine ratio of cases and controls are shown in table 2. It may be noted from table 2, that there was no statistically significant difference among controls, sub-clinical hypothyroid and overt hypothyroid cases regarding serum uric acid, serum uric acid/creatinine ratio and urinary uric acid / creatinine ratio.

**Discussion**

The present study was designed to investigate the relationship between the serum levels of uric acid and hypothyroidism. Serum uric acid, serum uric acid/serum creatinine ratio and urinary uric acid/urinary creatinine ratios were found to be insignificantly altered in both overt and sub clinical hypothyroid patients. These results are in conformity to the studies done by Raber et al\(^10\) which showed no relationship between serum uric acid level and \(T_4/TSH\) ratio in both overt and sub-clinical hypothyroid patients. While conflicting reports have been noted while reviewing the literature. Sato et al\(^8\) reported hypouricemia in hypothyroid patients whereas Yokogoshi\(^9\) et al showed hyperuricemia in subjects of hypothyroidism with myopathy. Urate acts as a chain breaking antioxidant. While studying oxidative stress in hypothyroidism Dumitriu et al\(^12\) observed both increase of malondialdehyde and uric acid in serum and decrease of serum ceruloplasmin level. The reason is that due to increased free radical formation, there is muscle necrosis leading to increased creatinine and uric acid levels.
Increased free radicals also cause a decrease of NADH synthesis causing depressed metabolism leading to decreased conversion of ADP to ATP; thereby there is increased levels of ADP leading to increased uric acid synthesis. In hypothyroidism, there is a decrease of BMR with less ATP requirement. Dumitriu et al.\textsuperscript{(12)} suggested that in hypothyroid subjects there is ADP excess which is degenerated to xanthine. As a result, xanthine oxidase activity is increased with consequent generation of superoxide anion and uric acid. Super oxide anion in turn is converted into hydrogen peroxide. As a result oxidative stress is increased leading to increased serum MDA. It has also been suggested that low ceruloplasmin may aggravate the condition.

In our study we could not find any alteration of serum uric acid and urinary excretion of uric acid in subclinical and overt hypothyroid subjects. Furthermore, in the presence of similar serum uric acid, unaltered urinary urate/creatinine ratio implied that uric acid production was not increased in our patients. Taken together, this suggested that uric acid metabolism (both production and renal clearance) had remained unaltered in both subclinical and overt hypothyroid subjects. This implied that in our patients the above mentioned mechanism suggested by Dumitriu et al.\textsuperscript{(12)} is not operative to the extent to alter uric acid metabolism. It is to be noted that both subclinical and overt hypothyroidism were of short duration before detection. This is reflected in the similar BMI in subclinical and overt hypothyroid subjects compared to euthyroid subjects. This may explain unalteration of uric acid metabolism in hypothyroids in our study. Further studies with large number of patients may be undertaken in future to substantiate our observation.

**Conclusion**

In our subjects we found unaltered serum uric acid and urinary urate clearance both in subclinical and overt hypothyroid subjects compared to euthyroid subjects.

**Acknowledgements** Authors to thank Mr. Chandan Sinha and Mr. Anupam Banerjee for estimation of thyroid hormones by ELISA.

**Table 1 : Demographic profile and Thyroid profile of controls and cases**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 30)</th>
<th>Sub clinical Hypothyroid (n = 31)</th>
<th>Overt Hypothyroid (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.65 ± 10.13</td>
<td>29.67 ± 13.02</td>
<td>32.44 ± 8.09</td>
</tr>
<tr>
<td>BMI (Kg/M(^2))</td>
<td>23.35 ± 5.45</td>
<td>22.59 ± 5.65</td>
<td>24.37 ± 4.34</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>T(_{3}) ((\mu\text{g/dl}))</td>
<td>7.96 ± 1.66</td>
<td>6.10 ± 2.71</td>
<td>1.22 ± 0.66</td>
</tr>
<tr>
<td>TSH ((\mu\text{U/ml}))</td>
<td>2.19 ± 1.35</td>
<td>7.18 ± 0.50</td>
<td>14.13 ± 6.91</td>
</tr>
</tbody>
</table>
Table 2: Uric acid Status of controls and cases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n =30)</th>
<th>Sub clinical Hypothyroid (n = 31)</th>
<th>Overt Hypothyroid (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum uric acid (mg/dl)</td>
<td>5.05 ± 1.04</td>
<td>5.69 ± 2.37</td>
<td>5.98 ± 1.51</td>
</tr>
<tr>
<td>SUA/mg of serum creatinine</td>
<td>8.13 ± 2.07</td>
<td>9.37 ± 3.74</td>
<td>7.65 ± 2.77</td>
</tr>
<tr>
<td>UUA/ mg of U_σ</td>
<td>0.55 ± 0.17</td>
<td>0.40 ± 0.05</td>
<td>0.52 ± 0.12</td>
</tr>
</tbody>
</table>

- p > 0.05
- SUA Serum Uric Acid; UUA = Urinary Uric Acid. U_σ = Urinary creatinine

References
ANEMIA DURING PREGNANCY: A HOSPITAL BASED SURVEY OF PREGNANT WOMEN IN DIBRUGARH DISTRICT, ASSAM

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College of Medicine, Abha, Saudi Arabia,
²Department of Biochemistry, Assam Medical College & Hospital
Dibrugarh-786102, Assam

Abstract
This study was carried out in order to obtain a prevalence data amongst pregnant women receiving antenatal care in Assam Medical College and Hospital, Dibrugarh. One hundred and twenty (80%) out of the 150 pregnant women studied were found to be anemic. Anemia was more prevalent among primigravidae (87.57%) than the multigravidae (75.57%) (p<0.05). Among all anaemic patients, 63 (52.5%) had moderate anemia while 48 (40%) had mild anemia and 9 (7.5%) were severely anemic, out of which 6 (66.7%) were primigravidae. Most severely anemic women were under 30 years old and having birth interval of more than 3years. Absence of symptoms of ill health and non-compliance with taking of iron and folic acid tablets were the major reason for these women neglecting their health. Anemia was higher among tea estate labourers and domestic servants, proving the role of poverty and ignorance in anemia of pregnancy. Iron deficiency (90%) was the predominant cause of anemia, with few cases of hookworm infestation (5.8%) contributing to iron deficiency anemia, and megaloblastic anemia occurred in only 12 (10%) cases.
Educating women on early antenatal care, adequate haematinc supplementation and improvement of health conditions is necessary to reduce the problem of anemia of pregnancy in Assam.

