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Remembrance

With profound grief and sorrow, we regret to inform the untimely sad demise of Prof. Sudipta Chatterjee, a frontline Covid Warrior. He was one of the best Nurosugeons in this part of the country. He was associated with the prestigious Bangur Institute of Neuroscience, Kolkata for many years.

This is a great loss for the fraternity and the general public at large.

JIMA will always be indebted for his substantive contribution as a Member of JIMA Editorial Advisory Board. His work and devotion will be felt by us for many years. We will miss an ever smiling, soft spoken, friendly personality.

On behalf of JIMA we extend deep condolences and sympathies to the family of the bereaved.

Om Shanti !

Dr Sudipto Chatterjee
Neuro Surgeon
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Largest Eye Care System in Eastern India

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Prof. Tamonas Chaudhuri
Hony. Editor
MBBS, MS, FAIS, FMAS, FACS, FACRSI (Hony)

Editorial

Enough is enough.....

Offence is the best means of Defense — this is a hackneyed idiom used by many to defend their violence rightfully or otherwise.Well, if this is an axiom then whom are we trying to defend when we take arms against the doctors. At this point of time when the molestation of doctors has become a trend and the molester is at times given the status of a paladin I think this would be the most relevant subject to discuss in this editorial. The incidents of violence against doctors, leading to grievous injury and even death, seem to be on an increasing trend in recent years. Still there is a paucity of studies on workplace violence against doctors and its effect, in India.Let us discuss in detail with the limited data we have in our disposal which is enough to bring out the horrific scenario.

According to the WHO framework Guidelines (2002), "Workplace violence is defined as the situations where staffs are ill-treated, intimidated or attacked in conditions linked to their workplace, including commuting to and from the workplace, involving an explicit or implicit challenge to their safety, well-being or health". Physical and psychological violence to the workers in the health care sector is many times higher as compared to the other sectors. Compared to all other workers, workplace violence seen in healthcare workers is four times higher and hence requires a longer time away from work[1]. The Indian Medical Association (IMA) reportedly said, "Healthcare violence has become an alarming phenomenon across the country. The real size of the problem is largely unknown and recent information shows that the current knowledge is the tip of the iceberg[2]." Many reports quote an IMA survey, which claims 80% of doctors in India are stressed in their profession, while 75% of doctors have dealt with some form of violence during their practice. This includes verbal, emotional, sexual, psychological, physical and cyber intimidation, threats, abuse, and occasionally even extreme bodily harm and injury caused by patients, patient-attendants, or even mobs of 'miscreants'. As many as 62.8% of doctors are unable to see their patients without any fear of violence; 13.7% fear criminal prosecution most days of the week; and 57.7% of doctors have thought of hiring security in their premises[2]. The data given above is enough to prove that the violence against the health care workers are increasing in leaps

and bounds in the recent years. However the purpose of this article is not to showcase statistical data on the increasing violence but to analyze in an unbiased manner the reason for such surge in molestation and finding out a plausible solution to alleviate such violence.

According to me, amongst many reasons, the prime most reason for violence is the lack of liaison between the patient families and the healthcare workers. Where there is darkness there is suspicion and where there is skepticism there is misunderstanding and where there is misunderstanding there is violence. The vindictive attitude brews among the families and leads to a sudden outburst. On one hand the doctors should be more patient and lenient in listening to the patients and the patient families since they are the poor souls struggling to fight against the odds.The patient families must also understand clearly that doctors are ultimately human beings and they would be most satisfied and happy when they see their patients recovering and going back with a smile back to their homes. Uncertainly and unpredictability however is the law of existence and not all are fortunate,in spite of best efforts from the doctors and other stuffs, to survive. Another glaring reason for such incidence of violence is the lack of infrastructure in health care. As the current pandemic has revealed, there is serious shortage of ITU or ICU units in private as well as government hospitals which prevents the seriously ill patients to get timely care. The helpless kith and kins of the patients on the verge of death lose their patience and find the front lineworkers, that are the doctors and the nurses, to vent out their vengeance scarcely realizing that they too are the victims of the faulty inadequate system. The snaking queue in front of the outpatient departments also reveals huge difference in the doctor to patient ratio.

"Don't lose the golden hour" – this line is displayed in huge fonts on huge bill boards as part of the advertisement campaign for a well know corporate hospital. The first hour after the onset of a heart attack is called the golden hour and appropriate action during this golden hour can spell the difference. Well we all know that but this sudden calamity also comes with a rider. The threat of a massive medical bill with astronomical figures. Now where will the patient's relative take the patient? Take him to a state of art hospital and become a pauper in a matter of hours or take him to a government hospital , with tremendous agony to get a timely care. If anything happened wrong, the end result – molestation of the health care workers. This socio economic problem can only be solved with the positive intervention of the State and establishing Health as the right of the People.

The doctors should also be vocal and raise their voices against the corporate autocracy which stops them from doing the right things at the right time. They become the victim of pubic rage as a result. As even the moon has blemishes, the doctors even though being grossly dedicated and loyal, do have among them unscrupulous peers camouflaged behind the white coats. These miscreants and outlaws should be isolated and ousted and weeded off to make the system clean. Because of these miniscule section the ethical doctors get molested by the public.

The Apex court, inJerrylBanait vs Union Of India on 8 April, 2020 dealt with a matter wherein the doctors who had gone to screen certain patients were attacked and faced stone-pelting. The Supreme Court observed and directed:

*"*The pandemic which is engulfing the entire country is a national calamity. In wake of calamity of such nature all citizens of the country have to act in a responsible manner to extend helping hand to the government and medical staff to perform their duties to contain and combat the COVID-19. The incidents as noted above are bound to instill a sense of insecurity in Doctors and medical staff from whom it is expected by the society that they looking to the call of their duties will protect citizen from disease of COVID-19. It is the duty of the State and the Police Administration to provide necessary security at all places where patients who have been diagnosed coronavirus positive or who have been quarantined are housed. The Police

security is also provided to Doctors and medical staff when they visit places for screening the people to find out the symptoms of disease."

In Abdul Naser v. State of Kerala, the Kerala High Court observed that apart from subjecting doctors to agony and anguish, attacks and violence on them adversely affect the treatment of all patients. It practically leads to a halt in functions, jeopardising the health of many persons, which is a matter of grave concern.

Observing the growing violence against doctors especially in the last year the Epidemic Diseases (Amendment) Act 2020 is an amendment to the Epidemic Diseases Act 1897. The principal Act comes under the State List under Schedule VII and is pre-independence legislation.

The 2020 Amended Act defines 'acts of violence' committed by any person against the healthcare service professional serving during an epidemic as one, which may cause, harassment, hurt, injury, a hindrance to services, damage to property or documents in custody.

The statute also defines 'health care professional' and 'property', providing a wide ambit for better protection. Section 2B provides that no person shall indulge in any act of violence against a healthcare service professional or cause any damage or loss to any property during the epidemic.

Section 3(2) provides punishment for commission or abetment of commission of an act of violence. Section 3(3) deals with committing an act of violence against a healthcare service professional, causing grievous hurt as defined in section 320 IPC. Section 3A of the statute provides that the inquiry or trial must conclude within a year[3].

Waukesha County, Wis. has a Zero Tolerance Workplace Violence Policy and Procedure. Waukesha County Risk Manager Laura Stauffer explains, "While we can't guarantee the protection of employees against acts of violence, we do have the ability to regulate and direct the conduct of our employees in an effort to prevent or minimize the severity of violent incidents. The policy has both emergency and non-emergency information, telling employees to "become aware of escape routes," "seek safety by leaving area if possible" and "do not attempt to control a violent individual." policies cannot protect against every situation, but they can open lines of communication so employees feel empowered to take action in a violent situation. Discussion can help employees to recognize or diffuse situations. Training can help employees feel less anxious when talking about workplace violence or participating in drills[4].