Introduction
According to World Health Organization (WHO), more than 50% pregnant women in the World have a hemoglobin level indicative of anemia (< 11.0 g/dl), the prevalence may however be as high as 56 or 61% in developing countries. Anemia becomes more prominent during pregnancy because the demand for iron and other vitamins is increased due to physiological burden of pregnancy. The inability to meet the required level for these substances either as a result of ignorance, dietary deficiencies or infection gives rise to anemia. Anemia, which is frequently observed during pregnancy, exposes women to an increased risk of blood transfusion during the peripartum period because it becomes difficult for the parturient to cope with the physiologic blood losses of delivery. The risk is equally increased by conditions that increase chronic bleeding during gestation, such as placenta praevia. Anemia in pregnancy is considered one of the major risk factors contributing to maternal deaths in developing countries, hemorrhages, eclampsia and infections being the three major causes. Association of anemia with adverse maternal outcome such as puerperal sepsis, antepartum hemorrhages, postpartum hemorrhages and maternal mortality is no longer a debatable issue. In pregnancy, anemia has a significant impact on the health of the fetus as well as that of the mother. Fetuses are at risk of preterm deliveries, low birth weights, morbidity and perinatal mortality due to the impairment of
Prevalence of anemia during pregnancy

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Oxygen delivery to placenta and fetus. (9,10) Iron Deficiency Anemia (IDA) is known to be an important factor in maternal death, (11) the poor cognitive development of children and decreased work capacity of the mother. (12) The pregnant women suffering from iron deficiency is less able to tolerate haemorrhage during labour and is more susceptible to infection. Association of anemia with hookworm infestations has been seen earlier in various studies done across the globe. (13) The WHO has emphasized the need of epidemiological studies where up-to-date information is not available. (14) Therefore, this study was aimed:

1. To study the prevalence of anemia among the subjects attending the Obstetrics and Gynecology outpatient department of Assam Medical College and Hospital.
2. To find out its association with age at pregnancy, birth interval, iron deficiency, worm infestation and megaloblastic anemia.
3. To assess the effectiveness of antenatal care in preventing anemia among pregnant women in the North-Eastern parts of India.
4. To suggest measures for control and prevention of anemia if prevalence is more.

Methods
The study was conducted in Obstetrics and Gynecological Department, Assam Medical College and Hospital, Dibrugarh District, Assam State, India, from September 2004 to August 2005. Ethical clearance was obtained from the ethical committee in the hospital, and also, informed consent was taken from the women after explaining the study to them. The women with multiple pregnancies and bleeding disorders were excluded from the study. The study was first introduced to the pregnant women in their first visit to the antenatal clinic in order to obtain their consent. There was a continuous enrolment into the study for twelve months; as many as consented were enrolled in the study. One hundred and fifty (150) women were enrolled in this study, consisting of 56 primigravidae and 94 multigravidae. Questionnaires were administered to obtain demographic information, use of folate and birth interval. For blood pressure estimation, an average of three readings measured thrice at an interval of 15 minutes was taken with subjects in a sitting position. The average of three measurements of Korotkoff phase I was considered as systolic blood pressure (SBP), and the average of three values of phase IV was recorded for diastolic blood pressure (DBP). (15) Determination of anemia was carried out using WHO criteria. (16) Anemia ranges from mild, moderate to severe cases and according to WHO, the hemoglobin level for each of these types of anemia in pregnancy are: 10.0–10.9 g/dl (mild anemia), 7–9.9 g/dl (moderate anemia) and < 7 g/dl (severe anemia). (16) Gestation age was estimated from interview based on the date of last menstruation and estimation of fundal height. Caution was taken to see that almost same age group primigravida and multigravida were compared. Blood samples were collected from the pregnant women and hematological investigations were carried out to determine hemoglobin by the Cyanmethemoglobin method, and relevant blood investigations done such as Packed cell volume, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, serum iron, serum total iron binding capacity and serum ferritin. (17) Fecal occult blood test was done to exclude gastrointestinal bleeding. Typing of anemia was done as per standard peripheral blood smear examination method. (18) Examination of stool samples for parasitic infestation was done initially. The presence of hookworm was recorded as positive or negative, depending on whether hookworm ova were detected. The subjects were subsequently examined periodically for hemoglobin and PCV until delivery.

Results
Mean age of the primigravidae was 24.2 years...
and multigravidae was 26.6 years. Anemia was recorded in 120 (80%) of the 150 enrolled women at one trimester of pregnancy or the other; Of these, 49 were primigravidae and 71 multigravidae, constituting a prevalence of 87.5% and 75.5% anemia among primigravidae and multigravidae respectively. Severe anemia was recorded in 9 (7.5%) women, 6 of whom were primigravidae, [Table 2]. Women in the 15-19 years age group constituted the highest percentage of anemic cases (92.3%) followed by 20-24 years age group (85.7%) compared to the other age groups, [Table 2]. All cases of severe anemia were recorded in women less than 30 years of age. Anemia were found to be more prevalent (96.3%) among tea estate labourers than others. Prevalence of 2nd and 3rd trimester anemia seen was only slightly less to that of the first trimester anemia during follow up as shown in table 3. Care was taken to see that same group of patients were studied in the 2nd and 3rd trimesters during follow up. However prevalence of anemia was generally higher among primigravidae (87.5%) than multigravidae (75.5%) (p<0.05). Severity of anemia was significantly higher in those with birth interval more than 3 years (p<0.05) [Table 4]. Normocytic hypochromic and microcytic hypochromic type of blood picture (a characteristic of iron deficiency anemia) was present in 108 (90%) of cases. Out of these hookworm infestations were detected in 7 cases (5.8%). Macrocytosis was detected in 12 (10%) cases only.

**Discussion**

The prevalence of anemia recorded in this study (80%) is an indication that anemia during pregnancy is a major problem in North-East region especially among Assamese people. This study confirms that severe anemia is more common among primigravidae compared to multigravidae as also recorded by Nagaraj [19]. This is an indication that primigravidae are more at risk of maternal death as a result of severe anemia. A higher prevalence (92.3%) of anemia recorded among teenage mothers (15-19 years) conforms to the observation of Thangaleela T and Vijayalakshmi P [20]. The high level of anemia recorded in this study among tea estate laborers may indicate that poverty and ignorance born out of unemployment may have contributed significantly to the high level of anemia as the women cannot afford to book early for antenatal care, eat nourishing food and prevent possible infection. The prevalence of anemia recorded in this study shows no significant difference in any trimesters. This is an indication that these women do not necessarily overcome anemia but only slip from one type of anemia to another either as a result of hemodilution, infection, and dietary deficiencies or improve as a result of appropriate medical management of anemic cases. As normocytic hypochromic and microcytic hypochromic blood pictures were predominant, it indicates deficient iron intake/or absorption irrespective of age, type of family, caste, religion or number of children. Megaloblastic anemia did not significantly contribute to the high prevalence of anemia. The non-compliance of the pregnant women to the iron supplementation necessary to prevent deterioration of anemic condition during increased physiological burden of pregnancy may have contributed to the higher prevalence recorded in this region [21,22]. According to WHO guidelines, a deworming tablet is recommended during the second and third trimesters of pregnancy [23]. However this recommendation is not often implemented owing to concerns over its potential teratogenicity.

**Conclusion**

This study has shown that anemia in pregnancy is still a major health problem in North-East region of Assam identifying primigravidae as being more at risk than multigravidae. So also are pregnant teenagers and women that book late for antenatal care. Clinicians should enlighten health providers on the use of iron and folate supplements in the management of their pregnant
patients. Educating women on early ANC booking and compliance with the use of prescribed medications should also be emphasized. In order to improve maternal health, and the health of the fetus during pregnancy, information on the prevalence or incidence of pregnancy related conditions (anemia in this case) would be useful for the managers of health institutions, and for district, provincial and national maternal, child and women's health programme development.

**Acknowledgment**

The authors greatly acknowledge the staff of the Biochemistry Dept., Assam Medical College and Hospital (A.M.C.H.), Dibrugarh, Assam for their help during the study. The authors also acknowledge Prof. H.H. Choudhury, Prof. & Head Dept. of Obstetrics and Gynecology, A.M.C.H., Dibrugarh, Assam for allowing to collect blood samples from the patients. Last, but not the least, we would like to thank all those people whose names have not been mentioned here.

<table>
<thead>
<tr>
<th>Table 1: Prevalence of anemia by age and occupation</th>
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<tbody>
<tr>
<td><strong>Characteristics</strong></td>
</tr>
<tr>
<td><strong>Age [in years]</strong></td>
</tr>
<tr>
<td>15-19</td>
</tr>
<tr>
<td>20-24</td>
</tr>
<tr>
<td>25-29</td>
</tr>
<tr>
<td>30-34</td>
</tr>
<tr>
<td>40+</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
</tr>
<tr>
<td>Housewife</td>
</tr>
<tr>
<td>Tea Estate labourer</td>
</tr>
<tr>
<td>School Mistress</td>
</tr>
<tr>
<td>Domestic Servant</td>
</tr>
<tr>
<td>Bank Employee</td>
</tr>
<tr>
<td>Student</td>
</tr>
<tr>
<td>Doctor</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 2: Distribution of anemia types between gravid types</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parity</strong></td>
</tr>
<tr>
<td>Primigravidae</td>
</tr>
<tr>
<td>6[66.7%]*</td>
</tr>
<tr>
<td>[12.2%]**</td>
</tr>
<tr>
<td>Multigravidae</td>
</tr>
<tr>
<td>3[33.3%]*</td>
</tr>
<tr>
<td>[4.2%]**</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* % within anemia type    ** % within gravid type
Table 3: Anemia in the various trimesters of pregnancy

<table>
<thead>
<tr>
<th>Subjects</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
<th>Total</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primigravidae</td>
<td>92.8</td>
<td>85.3</td>
<td>76.3</td>
<td>49</td>
<td>87.5</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>78.6</td>
<td>76.6</td>
<td>73.9</td>
<td>71</td>
<td>75.5</td>
</tr>
<tr>
<td>All</td>
<td>85.9</td>
<td>79.6</td>
<td>74.1</td>
<td>120</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 4: Distribution of subjects according to degree of anemia by birth interval (in multigravidae)

<table>
<thead>
<tr>
<th>Birth interval (in months)</th>
<th>Mild (%)</th>
<th>Moderate (%)</th>
<th>Severe (%)</th>
<th>Total anemic subjects (%)</th>
<th>Normal subjects (%)</th>
<th>Total No. Multi gravidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18</td>
<td>5(18.5)</td>
<td>16(59.3)</td>
<td>3(11.1)</td>
<td>24(88.9)</td>
<td>3(11.1)</td>
<td>27</td>
</tr>
<tr>
<td>18-36</td>
<td>8(22.9)</td>
<td>15(42.9)</td>
<td>6(17.1)</td>
<td>29(82.9)</td>
<td>6(17.1)</td>
<td>35</td>
</tr>
<tr>
<td>&gt;36</td>
<td>3(15)</td>
<td>6(30)</td>
<td>9(45)</td>
<td>18(60)</td>
<td>2(10)</td>
<td>20 p&lt;0.05</td>
</tr>
<tr>
<td>All</td>
<td>16(19.5)</td>
<td>37(45.1)</td>
<td>18(22)</td>
<td>71(86.6)</td>
<td>11(13.4)</td>
<td>82</td>
</tr>
</tbody>
</table>

References

15. Fuller Group. Prevalence of hypertension among...
Prevalence of anemia during pregnancy

ASSESSMENT OF OXIDATIVE STATUS AND ERYTHROCYTIC MEMBRANE DAMAGE IN ASPHYXIATED TERM NEONATES

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R G Kar Medical College & Hospital, 1, Khudiram Bose Sarani, Kolkata- 4
Department of Biochemistry, Medical College & Hospital, Kolkata- 73
Department of Obstetrics & Gynaecology
R G Kar Medical College & Hospital, 1, Khudiram Bose Sarani, Kolkata- 4

Abstract
In this hospital based cross sectional study we have determined serum NO metabolites, RBC membrane osmotic fragility and RBC reduced glutathione level in asphyxiated term neonates. It was conducted in Department of Pediatrics (Neonatology unit) and Department of Biochemistry of a tertiary care hospital in Kolkata. 31 asphyxiated term neonates (17 boys, 14 girls) and 20 non asphyxiated term neonates were included in the study as case and control population, respectively. Both groups were matched in respect to maternal age, parity, gestational age, sex and mode of delivery. Cord blood samples were analyzed for NO metabolites (Nitrite), osmotic fragility and reduced glutathione. Results showed statistically significant (p value <0.05) increase in serum Nitrite level, RBC membrane osmotic fragility and decrease in RBC reduced glutathione. Thus we can conclude that increased generation of NO and its metabolites in hypoxic ischemic encephalopathy leads to membrane damage as a result of oxidative injury in term neonates.

Introduction
Perinatal asphyxia is one of the major factors leading to cerebral damage during the postnatal adaptation to extrauterine life. Hypoxia-ischemia increases the extracellular concentration of endogenous excitatory amino acids which trigger a cascade of biochemical events such as activation of protease, lipase and protein kinase, free radical formation, and uncoupling of oxidative phosphorylation within mitochondria via an increase in cytosolic calcium ion. This cascade leads to neuronal death in which N-methyl D-aspartate (NMDA) receptors play a major role.

Nitric Oxide (NO), a free radical gas is synthesized by the vascular endothelium by the conversion of Larginine to Lcitruline by the enzyme NO synthase. NMDA stimulates NO synthesis and NO mediates glutathione induced neurotoxicity in vitro. NO diffuses to the target site and mediates the cytotoxic activity of macrophages, causes blood vessel relaxation and helps to release neurotransmitters in central and peripheral nervous system. NO lasts only for few seconds in blood, forms nitrate and nitrite, and excreted in urine. Nitrate and nitrite are considered to be the markers of NO production. Oxidative stress which occurs due to hypoxia reperfusion injury is an important phenomenon in pathogenesis of hypoxic ischemic encephalopathy (HIE). It causes membrane lipid peroxidation and iron release in reactive form. Reactive iron causes membrane protein damage and predisposes erythrocytes to lysis. Tissue ischemia followed by reperfusion generates excess free radicals, which combines...
with NO to produce peroxinitrite and induces membrane damage. The alteration of membrane structure results in impairment of membrane bound ionic pump chiefly Na⁺K⁺ATPase. Vasortelyi B et al.(4) reported an 80% decrease in the Na⁺K⁺ATPase activity of brain tissue during the re-oxygenation phase of previously asphyxiated newborn piglets, simultaneous with the progression of cerebral morphological changes.

The aim of our study is to determine whether generation of NO metabolites in HIE causes RBC membrane damage. We have measured serum NO metabolites (Nitrite), RBC membrane osmotic fragility and RBC reduced glutathione. Our hypothesis is that increased generation of NO and its metabolites in HIE leads to erythrocytic membrane damage as a result of oxidative injury in term neonates.

Materials and Methods
This in-hospital cross sectional, study was conducted in the Department of Pediatrics, R G Kar Medical College & Hospital and Department of Biochemistry, Medical College, Kolkata over a period of 6 months. 31 intramural, term,(5) asphyxiated (who failed to initiate or sustain spontaneous respiration at birth) babies weighing >2.5 kg were enrolled as study population. 20 term, normal, non-asphyxiated neonates served as control population. Neonates, whose mothers were not booked, having any chronic medical illness or any pregnancy related complications, were excluded from the study. Babies, who died within 12 hours of birth, were also excluded. Clinical data regarding maternal & fetal status in antenatal and perinatal period were collected from bed head tickets. Thorough clinical examination of each newborn was done at birth, 12 hrs and 24 hrs of age. Informed consent was obtained from the parents and the study was approved by the institutional ethical committee. For each enrolled baby, 2 ml of clotted and 2 ml of heparinised cord blood were collected without squeezing, just after delivery. Biochemical analysis was done within 3 hours of collection of sample. Results were expressed in mean ± 2SD.