The discussion burns down to a solid conclusion that something must be done with urgency to alleviate if not annihilate workplace violence. Both the health care professionals and the common man should be trained to understand each other so that each understands the other and a relationship of mutual respect develops between the two. The enacted laws of the land for the protection of health care workers should not only be written in the books of law but should be aptly executed to prevent such molestations and help the workers to work safely and effectively.

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Original Article

Study of Genetic Mutation Exhibiting Resistance to Rifampicin and Isoniazid in the Tuberculosis Cases of Eastern Region of Bihar

D P Singh[1], S K Ghosh[2], Pratik Kumar[3], Ajay Kumar Singh[4], Krishna Kumar[5]

Studies regarding epidemiology of mutations associated with Anti-TB drug resistance in Bihar are sparse. The present study analyzes the presence and prevalence of different genetic mutations associated with resistance to Rifampicin and Isoniazid. Specimens from presumptive MDR TB patients were received and LPA were performed to study the mutation patterns. Samples of 3322 patients were incorporated in this study. Processing of samples followed by DNA extraction, PCR and reverse hybridization were carried out and results were interpreted and analyzed. The prevalence of rifampicin resistance observed was 4.54% which includes 119 MDR TB cases and 32 rifampicin mono resistant cases. Similarly, the prevalence of isoniazid resistance observed was 7.6%. The mutation pattern depicts the presence of S531L, S315T1and C15T as the most prevalent mutations for *rpoB*, *katG* and *inhA* promoter region respectively. These findings resemble with other national and international reports.

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Key words **: Tuberculosis (TB), Multi-drug resistant (MDR) TB, Rifampicin, Isoniazid, *rpoB*, *katG*, *inhA*.**

Tuberculosis (TB) is predominantly an infectious disease caused by a group of mycobacterial species called *Mycobacterium tuberculosis* complex. One-third of the global population has been l infected with latent TB[1]. India carries 30% of the global TB burden[2]. The incidence of drug resistance against the key Anti-TB drugs is the leading challenge for the global TB control[3]. The combination therapy with Anti-TB drugs was a brilliant initiative to prevent the emergence of drug-resistant TB[4]. Unsuccessful treatment and dissemination of resistance have emerged as two of the biggest threats for the fight against drug resistant TB[5]. This situation could lead to multiply the number of Multi drug resistance (MDR) TB cases, where the bacilli resist to both rifampicin and isoniazid, which has been reported in all settings. The second-line anti-TB drug treatments for MDR-TB cases, however, are comparatively less effective with more side effects[6]. The estimated global MDR/RR TB burden was 4.1% in new cases and 19% in previously treated cases for the year 2016[7]. The rapid diagnostics of drug resistant TB plays an important role both in suppressing emergence of resistance with other prescribed drugs and better patient management. The WHO has endorsed the use of GenoType MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) also called as line probe assay (LPA) for molecular based rapid diagnostics of drug resistant TB[8]. The principle of LPA is based on the amplification of genetic regions that bears resistance associated mutations[9]. It is a DNA strip based technology and method includes processing of samples, DNA extraction, master-mix preparation, amplification with biotinylated primers and detection with reverse hybridization process. The LPA for rifampicin and isoniazid can confirm the presence of MTB with presence or absence of the most common mutations associated with resistance with Rifampicin and Isoniazid. *rpoB* gene was the target for identification of rifampicin resistance. However, *katG* and promoter region of *inhA* gene have been identified to confer resistance against isoniazid. There is not enough

Editor's Comment :

- Rapid Diagnostics of Drug resistant TB play an important role both in suppressing emergence of resistance and better patient management.
- It is of great significance to attain the knowledge of mutation patterns of key anti TB drugs Rifampicin and Isoniazid by Line Probe Assay in all TB patients.
- rpoB gene is the target of Rifampicin resistance while KatG and inhA genes are associated with INH resistance.
- Detection of mutation patterns associated with Rifampicin and INH resistance helps in formulation of accurate different Regimens for mono resistance , MDR resistance and different level of mutation for INH resistance.
- Effect of common and uncommon mutations associated with these anti TB drugs may pave way for prospect of future research.

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published epidemiological studies available for mutations associated with anti-TB drug resistance in Bihar. The present study analyze the presence and prevalence of different mutations associated with resistance to Rifampicin and Isoniazid in the Eastern Bihar.

Materials and Methods

Setting :

The tuberculosis culture and drug susceptibility testing (DST) laboratory functioning in the premise of Jawaharlal Nehru Medical College and Hospital, Bhagalpur, has been certified by National Mycobacteriology Certification System of Central TB Division, New Delhi, for performing line probe assay based molecular DST as well as liquid culture based DST for first and second line Anti-TB drugs. The laboratory is serving nine districts of Bihar, *ie*, Araria, Banka, Bhagalpur, Begusarai, Khagaria, Kishanganj, Katihar, Munger and Purnia. The study was performed on clinical samples received from July, 2016 to June, 2020.

Study design and sample size :

Specimens from presumptive MDR TB patients were received and LPA were performed to study the mutation patterns. Samples of 3322 patients were incorporated in this study.

Sample processing :

The specimens were processed by using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method[9,10]. Equal volumes of NALC-NaOH solution were gently added to the samples and allow the samples to liquefy properly by intermittent vortexing. The reaction time for NALC-NaOH was 15 minutes. After 15 minutes, phosphate buffer saline (PBS) was added to the 45 ml mark of the 50 ml falcon tube to stop the action of NaOH. After that, samples were centrifuged at 3000x g for 15 minutes at 4°C. After the centrifugation, the pellets were saved for further DNA extraction.

DNA extraction :

Further steps of DNA extraction, PCR and reverse hybridisation were carried out as per the manufacturer's instructions[11]. The pellets were resuspended with 2 ml PBS and 500 µl processed specimens were transferred into cryovials. These processed specimens were centrifuged at 10000xg for 15 minutes. After centrifugation, pellets were saved and 100 µl of lysis buffer were added to each of the samples and mixed properly. Samples were then incubated at 95°C for five minutes inside hot air oven to disrupt the cell wall. Again, 100 µl neutralization buffers were added to each sample and centrifuged at 13000 x g for 5 minutes after proper mixing. Finally, supernatant containing DNA was isolated in a fresh cryovial for each sample.

Multiplex PCR :

45 µl master-mix were prepared for each sample as per the kit instructions and aliquoted in PCR tubes. 5 µl of extracted DNA were added into the master mix prepared and PCR were performed with following reaction conditions - initial denaturation at 95°C for 15 minutes, followed by 20 cycles of denaturation at 95°C for 30 seconds and elongation at 65°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 25 seconds, annealing at 50°C for 40 seconds, elongation at 70°C for 40 seconds, followed by a final extension at 70°C for 8 minutes.

Reverse hybridization and result interpretation :

The amplicons were denatured and hybridized with probes attached on the LPA strips and following a series of washing, conjugate and substrate treatment, bands develop on the strips. Interpretations of bands were done and results were analyzed.

Results

A total of 3322 LPA tests have been performed in the study. Out of which, 3037 (91.42 %) detected as susceptible for both rifampicin and isoniazid. 119 (3.5%) cases have been detected as MDR TB. However, apart from MDR cases, additional 32 (0.96%) cases have been detected as resistant with Rifampicin and susceptible with Isoniazid. On the other hand, 134 (4.03%) cases have been identified as resistant with Isoniazid and susceptible with rifampicin. The details of mutation patterns for Rifampicin and Isoniazid are depicted in Table 1 & 2 respectively.