Erythrocytic membrane osmotic fragility was determined by method described by Parpart and co-workers.(6) In this method, small volume of heparinised blood was mixed with serial dilutions of buffered saline solution in large excess and hemolysis observed. Reduced glutathione was estimated by method described by Beutler et al.(7) Metabolites of NO, in the form of nitrite were measured by modified Griess reaction,(8) which is a modification of Cadmium reduction method.

Statistical analysis
Categorical variables were compared by chi-square test and continuous variables were compared by Student t test. The cut off value for p was 0.05. Open Epi version 2 software was used for data analysis.

Results
Mean birth weight of the cases was 2.78(± 0.28) kg and the controls 2.72 (±0.21 kg), p value being >0.05. Mean gestational age of the cases was 38.03(± 1.14) weeks and that of the controls was 38.15(± 1.03) weeks (p value >0.05).

Comparison of maternal data showed that the mean maternal age of asphyxiated neonates was 22.6(±3.56) years and that of the controls was 24.05(±1.79) years. 52Vo were primipara and 48Vo were multipara amongst the cases whereas 60Vo were primipara, and 40Vo multipara amongst the controls. In asphyxiated group, 32.2Vo were delivered by normal vaginal route, 51.2Vo by caesarean section and 16.1Vo with forceps; whereas in control group 30Vo were primipara, and 40Vo were delivered by normal delivery, 60Vo by caesarean section and 10Vo with forceps.

31 hypoxic ischemic encephalopathy cases were sub-divided according to Sarnat staging criteria; 48.4Vo were in stage I, 41.9Vo in stage II and 9.7Vo in stage III.

In Table 1 levels of the laboratory parameters are
compared. Levels of NO metabolites, RBC osmotic fragility and reduced glutathione were $14.23(\pm 4.63)$ $\mu$mol/L, $0.36(\pm 0.03)$ % and $2.34(\pm 0.25)$ $\mu$mol/g of hemoglobin respectively whereas that of control population were $6.31(\pm 1.49)$ $\mu$mol/L, $0.32(\pm 0.02)$ % and $3.74(\pm 0.23)$ $\mu$mol/g of hemoglobin in order. Statistical analysis showed significant difference between the two groups ($p < 0.05$).

We have divided total 31 asphyxiated neonates in two groups. The first group ($N_1=16$) were those, who needed endotracheal intubation, chest compression and/or injection adrenaline for resuscitation. Neonates in the second group ($N_2$) were mildly asphyxiated and needed only oxygen or bag & mask ventilation (BMV) for less than 2 minutes. Table 2 shows the biochemical parameters in both the groups in contrast to control population.

NO metabolites, RBC membrane osmotic fragility and reduced glutathione in the first group ($N_1$) were $16.23(\pm 4.21)$ $\mu$mol/L, $0.37(\pm 0.03)$ % and $2.21(\pm 0.34)$ $\mu$mol/g of hemoglobin respectively. The same parameters in the second group ($N_2$) were $12.48(\pm 4.39)$ $\mu$mol/L, $0.35(\pm 0.04)$ % and $2.47(\pm 0.12)$ $\mu$mol/g of hemoglobin in order. The study finds statistically significant ($p < 0.05$) decrease in reduced glutathione level and increase in serum NO metabolites level. There was no significant difference ($p > 0.05$) in RBC membrane osmotic fragility.

A bivariate analysis has been done between the levels of serum nitrite and RBC reduced glutathione level in asphyxiated neonates. Correlation coefficient of this study is $-0.67$ and p value is $<0.05$ implying that there is statistically significant negative correlation between concentration of Nitrite and reduced glutathione level in asphyxiated neonates (figure 1). We have also analyzed the correlation between RBC membrane osmotic fragility and serum nitrite (figure 2), which does not show any correlation (correlation coefficient $+0.25$, p value being $<0.05$).

Discussion

Birth asphyxia and resulting hypoxia-reperfusion injury leads to various biochemical and metabolic alterations in almost all organ systems in body. There is free radical production, generation of nitric oxide (NO) and its metabolites and activation of antioxidant system. This hypoxia-ischemia is typically initiated by a compromised placental or pulmonary gas exchange which leads to systemic hypoxemia/ anoxia followed by a phase of reperfusion. With neuronal glucose and oxygen debt arising from ischemia, oxidative metabolism shifts towards anaerobic glycolysis with its inefficient generation of high energy phosphates necessary to maintain cellular ionic gradients and other metabolic processes. Ultimately cellular energy failure occurs, which if not promptly reversed results in cell death.

NO and its metabolites play the pivotal role in hypoxia-reperfusion induced neurotoxicity. NO synthase (three types, eNOS, nNOS, and iNOS) is activated by calcium mediated NMDA and amino 3 hydroxy 5 methyl 4 isoxazole propionic acid (AMPA) receptors in brain. The generated NO diffuses to the site of action and reacts with superoxide molecule immediately to form stable peroxynitrite anion. Once protonated this peroxynitrite decomposes and generates a strong oxidant with reactivity similar to that of hydroxyl radical, along with membrane lipid peroxidation, direct DNA damage and increased release of glutathione. NO inhibits mitochondrial enzymes including complex I, complex II and aconitase, leading to subsequent inhibition of mitochondrial respiratory chain. It also inhibits the rate limiting enzyme in DNA replication, ribonucleotide reductase, resulting in inhibition of DNA synthesis in macrophage effector cells. NO has some neuro-protective role also by causing vasodilatation and inhibiting platelet activation. It also helps in circulatory decentralization during fetal hypoxia. Thus NO is the key molecule in the process of cellular damage in HIE.
Yuan Shi et al\(^{(13)}\) showed in their study that, NO level is increased in HIE in contrast to controls and the level is more in HIE grade II and III than grade I. Tamer Gunes et al\(^{(14)}\) and Cacha Peeter S Johana K et al\(^{(15)}\) showed that inhibition of NO synthase by 2-imino-biotin showed better immediate and post-asphyxial neuro-developmental outcome. In our study we have found increased levels of NO in asphyxiated neonates in comparison to control population and the level is more in severely asphyxiated (\(p < 0.05\)) ones.

Osmotic fragility of erythrocytic membrane reflects its ability to maintain structural integrity. It is related to their shape, deformability, surface area and volume ratio and intrinsic membrane properties. It is affected by maternal medication, disease, gestational age, maturity of the neonate, hypoxia, and oxidative stress.\(^{(16)}\)

We have shown in this study that mean erythrocytic membrane osmotic fragility in asphyxiated neonates is significantly increased (\(p < 0.05\)) than control population. In HIE, oxidative stress leads to membrane lipid peroxidation and iron release in reactive form.\(^{(3)}\) Lipid peroxidation products (Malondialdehyde and 4 Hydroxynonenal) are raised in HIE and membrane proteins also suffer from oxidative stress (reflected by increase in Carboxyl compounds).\(^{(17,18)}\) These in turn makes RBC membrane more vulnerable to lysis.

The excess release of free radicals during the post-hypoxic phase might induce membrane damage. There is intracellular accumulation of cations, chiefly sodium and calcium. The elevated sodium content might contribute to the development of post hypoxic cerebral edema. Calcium activates NMDA mediated pathways to perpetuate further neurotoxicity.

In newborn piglets it was experimentally demonstrated that there was a 20% decrease in Na\(^+\)K\(^+\)ATPase activity of RBCs during the post-hypoxic period.\(^{(4)}\)

Mean RBC osmotic fragility in adults is around 0.45 to 0.55% but it is much lower in neonates (control : 0.32± 0.02%). As neonatal RBC contains more amount of fetal hemoglobin (around 30% at term), it is osmotically more resistant. Gouri Rao Pasi and Schroter et al\(^{(19)}\) showed that cord blood and neonatal RBCs are osmotically less fragile than adult RBC. Levi J et al\(^{(20)}\) also had same observations on rabbit RBCs.

Role of glutathione is well established in HIE. Our observation supports previous reports in this context. Glutathione is the master antioxidant in our body. Hypoxia reperfusion activates glutathione peroxidase (GPx) which converts reduced glutathione in oxidized form. Significant decrease in reduced Glutathione level is observed in our study in asphyxiated neonates and the level is further decreased in severely asphyxiated ones (\(p < 0.05\)).

Nangia S, Salli A et al\(^{(21)}\) showed increased GPx and superoxide dismutase (SOD) levels in CSF of asphyxiated neonates. Schmidt et al\(^{(17)}\) showed decreased reduced: oxidized glutathione ratio in perinatal asphyxia.

Our study is constrained by the sample size (n=31) and many of them are HIE grade I. So there is scope for further studies in this aspect with larger sample size. There is also scope for further studies (in fact ongoing worldwide) regarding interventions to reduce HIE and its impact over neurodevelopmental outcome with use of NO synthase inhibitors, free radical scavenger molecules etc.

**Conclusion**

We can conclude from our study that birth asphyxia and resulting hypoxia-reperfusion injury causes increased generation of NO and its metabolites which results in decreased RBC glutathione concentration. The decreased concentration of RBC antioxidant gives rise to increased RBC membrane damage, probably due to increased membrane lipid peroxidation and cytoskeletal protein carbonylation. This is reflected in the increased osmotic fragility of RBC membrane. The direct effect of NO on RBC membrane is absent; the same mechanism i.e.
decreased intracellular antioxidant concentration is likely to be responsible for neurotoxicity.

Acknowledgement
The authors acknowledge the inspiration of Prof S Majumder, Vice Chancellor, WBUHS in taking up such subject as study matter. We also express our sincere thanks to the Principal, R G Kar Medical College & Hospital and Principal, Medical College, Kolkata for permitting us to carry out the task.

Table 1: NO metabolites, RBC membrane osmotic fragility and reduced glutathione levels in asphyxiated and non-asphyxiated neonates.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (mean± 2SD)</th>
<th>Control (mean± 2SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite (μmol/L)</td>
<td>14.23±4.63</td>
<td>6.31±1.49</td>
<td>0.01</td>
</tr>
<tr>
<td>RBC membrane osmotic fragility (%)</td>
<td>0.36±0.03</td>
<td>0.32±0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Reduced glutathione (μmol/g of hemoglobin)</td>
<td>2.34±0.25</td>
<td>3.74±0.23</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2: Laboratory parameters in N1 and N2 groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reduced glutathione in μmol/g of hemoglobin (mean±2SD)</th>
<th>Mean erythrocytic membrane osmotic fragility (mean±2SD)</th>
<th>NO metabolites in μmol/L (mean±2SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intubation and/or chest compression (N1=16)</td>
<td>2.21±0.34</td>
<td>0.37±0.03</td>
<td>16.23±4.21</td>
</tr>
<tr>
<td>O2, bag &amp; mask ventilation (N2=15)</td>
<td>2.47±0.12</td>
<td>0.35±0.04</td>
<td>12.48±4.39</td>
</tr>
<tr>
<td>Control (20)</td>
<td>3.74±0.23</td>
<td>0.32±0.02</td>
<td>6.31±1.49</td>
</tr>
<tr>
<td>P value</td>
<td>0.01</td>
<td>0.12</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Figure 1

Correlation between NO metabolites and RBC glutathione

Figure 2

Correlation between RBC osmotic fragility and NO metabolites (Nitrite)

References


6. Parpart AK, Lorenz PB, Parpart ER, Greg JR, Chase AM. The osmotic resistance (Fragility) of human red


GENDER BASED STUDY OF TOTAL CHOLESTEROL, TRIGLYCERIDES, HDL-C AND LDL-C IN HYPOTHYROID CASES

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Abstract
Numerous studies have observed a marked correlation between thyroid function indices and lipid metabolism. To examine the relationship between abnormal thyroid function as determined by thyrotropin and serum lipids, their levels in men and women were estimated. Aim of the study was to compare lipid abnormalities and hypothyroidism in both the genders. In this study we assessed 90 normal and 92 hypothyroid cases for T3, T4, TSH, Total cholesterol, Triglycerides (TG), HDL-C and LDL-C in men and women and statistically evaluated separately.

In the present study there is significant increase in total cholesterol, triglycerides and LDL-C is more in men than women but their increase is substantial in both the genders with elevated TSH, as compared to normal individuals (P<0.01, P<0.05, P<0.01). The HDL-C of hypothyroid men and women are not significantly different from the mean values for normal men and women.

Thus, the present study suggests that measurement of TSH may be useful in individuals with lipid abnormalities. The prevalence of abnormal TSH among individuals with lipid abnormalities was particularly very high and therefore screening for thyroid dysfunction may be warranted in high risk group.

Introduction
Correlation between blood levels of T3, T4, TSH and lipid metabolism in general is well established.(1) The data concerning a definite correlation between thyroid hormones and triglycerides is more controversial.(2,3) Hypothyroidism increases the oxidation of plasma cholesterol mainly because of an altered pattern of binding and to the increased levels of cholesterol, which presents a substrate for the oxidative stress. Cardiac oxygen consumption is reduced in hypothyroidism. This reduction is associated with increased peripheral resistance and reduced contractility.(4) We know that thyroid dysfunction is more common in females as compared to males. At the same time we also know that hypothyroid subjects are more prone to develop coronary heart disease.(5) To examine the relationship between abnormal thyroid function as determined by thyrotropin and serum lipids, their levels in men and women was estimated and statistically evaluated separately.

The aim of study was to compare lipid abnormalities and hypothyroidism in both the genders.

Material and methods
We studied 90 cases of normal patients for T3, T4, and TSH with serum total cholesterol and triglycerides. Institutional ethical committee approved this study and verbal informed consent was also taken from the patients. Among 90 normal cases studied, 63 were women and 27 were men, most of them belong to the age group of 20-40 years although age group varied between 20 and 55 years.

This study was conducted at S. Nijalingappa Medical College and HSK Hospital Bagalkot. We studied 92 cases of clinically diagnosed Hypothyroid patients, out of which 74 were women and 18 were men. We excluded patients with family history of dyslipidemia, smokers, patients with pharmacological history of intake of hypolipidemic drugs, corticosteroids and
in insulin. In the selected patients, we measured $T_3$, $T_4$ and TSH using Monobind Inc. USA-Lilac diagnostic kits. Readings were taken in ELISA reader. We also analysed serum levels of total cholesterol and triglycerides by adopting enzymatic methods, serum HDL-C was measured by phosphotungstic acid precipitation method by using CPC Identi Diagnostic kits, whereas LDL-C was calculated by using Friedewalds formula.(6) All the results were statistically evaluated.