When the mutation patterns were analyzed, it was observed that out of 151 (4.54%) rifampicin resistant cases, 105 (69.5%) cases had missing wild type 8 probe with presence of mutation 3 probe for *rpoB* gene. 14 (9.2%) cases had missing wild type 3 and wild type 4 probe with presence of mutation 1 probe. 10 (6.6%) cases had missing wild type 7 probe with no mutation band present. 6 (3.9%) cases had missing wild type 3 and wild type 4 probe with no mutation band present. 3 (1.9%) cases had missing wild type 7 probe with presence of mut 2B probe. 2 (1.3 %) cases had missing of wild type 2 probe with no presence of mutation band. In 2 (1.3 %) cases had missing wild type 2 and wild type 3 with no mutation band present. 2 (1.3%) cases had missing wild type 4 with mutation 1 band present. 2 (1.3%) cases had missing wild type 4 probe with presence of mutation 1 band. 2 (1.3%)

Table 1 — *Mutation pattern associated with Rifampicin resistance*

Rifampicin resistance					
Failing of wild type band	Codon analysed	Presence of mutation band	Specific mutation	Number of cases observed	Percent shared
wt 2	510-513	no common mutation		2	1.32%
wt 2 and wt 3	510-517	no common mutation		2	1.32%
wt 2 and wt 3	510-517	mut 1	Aspartate516Valine	1	0.66%
wt 3 and wt 4	513-519	mut 1	Aspartate516Valine	14	9.27%
wt 3 and wt 4	513-519	no common mutation		6	3.97%
wt 4	516-519	mut 1	Aspartate516Valine	2	1.32%
wt 7	526-529	no common mutation		10	6.62%
wt 7	526-529	mut 2A	Histidine 526 Tyrosine	2	1.32%
wt 7	526-529	mut 2B	Histidine 526 Aspartate	3	1.99%
no wt absent		mut 2B	Histidine 526 Aspartate	2	1.32%
no wt absent		mut 1	Aspartate516Valine	1	0.66%
no wt absent		mut 3	Serine 531Leucine	1	0.66%
wt 8	530-533	mut 3	Serine 531Leucine	105	69.54%

Table 2 — *Mutation pattern associated with Isoniazid resistance*

Isoniazid resistance										
katG					ihhA				Cases	Percent share
Locus	Failing of wild type band	Codon analysed	Presence of mutation band	Specific mutation	Failing of wild type band	Analysed nucleic acid position	Presence of mutation band	Specific mutation		
Present	1	315	1	Serine 315 Threonine	Mutation not detected				194	76.68%
Present	1	315	0		Mutation not detected				6	2.37%
Absent					Mutation not detected				4	1.58%
Present	1	315	1	Serine 315 Threonine	1	-15	1	Cytosine15 Thymine	5	1.98%
Present	1	315	1	Serine 315 Threonine	2	-8	3A	Thymine 8 Cytosine	1	0.40%
Present	Mutation not detected				1	-15	1	Cytosine15 Thymine	37	14.62%
Present	Mutation not detected				2	-8	3B	Thymine 8 Adenine	3	1.19%
Present	Mutation not detected				0		1	Cytosine15 Thymine	3	1.19%

cases had missing wild type 7 probe with presence of mutation 2A probe. 2 (1.3%) cases had no missing wild type with mutation 2 B band present. 1 (0.6%) case had no wild type probe absent with mutation 1 band present. 1 (0.6%) case had no wild type probe absent with mutation 3 probe was present.

Out of 253 Isoniazid resistance cases, 194 (76.6%) had missing *katG* wild type probe with presence of mutation 1 probe. 6 (2.3%) had missing *katG* wild type probe with no mutation probe present. However, 4 (1.5%) had missing *katG* locus with no wild type and mutation bands present. 37 (14.6%) had missing of *inhA* wild type 1 band with presence of mutation 1 band. 3 (1.1%) cases had missing *inhA* wild type 2 band with presence of mutation 3B band. 3 (1.1%) cases had no missing wild type probe with presence of mutation 1 band. However, 6 (2.3%) cases had both *katG* and *inhA* mutation. For *katG*, all of them had missing wild type probe and presence of mutation 1 probe but for *inhA* region, 5 cases had missing wild type 1 probe and presence of mutation 1 band and 1 case had missing *inhA* wild type 2 probe and presence of mutation 3A probe was observed.

Discussion

India shares 26% of the global TB burden and 27% of the global Rifampicin resistance TB burden[12]. The global TB data 2020 states the presence of Rifampicin

resistant TB in 3.3% new and 17.7 % previously treated cases[12]. Rapid diagnostics of drug resistance by detecting the genes associated with resistance plays a very important role in management of TB cases. Resistance with rifampicin has been identified as one of the main reasons behind the treatment failure of TB cases[13]. Rifampicin has bactericidal effects on both metabolically active as well as semi -dormant *M tuberculosis* Bacilli[14]. This effect of Rifampicin, in addition with the effectiveness of Pyrazinamide, has allowed reducing the TB treatment from one year to six months[14]. Rifampicin, basically targets the DNA dependent RNA polymerase of mycobacteria which inhibits the bacterial transcription. The 81 base pairs (27 codons) central region of the gene that encodes the β-subunit of RNA polymerase (*rpoB)* constitutes the resistance determining region. Mutation in this region may results to resistance with Rifampicin. Isoniazid, on the other hand, is a prodrug and it gets activated inside the mycobacterial cell. It enters the mycobacterial cytoplasm through passive diffusion and can kill only actively dividing bacilli[15,16]. It blocks the synthesis of mycobacterial cell wall mycolic acids. An interesting effect of isoniazid on M.TB was identified as loss of acid Fastness[17]. The prodrug isoniazid is activated by catalase-peroxidase enzyme (*katG*) and specific mutation in the *katG* gene confers high level resistance to Isoniazid. However, mutation in the promoter region of *inhA* gene, which codes for one of the main targets of Isoniazid - enoyl acyl carrier protein (ACP) reductase, confers low level resistance with Isoniazid. The mutation in promoter region of *inhA* gene results in over expression of this protein which generally counter-balances the effect of Isoniazid. The present study incorporate a huge sample size of 3322 LPA tests. The prevalence of Rifampicin resistance was 151 (4.54%) which includes 119 MDR TB cases and 32 Rifampicin mono resistant cases. Similarly, including the 119 MDR and 134 Isoniazid mono resistant cases, the prevalence of isoniazid resistance reached 7.6%.

When the mutation patterns were analyzed, it was observed that a huge 70.20% of specific mutation was detected in the 531 codon region of the *rpoB* gene, which was S531L missence mutation. However, 11.26% D516V mutation appears as the second most prevalent mutation in *rpoB* followed by 3.31% H526D and 1.32% H526Y. Similarly, for isoniazid, *katG* S315T1 covered 79.05% of total isoniazid resistance detected. These specific mutations were observed to be the most prevalent mutations detected by LPA across different regions. Singhal *et al* (2015) observed 59% prevalence of S531L mutation in *rpoB* gene and 88.3% prevalence of S315T1 in *katG* gene in New Delhi, India[18]. Aparna *et al* (2010) observed 40% of such mutation in *rpoB* locus in their study based in Hyderabad and Koraput in India[19]. Maurya *et al.* (2013) showed 62.3% and 93.3% prevalence of such mutations for Rifampicin and Isoniazid respectively in Northern India[20]. International studies showed mutation in the 315 region of *katG* was present in 93.3% of isoniazid resistant cases predominantly reported from Germany, Russia and other countries[21-24]. Sinha *et al.* (2020) reported 63.3% S531L mutation followed with 21.4% of D516V and 12.2% H526Y mutation for *rpoB* gene in a study carried out at Varanasi, India[25].