Results
The serum $T_3$, $T_4$ and TSH, Total cholesterol, Triglycerides in normal cases are shown in table 1. The mean value of $T_3$, in normal men was $1.49 \pm 0.81$ ng/ml and $1.48 \pm 1.15$ ng/ml in women. Mean $T_4$ was $9.26 \pm 3.28 \mu g/dl$ in men and $10.54 \pm 4.31 \mu g/dl$ in women. Mean value of TSH in men was $2.11 \pm 1.61 \mu IU/ml$ and $2.33 \pm 1.60 \mu IU/ml$ in women. Serum total cholesterol mean values were $173.06 \pm 34.26$ mg/dl in men and mean of $177.52 \pm 40.77$ mg/dl in women. Serum Triglycerides mean in men was $129.44 \pm 43.73$ mg/dl and $134.94 \pm 64.66$ mg/dl in women. Serum HDL-Cholesterol and LDL-Cholesterol in normal was $37.45 \pm 7.23$ mg/dl and $109.72 \pm 18.28$ mg/dl in men whereas in women it was $41.34 \pm 5.43$ mg/dl and $109.19. 19 \pm 22.41$ mg/dl respectively.

The results of hypothyroid cases are shown in table 2. In hypothyroid cases mean $T_3$, was $0.75 \pm 0.59$ ng/ml in men and $0.98 \pm 0.70$ ng/ml in women. $T_4$ mean was $3.93 \pm 3.79 \mu g/dl$ in men and $5.23 \pm 3.69 \mu g/dl$ in women. Mean TSH was $31.46 \pm 20.17 \mu IU/ml$ in male and $25.73 \pm 19.12 \mu IU/ml$ in women. The total cholesterol was $266.42 \pm 107.03$ mg/dl in men and mean of $224.38 \pm 38 \pm 70.39$ mg/dl in women. The Serum triglycerides levels is $259.68 \pm 192.19$ mg/dl in men and mean of $170.78 \pm 91.38$ mg/dl in women. Serum HDL-C and LDL-C in hypothyroid cases was $35.63 \pm 6.18$ mg/dl and $178.86 \pm 62.41$ mg/dl in men whereas in women it was $39.74 \pm 6.46$ mg/dl and $150.49 \pm 45.66$ mg/dl respectively. In hypothyroid cases the changes of serum cholesterol, triglycerides and LDL-C are more marked as the TSH level increases. In our study, increase in TSH level is more in men than women (P<0.01) in hypothyroid cases. It has also been observed that serum Total cholesterol, serum triglycerides and LDL-C are more marked as the TSH level increased. In our study, increase in TSH level is more in men than women (P<0.01, P<0.01, P<0.01) in hypothyroid cases as compared to normal subjects. The HDL-C of hypothyroid men and women are not significantly different from the mean values of normal men and women.

Discussion
Increase in serum cholesterol and triglycerides increases the risk of atherosclerosis and coronary artery diseases. At the same time we also know that hypothyroid subjects are more prone to develop coronary heart disease.(5) In the present study it was observed that the effect of elevated TSH on serum lipids was clinically significant in both men and women as compared with normal TSH. However the effect of elevated TSH was more in men than women. Serum Total cholesterol, Triglycerides and LDL-C were increased significantly more in men that in women. Many previous clinical studies have found that individuals with overt hypothyroidism have elevated Total cholesterol, triglycerides and LDL-C. Hypothyroidism is often accompanied by diastolic hypertension, and in conjunction with dyslipidaemia may promote atherosclerosis. Hypercholesterolemia in hypothyroidism is because of decreased hepatic cholesterol catabolism. Hypothyroidism is also associated with a decreased rate of catabolism of LDL i.e decreased fractional clearance of LDL by a reduced number of LDL receptors in the liver. Increased TG is attributable to decreased clearance. Hypertriglyceridemia of hypothyroidism is the result of decreased clearance of VLDL rather than of increased
Gender based dyslipidaemia in hypothyroid cases

hepatic production of VLDL from circulating FFA.\(^{(7,10)}\)

The present data of normal or slightly reduced HDL-C with cardioprotection may be misinterpreted. In fact, HDL-C of hypothyroid men and women are not significantly different from the mean values for normal men and women. It is the predominant LDL-C moiety that is responsible for the striking changes. Therefore the concentration of serum LDL-C with established atherogenic effect, should be given as much weight as the concentration of HDL-C with alleged cardioprotective effect, in the assessment of risk of coronary heart disease. The high density lipoprotein (HDL) levels are normal or even elevated in severe hypothyroidism because of decreased activity of cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) which are regulated by thyroid hormones. The low activity of CETP and more specifically of HL, results in reduced transport of cholesteryl ester from HDL to very low density lipoprotein (VLDL).\(^{(5,10)}\)

Moreover, hypothyroidism increases the oxidation of plasma cholesterol mainly because of an altered pattern of binding and to the increased levels of cholesterol, which presents a substrate for the oxidative stress.\(^{(1)}\)

In conclusion, we found that, the increase in total cholesterol, triglycerides and LDL-C is more in men than in women but their increase is more in both the genders as compared to normal individuals. Thus our data suggests that measurement of TSH may be useful in individuals with lipid abnormalities. The prevalence of abnormal TSH among individuals with lipid abnormalities was particularly very high and therefore screening for thyroid dysfunction may be warranted in high risk group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_3) (ng/ml)</td>
<td>1.49 ± 0.81</td>
<td>1.48 ± 1.15</td>
</tr>
<tr>
<td>(T_4) ((\mu)g/dl)</td>
<td>9.26 ± 3.28</td>
<td>10.54 ± 4.31</td>
</tr>
<tr>
<td>TSH ((\mu)IU/ml)</td>
<td>2.11 ± 1.61</td>
<td>2.33 ± 1.60</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>173.06 ± 34.26</td>
<td>177.52 ± 40.77</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>129.44 ± 43.73</td>
<td>134.94 ± 64.66</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>37.45 ± 7.23</td>
<td>41.34 ± 5.43</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>109.19 ± 22.41</td>
<td>109.19 ± 22.41</td>
</tr>
</tbody>
</table>
Table 2: Serum Levels of $T_3$, $T_4$, TSH, Total cholesterol, Triglycerides, HDL-C and LDL-C in Hypothyroid.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_3$ (ng/ml)</td>
<td>0.75 ± 0.59 *</td>
<td>0.98 ± 0.70</td>
</tr>
<tr>
<td>$T_4$ (µg/dl)</td>
<td>3.93 ± 3.73 *</td>
<td>5.23 ± 3.69 *</td>
</tr>
<tr>
<td>TSH (µIU/ml)</td>
<td>31.46 ± 20.17 *</td>
<td>25.73 ± 19.12 *</td>
</tr>
</tbody>
</table>
| Total cholesterol (mg/dl) | 266.42 ± 107.03 * | 224.38 ± 70.39 * *
| Triglycerides (mg/dl)     | 259.68 ± 192.19 * | 170.78 ± 91.38 **|
| HDL-C (mg/dl)             | 35.63 ± 6.18     | 39.74 ± 6.46      |
| LDL-C (mg/dl)             | 178.86 ± 62.41 * | 150.49 ± 45.66 *  |

* $P =<0.01$ ** $P =<0.05$, Values are in mean ± SD.