The presence of *inhA* mutations were observed in 19.37% of total Isoniazid resistance cases detected. In these, 2.37% have both *katG* and *inhA* mutation present. Singhal *et al* (2015) and other studies have similar findings over prevalence of *inhA* mutation[18]. The most prevalent mutation in *inhA* gene was observed as C15T and it covers 14.62% of total isoniazid resistant cases.

Conclusion

In the present era of precision medicine, it is of great importance to attain the knowledge of mutation patterns of Isoniazid and Rifampicin for all microbiologically confirmed TB patients. Different regimen could be prescribed based on the information of high and low level resistance of Isoniazid. The WHO has estimated that the COVID-19 pandemic and its associated effects could increase the TB burden by one million per year in the period 2020 to 2025. Both research and robust implementation of new findings are important for the National TB elimination programme to fight against TB. The present study is the first one to analyse the epidemiology of mutations associated with Rifampicin and Isoniazid resistance in the Eastern Bihar. With a huge sample size, the study concluded with the presence of S531L, S315T1and C15T as the most prevalent mutations for *rpoB*, *katG* and *inhA* promoter region respectively. These findings resemble with other National and International reports. However, the effect of common and uncommon mutations on the treatment with that particular drug with different doses could be the future prospect of research.

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Original Article

Comparative Analysis of Efficacy & Safety of Prostaglandin - Timolol Fixed Combination *versus* Adding Ripasudil to Prostaglandin in Primary Open Angle Glaucoma Patients with Insufficient IOP Control with Prostaglandin Analogue Monotherapy — An Open Label, Randomised Study

Sutapa Roy[1], Apala Bhattacharya[2], Nilay Kumar Majumdar[3], Anirban Dolui[4], Srijato Bhattacharya[5]

Purpose : To compare the efficacy & safety of either switching from topical Prostaglandin analogue monotherapy to topical Prostaglandin – Timolol fixed combination therapy or adding Ripasudil to Prostaglandin monotherapy in Primary Open Angle Glaucoma (POAG) patients with insufficient intraocular pressure control.

Methods : 36 POAG patients, experiencing insufficient IOP control while on a Prostaglandin analogue were enrolled for this study.The participants were divided into 2 treatment groups- PG/ Timolol fixed combination group (switched group) & Ripasudil 0.4% eye drop (added group). Blood pressure, IOP, pulse rate were measured at baseline, 2weeks, 1month, 2 months & 3 months of study. AP (24-2) test was done in all patients at baseline & at 3 months of the study. Data on adverse drug reactions & IOP were collected and analysed on first 3 months of treatment.

Results : In the switched group mean IOP after 3 months was 17.3±2.73 mm hg & 16.88±3.01mm hg in the added group, both of which were statistically significantly lower than baseline (switched group 19.55±3 mm hg & added group 19.29 ±3.13 mm hg). At 3 months IOP was reduced by 2.22±0.85 mm hg (11.38±4.04%) in the switched group & 2.41±0.77 mm hg (12.63±4.22%) in the added group. After 3 months of the study there was no significant change in systolic & diastolic BP & pulse rate in either of the groups. In the switched group 4 (22.22%) & in the added group 6 (35.29%) participants experienced adverse reactions.

Conclusion : In POAG patients on prostaglandin monotherapy with insufficient IOP control, adding Ripasudil was equally safe & effective to switching PG/ Timolol fixed combination in reducing IOP after 3 months of treatment.

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Key words **: Prostaglandin-Timolol fixed combination, Ripasudil, Intraocular pressure.**

Glaucoma is the second leading cause of blindness globally, accounting for 12.3% of the total blindness. WHO has estimated that glaucoma caused blindness to around 4.5 million people. In India, glaucoma is the leading cause of irreversible blindness with at least 12 million people affected and nearly 1.2 million people are blind from it[1]. Population based studies report a prevalence between 2 to 13%. Every eighth individual aged 40 years or above has glaucoma or is at risk in India. Among them, 74% have open angle glaucoma. From 2010 to 2020, the most detectable change in glaucoma worldwide will be an increase of the incidence of glaucoma in India.

Editor's Comment :

- Ripasudil is a new drug in the glaucoma armamentarium.
- With a novel mechanism of action and minimum systemic side effects it is showing promising results.
- It can be a safe & effective adjunct to Prostaglandin analogues in POAG patients.

Primary Open Angle Glaucoma (POAG) is the commonest form of glaucoma throughout world, accounting for about two-thirds of cases. Today for OAG, both pharmacologic and surgical treatment modalities are aimed at lowering IOP, which is the primary modifiable risk factor associated with glaucoma progression[2]. Despite the availability of the therapies and the newer surgical treatments of glaucoma, two variants of OAG—progressive POAG (despite achieving "target" IOPs) and NTG—are the main challenges. Progressive POAG is characterized by

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significant visual field loss and increased likelihood of glaucoma progression despite multiple medications, often combined with laser or surgical interventions.

The antiglaucoma agents act on the aqueous humor dynamics to reduce the intraocular pressure by various mechanisms. Miotics in angle closure glaucoma act by contraction of sphincter pupillae, which removes papillary block and reverses obliteration of the iridocorneal angle & in open angle glaucoma act by contraction of the ciliary muscle pulls on the scleral spur and improves trabecular patency. β-blockers and carbonic anhydrase inhibitors reduce aqueous humor secretion by the ciliary body. Prostaglandins increase uveoscleral outflow by changing the permeability or pressure gradients[3]. Prostaglandins reduces IOP by facilitating the aqueous outflow through the uveoscleral pathway. It is thought that they bind to the receptors of the ciliary body and upregulate the matrix metalloproteinases. By remodelling the extracellular matrix these enzymes make the area more permeable to aqueous humor, thereby increasing outflow[4].

Commonly prostaglandins are the drug of first choice but when a single therapy of prostaglandin is not sufficient to lower the IOP, a combined treatment is indicated. While selecting an agent for combination therapy, we should think of the drugs with complementary mechanisms of action. Fixed-combination products have the combined efficacy of two ocular hypotensive drugs and the convenience of a two-drug regimen in a single container, which is beneficial for treatment adherence. Studies show that around 30% patients on Prostaglandin require additive therapy. Compared to a prostaglandin monotherapy, the range of IOP reduction in various PG- Timolol combinations from baseline is around 13-37%[5].

Recently, new addition to these IOP-lowering drugs, Rho-associated protein kinase (ROCK) inhibitors are showing promising result. They work by changing the status of trabecular meshwork and Schlemm's canal endothelial cells. They inhibit the contractile tone of actin cytoskeleton, resulting in improvement in the conventional aqueous outflow. Because of these novel IOP-lowering mechanisms, different from the mechanisms of action of other anti-glaucoma drugs, ROCK inhibitors are showing promises to investigators. In addition they protect trabecular meshwork from oxidative stress, improve optic nerve head blood flow, facilitate corneal endothelial wound healing, increase ganglion cell survival & reduce scarring in glaucoma surgery[6].

Ripasudil (0.4%), a derivative of Fasudil, approved as an antiglaucoma agent for the first time in September, 2014 in Japan, is now available in Indian market also. It is a selective Rho-associated coiled-coil-containing protein kinase1(ROCK1) inhibitor, where ROCK1 is an important downstream effector of Rho guanosine trisphosphates (GTP), proteins that are significant in the contractile control of smooth muscle tissue. The S configuration at the 2 position on the 1,4-diazepane ring of Ripasudil is responsible for its characteristic effect. Ripasudil hydrochloride hydrate showed no binding affinity for receptors of the adrenergic, endothelin, angiotensin II, glutamate, histamine, muscarinic or prostanoid variety. It doesn't have any affinity for Ca+&K+ channels,HMG-Co A reductase, carbonic anhydrase. Ripasudil had no effect on respiratory or neurological function. It is believed to be non-carcinogenic due to its rapid elimination, as well as it lacks inflammatory response in the eye post-administration[7].