References
Case Report

ALKAPTONURIA- AN UNUSUAL PRESENTATION WITH STROKE

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Abstract
Alkaptonuria is an hereditary metabolic disease due to deficiency of key enzyme Homogentisic acid (HGA) Oxidase in tyrosine metabolism. It leads to degenerative arthritis and pigmentation of cartilage. Severe pigment deposition can compromise cardiovascular, renal or pulmonary function. Such a case of alkaptonuria presenting with stroke is reported here. Biochemical tests and radiological findings correlated with clinical diagnosis.

Introduction
Alkaptonuria is one of the rare primary aminoaciduria with incidence of 1: 2, 50,000. It is an hereditary metabolic disease inherited as an autosomal recessive disorder. It is caused due to deficient activity of the major enzyme in catabolic pathway of tyrosine metabolism namely Homogentisic acid Oxidase(fig. 1). Reduced activity of enzyme leads to accumulation of HGA in cells and body fluids. This binds to various connective tissues leading to different clinical manifestations. Though can be diagnosed in neonates, usually its not diagnosed until middle age when ochronosis and arthritis leads to suspicion(1). A simple test like darkening of urine on exposure to air can give clue to diagnosis of this disorder. Various reduction tests for demonstrating HGA can be used as diagnostic tool.

Case Details
History : A 55 yr old male patient came to medicine department with complaints of slurring of speech, poor comprehension and inability to move right lower limb since 15 days. Patient gave a past history of joint pains since 15 years with minimal limitation of movement. On detailed enquiry, patient revealed that he had noticed blackening of urine since child hood.

Examination : Black pigmentation was seen in sclera (Figure 2). Kyphosis of spine and features of arthritis were found in shoulder and knee joints. CNS examination showed that patient was conscious with poor comprehension, had global aphasia and right UMN facial palsy. In motor examination, power could not be tested due to poor comprehension. Tone was normal. Reflexes were brisk on right side. In sensory examination, he could appreciate pain in all four limbs.

Investigations :
ECG: Normal
Haematology : Hb- 13.6 gm%
TC- 8,800 cells/cc
DC- Neutrophils-60
Lymphocytes-32
Eosinophils-07
Monocytes- 01
Platelets- 2.7 lakhs / cc

Peripheral smear : Normocytic normochromic blood picture with mild eosinophilia and adequate platelets seen.
Radiology: Kyphosis in thoraco-lumbar region. Symmetrical reduction of disc spaces with calcification of discs. Extensive degenerative changes of thoraco-lumbar region (figure 3). Bones are extremely osteoporotic. Symmetrical reduction of joint spaces with loose bodies (figure 4). Soft tissue calcification in tendons (figure 4). CT brain (plain) revealed subacute to chronic infarct in the anterior and left middle cerebral territories.

Biochemistry:
Blood: Random blood sugar - 131 mg/dL
Blood urea - 42.3 mg/dL
Creatinine - 1.2 mg/dL

Urine:
Ammonical silver nitrate test: Brownish black precipitate of reduced elemental silver. (figure 5a)
Ferric Chloride test: Transient green colour intensified on exposure to air. (figure 5b)
Benedict’s Test: Yellow precipitate with dark supernatant. (figure 5c)

Paper chromatography using HGA as standard was done and spot corresponding to the standard was noted.

Discussion
Alkaptonuria is a rare metabolic disease characterized by triad of homogentisic aciduria, arthritis and ochronosis. The manifestations are that urine turns dark on standing and alkalinasation due to excretion of excessive amounts of homogentisic acid, large joint arthritis and black ochronotic pigmentation of cartilage and collagenous tissue.(2) Alkaptonuria and ochronosis affects various body systems which include cutaneous, ocular, skeletal, cardiovascular, genitourinary, CNS and endocrine system. Homogentisic oxidase is an enzyme in tyrosine metabolism.

On deficiency of Homogentisic acid oxidase enzyme, homogentisic acid accumulates in body fluids and connective tissues. This leads to darkening of urine on exposure to air, arthritis and connective tissue pigmentation due to oxidation of Homogentisic acid to benzoquinone acetate, which polymerizes and binds to connective tissue. HGA forms plasma soluble melanins by oxidative polymerization and co polymerization. Due to this reactive oxygen side products are generated during and after melanogenesis. HGA melanins (both soluble and deposited) and their intermediates have toxic chemical reactivities. This leads to patients with alkaptonuria to develop arthritis and suffer from other diseases including cardiovascular disease.(4) The urine turns black on standing or staining the diaper is the first feature of this disorder but majority of parents fail to note or attend with the complaint. Still few cases have reported with above complaint in early childhood. But an adult
patient with this disorder usually present after 4th decade with arthritis, ocular, cutaneous and cardiovascular ochronosis. There are reports of aortic valve stenosis and concomitant coronary artery disease.\(^{(5)}\) In another case during open heart surgery for aortic dissection with intramural haematoma, it was postulated that coronary artery disease as well as significant peripheral vascular disease found was in part due to intracellular pigment deposition in arterial intima.\(^{(6)}\) Ochronotic granules can cause valves to calcify and pigment deposits also can lead to formation of atherosclerotic plaques.\(^{(7)}\) In our case the patient presented with classical manifestation of arthritis involving various joints, ocular features with scleral pigmentation and stroke, possible cause for which may be due to cardiovascular involvement of the disorder. Hence though there are other risk factors for stroke like age of the patient, the cerebral infarct here could be due to atherosclerotic plaques involving cerebral arteries cause of which could be alkaptonuria.

The diagnosis can be confirmed by measurement of Homogentisic acid and its derivative benzoquinone acetic acid by enzymatic spectrophotometry or by using gas liquid chromatography or by high pressure liquid chromatography. But simple screening tests based on principle of reduction reactions of Homogentisic acid like Benedict's test, Ammoniacal silver nitrate test and test like Ferric chloride test done in routine laboratory can give a clue to the diagnosis.\(^{(8)}\) Here we have made a diagnosis by performing screening tests and confirmed by paper chromatography using HGA as standard. No treatment has been completely successful for this disorder. But dietary restrictions on intake of phenylalanine and tyrosine and providing vitamin C supplements are found to be helpful to some extent.\(^{(2)}\)

**Conclusion**

Alkaptonuria being a rare metabolic disorder, though present since early childhood, manifests at a later age. It can affect various body systems including skeletal, cardiovascular, genitourinary, respiratory and also central nervous system. These unusual presentations should be kept in mind when dealing with such cases. Simple screening tests done in a routine laboratory can help in early diagnosis of the disorder.

**Figure 2:** Black pigmentation seen in right sclera suggestive of ocular ochronosis

**Figure 3:** X-ray thoraco-lumbar spine lateral view showing features of Kyphosis, reduction in disc spaces and degenerative changes.
Figure 4: X-ray of Knee joint lateral view showing features of osteoporotic bones, decrease in joint space and calcification of tendon.