Ripasudil has shown IOP-lowering effects when used as monotherapy or in combination therapy with prostaglandin analogues or beta-blockers. In addition to it Ripasudil has a comparatively good safety profile in relation to the adverse drug reactions (ADRs). Commonly switching patients from PG to PG/Timolol FC therapy leads to a good patient compliance. There is proven literature support that both PG/Timolol FC eye drops and PG eye drops with Ripasudil (0.4%) eye drops cause safe & effective reduction of IOP.

Materials and Methods

This is a prospective, open-label, randomised comparative study investigating the safety and efficacy of either switching from topical PG analogue monotherapy to topical PG/Timolol fixed combination therapy or adding Ripasudil (0.4%) drops in patients with primary open angle glaucoma who had insufficient IOP control while on PGA monotherapy, done in a tertiary eye hospital in Kolkata, West Bengal. The total surveillance period for this study is from March, 2020 to August, 2020.

Patients were eligible to participate if they had primary open angle glaucoma & IOP control was insufficient with PG monotherapy for more than 3 months & had not previously received Ripasudil treatment. The data of interest included patient background, study treatment status, concomitant medication status, ocular surgery status, ophthalmic parameters (eg, IOP, visual field and corrected visual acuity and detailed slit lamp examination) and ADRs on all follow up visits. All the participants gave written informed consent before participation & the study was done in accordance with the tenets of the Declaration of Helsinki.

Inclusion criteria :

(1) Male & female patients aged 40-75 years of age

(2) POAG patients having insufficient IOP reduction after more than 3 months of treatment with PG analogue

(3) In cases where both eyes qualified for study inclusion, the eye with higher IOP was selected. If both eyes had same IOP, then the right eye was selected for the study.

Exclusion criteria :

(1) Patients having serious systemic illness (eg. Asthma, allergy, cardiac illness)

(2) Patients having history of drug hypersensitivity were excluded

(3) Subjects with advanced cases of Glaucoma, central visual field loss in either eye measured by perimetry

(4) Subjects who are blind in one eye

(5) Subjects who are scheduled to undergo any kind of eye surgery during the study period

(6) History of chronic, recurrent or severe inflammatory eye diseases (eg, scleritis, uveitis, keratitis) or current other severe ocular pathology such as diabetic retinopathy, retinal detachment, severe dry eye

(7) Subjects using the listed medications were excluded from the study- (a) oral antihypertensive agents, (b) systemic steroids or immunosuppressive agents, (c) high dose (>1 gm daily) of salicylates

In this study we included total 36 patients (20 men,16 women, total 36 eyes) with POAG who were being treated with PG analogue monotherapy administered daily at 9 PM at night. Among them 8 eyes Latanoprost, 10 eyes Travoprost and 18 eyes were using Bimatoprost as PG monotherapy and IOP reduction was insufficient after more than 3 months of treatment. The participants were randomly placed into one of the two groups -(1)switch from once daily PG monotherapy to PG/Timolol fixed combination therapy administered in the evening at 8 pm(switched group) or (2) addition of twice daily Ripasudil (0.4%) in the morning 8am & in the evening 8pm to once daily PG analogue monotherapy at 9 pm (added group).

In the switched group Latanoprost (0.005%) was replaced by Latanoprost (0.005%)/Timolol (0.5%) FC, Travoprost was replaced by 0.004% Travorost/0.5% Timolol FC & Bimatoprost 0.03% was replaced by 0.03% Bimatoprost/0.5% Timolol FC. In all participants only one eye was included in the study when the combination drop or Ripasudil was administered in both eyes. The eye with highest IOP at baseline was selected for study evaluation. If both the eyes had same IOP at baseline then the right eye was selected for evaluation.

At the baseline visual acuity of all patients were tested to rule out best corrected visual acuity worse than 0.6log MAR. Autoperimetry (24-2) was done to rule out the advanced case of glaucoma at baseline. It was also used as a safety assessment parameter & performed at baseline & at the end of the study at 3 months. Slit lamp biomicroscopy was done to assess the safety & side effects in all visits. Undilated fundoscopy was done during all follow up visits to rule out any fundus changes during the study. Goldman applanation tonometry was done in each visit by single observer to rule out observer dependant changes and at a definite time in the OPD (10 am to 12 pm) in all patients to avoid any diurnal variations. Gonioscopy was performed at the screening visit in all participants to rule out angle closure disease. All participants had their BP& pulse rate measured at each visit.

The number of adverse events & safety were examined & compared between the treatment groups at baseline, 3 weeks, 6 weeks & after 3 months of the study.

Statistical analysis :

ANOVA test was done to check the value of the data at baseline to find out any significant difference between the 2 groups. The IOP, BP & pulse rate were compared within groups by use of paired T- test & between the 2 groups by unpaired T test. The IOP reduction width & rate were compared between baseline & at 3 weeks, 6 weeks & 3 months after starting of treatment. Adverse drug reactions were examined & compared between 2 groups. P value lower than 0.05 were statistically significant.

Results

A total of 18 patients in the switched group & 18 patients added group were recruited in this study but there was 1 drop out in the added group, so total 18 & 17 patients were analysed in the switched group & added group respectively.

No significant difference was found between the two groups in age, male female ratio, number of pre-treatment medication & prostaglandin administration period, IOP, visual field mean deviation (decibel), systolic BP, diastolic BP, pulse rate at the baseline (Table 1, Fig 1).

In the switched group mean IOP after 3 months was 17.3±2.73 mm hg & 16.88±3.01mm hg in the added group, both of which were statistically significantly lower than baseline (switched group 19.55±3 mm hg & added group 19.29 ±3.13 mm hg). Mean IOP change noted after 3 months was 2.22±0.85 in the switched group & 2.41±0.77 in the added group & percentage change rate of IOP was 11.38±4.04 in the switched group & 12.63±4.22 which are statistically significant ($p<0.001$) but there was no statistically significant difference between the 2 groups in mean change of IOP & percentage change rate (Table 2, Fig 2).

At the end of 3 months study there was no significant change of systolic BP, diastolic BP & pulse rate in both the switched group & study groups, and the inter group difference was also not significant.

Table 1 — *Participants demographics & ocular characteristics*

Patient characteristics		Switched group	Added group	P value
Number		18	17	-
Sex ratio (M : F)		10 : 8	8 : 9	-
Age (Years)		61.22±7.62	64.65±9.12	0.25
Pretreatment medication	Latanoprost	4	3	-
	Travoprost	5	6	-
	Bimatoprost	9	8	-
Mean PG treatment period (months)		6.61±1.89	8.2±172	0.001
Mean IOP (mm Hg)		19.56±3.0	19.29±3.14	0.81
Visual field (Mean; MD, dB)		6.76±1.93	7.19±1.91	0.52
Mean Systolic BP (mm Hg)		127.56±9.27	127.18±9.68	0.91
Mean Diastolic BP (mm Hg)		79.67±3.54	77.18±4.66	0.92
Mean Pulse rate		79.11±6.05	75.41±5.30	0.07

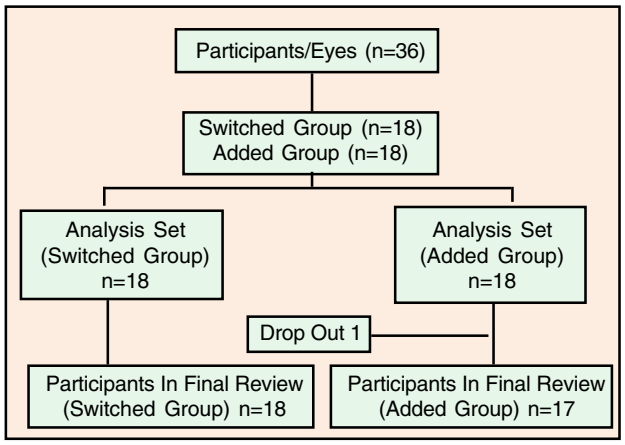


Fig 1 — Showing patient recruitment and randomisation

Systemic adverse events like palpitation, drowsiness, alteration of taste etc were also not statistically significant in our study participants in this interval. None of the study groups had any serious systemic side effects in our study (Table 3).

In the safety analysis total 4 patients had adverse reactions in switched group and 6 patients had adverse drug reactions in added group which was not statistically significant. Conjunctival hyperaemia was the commonest side effect in added group which was 23.53% which developed early after instillation of Ripasudil & resolved within few hours. However, in either of the groups, there was no discontinuation of treatment drugs due to any serious side effect (Table 4).

Discussion

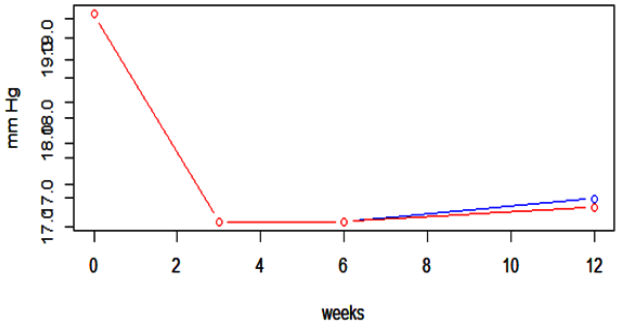
Ripasudil was first approved in Japan in 2014 for the treatment of glaucoma. There are many studies involving Japanese population showing efficacy of Ripasudil in various subtypes of glaucoma patients. Use of Ripasudil as adjunct may act via different mechanisms resulting in sufficient reduction of IOP without competition with other medicines. According to literature, it is the first drug to directly target the conventional outflow pathway. In the early phase (within few hours of instillation) it widens the trabecular meshwork by deforming the cytoskeleton of trabecular cells & loosening the intracellular junction within the schlemm's canal endothelium & in the late phase (within a few months of instillation) the extracellular matrix may reform , leading to reductions in tissue resistance[7,14].

Previously a 2 months study involving Japanese patients with POAG demonstrated that adjunctive treatment with Ripasudil resulted in significant reduction of IOP at the time of peak efficacy. Studies have reported positive effects from the use of Ripasudil combined with Latanoprost for treatment of POAG.

A one-year study on patients with POAG, ocular hypertension & pseudo exfoliation glaucoma in Japan revealed IOP reduction at the time of peak efficacy

Table 2 — *Efficacy evaluation – mean IOP reduction & change rate*

	Switched group	Added group	T test
Mean IOP change at 3 months(mm Hg)	2.22±0.85	2.41±0.77	P=0.25
Change rate%	11.38±4.04	12.63±4.22	P=0.19



Graph showing mean IOP (mm of Hg) of switched group & added group of patients from baseline to end of study (12 weeks)
[Blue : switched group mean IOP; Red: added group mean IOP]

Fig 2 — Combined plot of IOP change of 2 groups

Table 4 — *Adverse drug reactions*

Adverse reaction	Switched group	Added group	P value
Total no of ADR	4 (22.22%)	6 (35.29%)	0.19
Conj. hyperaemia	1 (5.56%)	4 (23.53%)	0.06
Blepharitis	-	1 (5.88%)	0.15
Allergic conjunctivitis	-	-	
Eye irritation	2 (11.11%)	1 (5.88%)	0.55
Eyelid pruritus	-	-	
SPK	-	-	
Palpitation	1 (5.56%)	-	0.16
Others	-	-	

was 3.7 mm Hg in patients with Ripasudil monotherapy & 1.7 to 3 mm Hg in patients having adjunctive therapy with PG & beta blocker[8].

Table 3 — *Blood pressure & pulse rate chart of the study groups*

Variables (Mean)		Baseline	After 3weeks	After 6weeks	After 3 months	P value
Systolic BP (mm Hg)	Switched group	127.56±9.27	128.89±6.97	130±8.11	127.78±10.48	P=0.07
	Added group	127.18±9.68	128.24±8.08	127.53±8.3	126.82±11.29	P=0.42
Diastolic BP (mm Hg)	Switched group	79.67±3.54	79.78±3.39	78.67±4.67	77.78±4.76	P=0.09
	Added group	77.18±4.66	77.76±4.66	76.35±6.07	78±6.25	P=0.21
Pulse rate (Bpm)	Switched group	79.11±6.05	80.06±4.22	77.56±5.23	77.44±5.23	P=0.13
	Added group	75.41±5.30	75.64±5.20	75.88±6.56	75.76±6.01	P=0.39

Three previous studies indicated Ripasudil had a significant IOP lowering effect even for POAG patients who were already undergoing maximal drug treatment[9,13].

When patients were divided in groups with respect to the time of IOP measurement, greater IOP reduction was observed in morning visit group nearly 1.9mm of Hg & no significant reduction in afternoon visit group. According to studies, IOP lowering effect of Ripasudil reaches peak level 2 hours after administration & then decreases to near trough level by 12 hour after administration[14]. This suggests chances of underestimation of efficacy of Ripasudil in clinical practice depending on time of IOP measurement relative to administration.

A previous study showed that 55.9% of the participants experienced conjunctival hyperaemia after initiating Ripasudil use, with other adverse reactions including ocular irritation, superficial punctate keratitis and nasopharyngitis[15].

In our study we measured IOP at a definite time of the day in each visit. Further studies should consider the measurement time relative to Ripasudil instillation for more appropriate evaluation of the efficacy. Our study group was small & only POAG patients were studied for 3 months duration & it is necessary to evaluate the IOP lowering effect of Ripasudil in long term, involving large number of patient of various glaucoma subtypes.

Ripasudil is showing a promising result as a new weapon in our glaucoma armamentarium & can be used effectively even in patients with systemic illness (eg, cardiac illness, asthma) as well as ocular comorbidities (eg, decompensated cornea, diabetic macular edema, uveitis etc) where other glaucoma medications are contraindicated.

Conclusion

From this study it can be concluded that switching from prostaglandin monotherapy to PG/Timolol fixed combination or adding Ripasudil therapy to prostaglandin are both equally safe & effective in control of IOP in short term in POAG patients with insufficient IOP control with prostaglandin monotherapy.

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Original Article

Report from a Trained Specialist Dependent ROP Screening Program in Two State Government Managed Special Newborn Care Units (SNCUs) of North Bengal

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Context : Retinopathy of Prematurity (ROP) is emerging as the leading cause of childhood blindness in India. Due to lack of specialists trained in ROP, many SNCUs in remote areas do not have a regular ROP screening program.

Aim : We organized a monthly, ROP screening program in two State Government managed SNCUs of North Bengal, to find the incidence of ROP and feasibility of running such a program.

Materials and Methods : A retrospective analysis of babies weighing ≤2000 g or ≤34 weeks of gestation screened from November 2017 till May 2020.

Results : Of the 508 babies screened 69 (13.6%) had ROP. 16 (3.2%) babies developed vision threatening ROP requiring Laser. The risk of any ROP increased with decreasing birth weight (Extended Mantel Haenszel Chi Square for linear trend: 15.4; p value: 0.00009). Of the pre-fixed 31 screening camps, only 19 (61.3%) could be conducted. Due to irregular screening, 180 (35.4%) of the babies underwent "Delayed screening" (first screening beyond 30 days of life). For these babies the odds of developing any ROP was 2.08 times (OR: 2.08; 95% CI: 1.25-3.48; p value: 0.002)higher and vision threatening ROP requiring Laser, 3.9 times (OR: 3.9; 95% CI: 1.1-13.7; p value: 0.015)higher, compared to those screened on time.