References

Figure 5: Test tubes showing the biochemical tests
a. Ammonical silver nitrate test showing brownish black precipitate of reduced elemental silver.
b. Ferric chloride test showing transient green colour which intensifies on exposure to air.
c. Benedict's test showing yellow precipitate with dark supernatant.
LACTATE AS MARKER OF PPROM

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Abstract
Preterm prelabour rupture of membrane (PPROM) before 37 weeks of gestation is sometimes a clinical problem due to associated perinatal morbidity and mortality. Clinical diagnosis from leakage of amniotic fluid cannot predict the time of delivery. This has been overcome by lactate strip test at the bedside. The lactate concentration if is equal to 4.5 mmol/l in the vaginal fluid, the labour time can be predicted as 13.6 hours and helps in clinical diagnosis of onset of labour.

Introduction
Rupture of fetal membranes prior to onset of labour in a patient who has gestational age of less than 37 weeks is known as preterm prelabour rupture of membrane (PPROM). This is a common problem accounting for about one-third of all preterm births. Often patients are presented at hospital emergency & refused admission on the ground that the delivery is still delayed. It has been seen in the newspapers that many such patients, after getting refusal from the hospital delivered the baby on their way to home. Thus PPROM associated risk of perinatal morbidity & mortality is our major concern.
The leakage of amniotic fluid, if scanty or intermittent, then a combination of factors, including mother's history, the amount of vaginal fluids, chance of infections are to be remembered to ensure the management of these patients and prevent further complications, if any.

Biochemical marker
Recent study(1) to search for the biochemical marker for PPROM was encouraging. The lactate concentration is reported to be about 4-6 times more in amniotic fluid than in fetal & maternal blood(2,3,4). Wiberg-Itzel E et al (1) has conducted a study at Stockholm during the period of 2002-2004 on the PPROM patients with a history of leakage of water like fluid from vagina during 34-36 weeks of gestation. All women included in their study did not have any symptoms or signs of infection; however bacterial culture was not done. They have shown that the vaginal fluid collected from the posterior fornix was lactate positive by strip test. Later on lactate concentration was measured by electrochemical test strip method. This test was carried out at bedside & the report was available after 60 seconds. It has been seen that the median time between examination & onset of labour was 13.6 hours in those patients where the lactate concentration was $> 4.5$ mmol/l than those who has lactate concentration $= 4.5$ mmol/l. In contrast, women with lactate concentration $< 4.5$ mmol/l appeared to have much a longer time to spontaneous onset of labour (about 48 days). Therefore, Wiberg suggested that if the lactate concentration is $\geq 4.5$ mmol/l in the vaginal fluid,
the patient should be considered to have delivery within 48 hours. In their study they have not found any dose-response relationship between lactate & time to spontaneous onset of labour, either in woman at term\(^{(5)}\) or in woman at PPROM. Quenby et al\(^{(6)}\) have suggested that the main producer of lactate during labour is myometrium. If myometrial activity is the source of lactate during the latent phase, a dose-response relationship would be expected, but it was not obtained. Wiberg et al in their study hypothesized that the lactate concentration in vaginal fluid is useful tool in diagnosis of ruptured membranes, which in turn predicts onset of labour.

**Conclusions**

Determination of lactate in vaginal fluid seems promising as a tool to predict onset of labour within 48 hours in women with suspected PPROM. A positive LAC test (≥ 4.5 mmol/l) is more strongly associated with spontaneous onset of labour than visible amniotic fluid.

**References**

News Update

News of Nobel Prize, 2009 & winner scientists

Nobel Prize in chemistry shared by an NRI Indian

This year's Nobel Prize in Chemistry awards Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath for having showed what the ribosome looks like and how it functions at the atomic level. All three have used a method called X-ray crystallography to map the position for each and every one of the hundreds of thousands of atoms that make up the ribosome.

Venkatraman Ramakrishnan is an NRI Indian, (US citizen) was born in 1952 in Chidambaram, Tamil Nadu, India. He studied B.Sc.( Physics) at Baroda University.

He did Ph.D. in Physics in 1976 from Ohio University, USA. He then studied biology at the University of California, San Diego and started conducting research.

In the September 27, 2000 issue of Nature, Ramakrishnan published two papers on the 3 Angstrom structure of the 30S ribosomal subunit. He joined the University of Utah in 1995 as a professor in the Department of Biochemistry where he initiated his studies on protein-RNA complexes and the entire 30S subunit.

He is Senior Scientist and Group Leader at Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK.

Work of Venkatraman Ramakrishnan et al in a nutshell

Inside every cell in all organisms, there are DNA molecules. They contain the blueprints for how a human being, a plant or a bacterium, looks and functions. But the DNA molecule is passive. If there was nothing else, there would be no life. The blueprints become transformed into living matter through the work of ribosomes. Based upon the information in DNA, ribosomes make proteins: oxygen-transporting haemoglobin, antibodies of the immune system, hormones such as insulin, the collagen of the skin, or enzymes that break down sugar. There are tens of thousands of proteins in the body and they all have different forms and functions. They build and control life at the chemical level.

An understanding of the ribosome's innermost workings is important for a scientific understanding of life. This knowledge can be put to a practical and immediate use; many of today's antibiotics cure various diseases by blocking the function of bacterial ribosomes. Without functional ribosomes, bacteria cannot survive. This is why ribosomes are such an important target for new antibiotics.

This year's three Laureates have all generated 3D models that show how different antibiotics bind to the ribosome. These models are now used by scientists in order to develop new antibiotics, directly assisting the saving of lives and decreasing humanity's suffering.
Nobel Prize in Physiology & Medicine

Americans Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak won the 2009 Nobel Prize in medicine for discovering a key mechanism in the genetic operations of cells, an insight that has inspired new lines of research into cancer. The trio solved the mystery of how chromosomes, the rod-like structures that carry DNA, protect themselves from degrading when cells divide.

The prize-winners' work, done in the late 1970s and 1980s, set the stage for research suggesting that cancer cells use telomerase to sustain their uncontrolled growth. Scientists are studying whether drugs that block the enzyme can fight the disease. In addition, scientists believe that the DNA erosion the enzyme repairs might play a role in some illnesses. "The discoveries by Blackburn, Greider and Szostak have added a new dimension to our understanding of the cell, shed light on disease mechanisms, and stimulated the development of potential new therapies," the prize committee said in its citation.

Some inherited diseases are now known to be caused by telomerase defects, including certain forms of congenital aplastic anemia, in which insufficient cell divisions in the stem cells of the bone marrow lead to severe anemia. Certain inherited diseases of the skin and the lungs are also caused by telomerase defects.

Elizabeth H. Blackburn has US and Australian citizenship. She was born in 1948 in Hobart, Tasmania, Australia. After undergraduate studies at the University of Melbourne, she received her PhD in 1975 from the University of Cambridge, England, and was a postdoctoral researcher at Yale University, New Haven, USA. She was on the faculty at the University of California, Berkeley, and since 1990 has been professor of biology and physiology at the University of California, San Francisco.

Carol W. Greider is a US citizen and was born in 1961 in San Diego, California, USA. She studied at the University of California in Santa Barbara and in Berkeley, where she obtained her
PhD in 1987 with Blackburn as her supervisor. After postdoctoral research at Cold Spring Harbor Laboratory, she was appointed professor in the department of molecular biology and genetics at Johns Hopkins University School of Medicine in Baltimore in 1997.

**Jack W. Szostak** is a US citizen. He was born in 1952 in London, UK and grew up in Canada. He studied at McGill University in Montreal and at Cornell University in Ithaca, New York, where he received his PhD in 1977. He has been at Harvard Medical School since 1979 and is currently professor of genetics at Massachusetts General Hospital in Boston. He is also affiliated with the Howard Hughes Medical Institute.