Conclusions : This is a first of its kind report from any SNCU located in North Bengal. It highlights the importance of regular ROP screening and also exposes the limitations of a screening program dependent on physical screening by a trained specialist.

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***Key words* : Retinopathy of Prematurity, Retina, Childhood Blindness, Special Newborn Care Units, North Bengal.**

Nearly 2% of total live births in India are infants with birth weight ≤2000 grams and gestational age ≤34 weeks[1]. All these babies are at risk of developing Retinopathy of Prematurity. Although the exact prevalence of ROP in India is not known, the incidence reported is between 22-52%[2,3]. In 20% of these babies have severe ROP and can go completely blind, if not diagnosed and treated at the right time[4]. With improved survival rate of premature babies across the country the number of infants who need ROP screening is bound to increase[5].

A premature child is not born with ROP[6]. Usually the disease develops over time. Hence, by proactively screening for ROP, one can detect the disease early and intervene[7]. Timely intervention with Laser can reduce the risk of progression of ROP to retinal detachment[8]. The National Guidelines for ROP screening mandates that all babies weighing ≤2000 g or ≤34 weeks of gestation need to be screened[9]. The guidelines also mandate that at least one screening should be completed within 30 days of life.

Ophthalmologists need to undergo special training to screen for and treat ROP. In a country like India with over 20,000 ophthalmologists, less than 150 actively practice ROP management[10]. The distribution of these specialists is also uneven. Though there are over 700 "Special Neonatal Care Units (SNCUs)" in India with one in nearly all district headquarters, most

Editor's Comment :

- This first of its kind study based on data from regular ROP screening program in government SNCUs of a district in North Bengal, highlights the urgent need to address the threat of blindness from ROP.
- Development of SNCU facilties have improved survival rates of premature babies. However, due to lack of ophthalmologists trained to do ROP screening in the periphery, many of those at risk are not being screened.
- To overcome this obstacle government needs to consider Retinal camera based tele-screening programs.

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of these units do not have regular ongoing ROP screening programme[11].

Though there are functional SNCUs under State government Health Services, in all the district towns in Northern part of Bengal, to the best of our knowledge none of them have an ongoing regular ROP screening programme. The Two-Government run SNCUs in (blinded) district have been an exception. Since 2017, regular ROP screening programme is being conducted at the SNCUs of (blinded) district hospital and (blinded) State General Hospital, jointly by a specialized private Retina institute in (blinded), licensed under the West Bengal Clinical Establishments (Registration, Regulation & Transparency) Rules, 2017 and the District Health & Family Welfare Samiti (DH & FWS), (blinded). We analysed the data from that screening program to assess incidence of ROP in the district and requirements for establishing such an operational screening programme in all the SNCUs of North Bengal.

Material and Method

This is a retrospective analysis of data from the monthly ROP screening programme carried out at SNCUs of (blinded) District Hospital and (blinded) State General Hospital, in (blinded) district.

One of the authors examined all eligible infants subsequent to detailed history, including birth weight, gestational age at birth and adverse events during stay in the SNCUs, using a binocular indirect ophthalmoscope and +20 D lens under topical anaesthesia using 2% proparacaine eye drops. The eyelids of the babies were separated with an infant wire speculum and a wire vectis was used as a scleral depressor. The pupils were dilated using 0.4% tropicamide +2.5% phenylephrine eye drops three times till full dilatation occurred. Any ROP if present was graded into stages and zones as per the International Classification of Retinopathy of Prematurity (ICROP)[12]. Repeated examinations were scheduled separately, for babies with any stage of ROP till the ROP completely regressed or reached high risk pre-threshold or threshold ROP; at which stage immediate laser treatment was conducted under topical anaesthesia, under anaesthetist supervision, using doubled-frequency Nd: YAG laser and Laser Indirect ophthalmoscope.

We abstracted data of all babies weighing ≤2000 g or ≤34 weeks gestation screened between November, 2017 and May, 2020, from the hospital records.

As Postconceptional age at birth could not be assessed reliably in all cases, we further analyzed the data based on the birth weight of the babies. The babies were accordingly grouped based on birth weight into three groups as Group A with birth Weight of ≤1000 g, Group B with birth weight of >1000g but <1500g and Group C with birth weight of ≥1500g to 2000g and assigned to those groups as number of babies with any ROP and number of babies with vision threatening ROP (Table 1).

We calculated the Chi square for linear trend for examining the linear trend in incidence of ROP in babies with reducing birth weight. We also calculated the Chi square for linear trend for examining the incidence of vision threatening ROP in babies with ROP with reducing birth weight. We used EpiInfo version 2007 for the data analysis.

We also calculated proportions and measured the association in terms of Odds Ratio (OR) for estimating the higher risk of ROP among those eligible babies, who underwent "Delayed screening" of beyond 30 days after birth as against those screenedtimely i.e. within 30 days of birth.

Results

We conducted 19 (61.3%) ROP screening against a planned and pre-fixed schedule for 31 camps during the study period (November'17 to May'20). Of the 12 missed camps, 10 (83.3%) were due to unavailability of the retina specialist and 2 (16.7%) due to the national lockdown consequent to the COVID-19 pandemic.

We screened 508 babies for ROP during the study period and detected ROP in 69 (13.6%) babies. Out of 508 babies screened, 328 (64.6%) babies were screened within 30 days of birth while 180 (35.4%) babies were beyond 30 days of birth. ROP was detected in 34 and 35 cases in among babies screened within 30 days of birth and beyond 30 days of birth, respectively.

Of the 69 babies with ROP, 53 (76.8%) babies had spontaneous regression of ROP, while 16 (23.1%) babies developed vision threatening ROP requiring laser treatment. All the babies treated with laser recovered. No one needed surgery or anti-VEGF injections.

We detected 8 (66.7%) cases of ROP in Group A, 25 (16.3%) in Group B and 36 (10.5%) in Group C. We also detected 6 (50%),13 (2.6%) and 6 (1.7%) vision threatening ROP in Groups A, B and C respectively (Table 1).

We estimated an Odds Ratio of 2.08 (95% Confidence Interval: 1.25-3.48; p value: 0.002) risk of ROP among the babies with delayed ROP screening compared to those with screening within 30 days of birth (Table 2).

We also estimated an Odds Ratio of 3.9 (95% Confidence Interval: 1.1-13.7; p value: 0.015) risk of developing vision threatening ROP requiring Laser among the babies with delayed ROP screening

compared to those with screening within 30 days of birth (Table 3).

We estimated the Chi Square for linear trend (Extended Mantel Haenszel) of 15.4 (p value: 0.00009) with respect to increasing trend of incidence of ROP in high risk babies with reduced birth weight (Table 4).

We also estimated the Chi Square for linear trend (Extended Mantel Haenszel) of 9 (p value: 0.0026) with respect to an increase in incidence of vision threatening ROP requiring laser with decreased birth weight (Table 5).

Discussion

Of the 508 babies screened during the study period, we detected ROP in 69 cases with an incidence of 13.6%. This incidence is lower than the incidence of ROP (22-52%) reported in other studies from India[2,3]. Based on ROP screening conducted in the SNCU of a Tertiary Care Hospital located in Southern India, Le *et al* have also reported a low incidence of ROP (2.3%) ascribing it to the proportion of "large babies" (33% had a birth weight of ≥1500g), in their study sample[13]. In our study too, 67.5% of the babies had birth weight between ≥1500g to 2000g, which may explain the lower incidence of ROP of in our series.

The incidence of ROP was not uniform among the three age groups in which we had divided the babies. Rather the incidence of ROP was found to be relatively higher with decrease in birth weights. Further, the risk of ROP cases developing vision threatening ROP requiring Laser therapy was also found to be higher with decrease in birth weights. Analysis for linear trend among high risk babies using Chi Square for trend showed statistically significant rise in incidence of ROP with reduction in birth weight, as well as, rise in vision threatening ROP among ROP cases with reduction in birth weight. Dhingra et al have earlier reported that the mean birth weight and gestational age of the infants who developed ROP were significantly lower than those who did not develop ROP[14]. The authors found that, compared to the birth weight category >1500 gms (which was the reference category), birth weight categories of ≤1000 gms and 1001-1250 gms independently had

Table 1 — *Overview of the babies screened for Retinopathy of Prematurity (ROP), any ROP babies and babies with visioning threatening ROP grouped birth weight wise, A## district, West Bengal, India, 2017 – 2020 (May)*

Weight at birth (gms)	# ROP screening within 30 days	# ROP screening beyond 30 days	# babies with ROP	# of babies with vision threatening ROP
< 1000	6 (1.2%)	6 (1.2%)	8 (66,7%)	6 (50%)
>1000-<1500	80 (15.7%)	73 (14.4%)	25 (16.8%)	4 (2.6%)
≥1500	242 (47.6%)	101 (19.9%)	36 (10.5%)	6 (1.7%)
TOTAL	328 (64.6)	180 (35.4%	69 (13.6%)	16 (3.2%)

Table 2 — *Measure of Association (Odds Ratio) between detection of ROP among babies with delayed screening > 30 days after birth, A##, West Bengal, India, 2017 – 2020 (May)*

Exposure	Outcome	ROP	NO ROP	TOTAL
	# Babies screened for ROP > 30 days after birth	35	145	187
	# Babies screened for ROP < 30 days of birth	34	294	328
Total		69	439	508

Odds Ratio: 2.08
95% Confidence Interval (CI): 1.7 – 3.8, p value : 0.0000538

Table 3 — *Measure of Association (Odds Ratio) between detection of vision threatening ROP among babies with delayed screening > 30 days after birth, A##, West Bengal, India, 2017 – 2020 (May)*

Exposure	Outcome	# Vision threatening ROP	# Any ROP	Total
	# Babies with ROP screened >30 days after birth	12	23	35
	# Babies with ROP screened < 30 days of birth	04	30	34
Total		16	53	69

Odds Ratio: 3.9
95% Confidence Interval (CI): 1.1 – 13.7, p value: 0.015

Table 4 — *Analysis of linear trend in babies of RDP with reducing birth weight, A##, West Bengal, India, 2017-2020 (May)*

Weight at birth (gms)	# High Risk Babies	RDP	No RDP	Odds Ratio
<1000	12	08	04	1
>1000-<1500	153	25	128	0.098
>1500	343	36	307	0.059

Chi Square for trend (Extended Mantel Haenszel) : 15.4
p value: 0.00009

Table 5 — *Analysis of linear trend in babies with any ROP of vision threatening ROP with reducing birth weight, A##, West Bengal, India, 2017 – 2020 (May)*

Weight at birth (gms)	ROP	Vision threatening ROP	No Vision threatening ROP	Odds Ratio
< 1000	08	06	02	1
>1000 - <1500	25	04	21	0.063
≥1500	36	06	30	0.067

Chi Square for trend (Extended Mantel Haenszel): 9
p value: 0.0026

increased risk of ROP.

In the West, treatable ROP is not seen in babies with birth weight ≥1500g[15]. Experts there do not recommend ROP screening in babies with birth weight ≥1500g, regardless of exposure to supplemental oxygen[16,17]. In contrast, cases of ROP, even of the aggressive variety; have been reported in babies with birth weight ≥1500g, in India[18]. As a result, the National Guidelines for ROP screening in India, mandates screening for all infants weighing ≤2000. Our study confirms the importance of following this guideline, as ROP was seen in 36(10.5%) of the 343 babies with birth weight ≥1500g to 2000g (Group C). 6 (1.7%) of these 343 babies progressed to vision threatening ROP and had to be treated with Laser.

With a recent Supreme Court of India judgment bringing ROP screening into the medicolegal focus, it has now become imperative for all neonatal units to provide for appropriate ROP screening[19]. However, there are few ROP trained specialists available in remote parts of the country, like the northern part of West Bengal, leading to lack of any ROP screening program in most SNCUs. Ideally the ROP screening program should be repeated on a weekly basis. As there was only one trained ROP specialist available to the group, we agreed to initiate the program by conducting at least one screening camp every month.

Of the 31 pre-fixed ROP screening camps, we could conduct only 19 camps mostly due to the unavailability of the retina specialist. Similar irregularity in screening has been noted in 1/3rd of the SNCUs in Mexico, due to unavailability of the screening doctor[20].

Irregularity in the screening schedule lead to delayed screening of 180 (35.4%) babies, who did not receive their first ROP screening within 30 days of life. The odds of detecting more ROP cases among the babies screened later than the mandated 30 days of birth was 2.08 times higher than in those screened within 30 days. Timely screening is of paramount importance to avoid blindness due to ROP. Delayed screening has been reported to be associated with a higher possibility of any stage of ROP or progression to vision threatening ROP[21]. Lack of timely screening has been also reported to be a major cause of stage 5 ROP blindness in a tertiary eye care setup from India[22].

Lack of alternate trained ROP specialists in the region covered by the study and remoteness of the study SNCUs from (blinded), (location of the Retina Institute) makes us believe that it is not feasible to successfully conduct a physical specialist dependent screening program regularly, over a long period. Given these facts, we highly recommend a technology driven, ROP fundus camera-based screening programme like the Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity (KID-ROP) programme[11]. The KID-ROP programme has successfully screened over 45,000 infants from 126 centres spread across Karnataka and treated over 2250 babies[23]. A possible objection to a KIDROP like model of ROP screening program in our region can be that, it requires significant financial and non-financial resources[24]. Such a model requires 3-4 trained ophthalmic staff, a specialised fundus camera and a laptop equipped with additional special software to record ROP images which are assessed by ophthalmologist elsewhere. However, in our mind these additional burdens are offset by the fact that the KID-ROP program was seen to have the scope of preventing blind-person years (BPY) accounting for over 200 million USD annually in ten states of the country[25]. Based on our experience we feel that the for a successful, sustained ROP screening programme in North Bengal a ROP-trained ophthalmologist should act as the central resource person. But he or she should not have to travel physically to all the SNCUs. Trained operators can visit the SNCUs under the programme, once a week, along with the ROP fundus camera and upload images over internet for grading by the trained specialist. This way we are unlikely to miss out most of the eligible neonates admitted in the SNCUs.

Our study had some limitations. Firstly, our study covered only two SNCUs out of the 11 present in the public sector in North Bengal and our findings may not be representative of the other districts. However, it does highlight the existence of the problem of ROP in the region, its underreporting and the highlights the risk of childhood blindness, associated with the near absence of any regular screening program in the region. Secondly, our program did not cover the privately managed SNCUs in (blinded) district, thus limiting our ability to give a true estimate of the incidence of ROP in the district. However, we have been able to highlight the problem of ROP in the region. Thirdly, a monthly screening program, as attempted by us is less than ideal. However, our intention was to provide some access to ROP screening, where none previously existed, in which we succeeded to some extent. Fourthly, we had only 12 babies (2.4%) in the extremely low birth weight group (Birth weight ≤1000g), which is low compared to other studies. However, this is explained by the fact that these extremely premature and sick babies either did not survive or were often referred by the paediatricians to the tertiary level government managed SNCUs at North Bengal Medical College, Siliguri or Coochbehar Medical College, Coochbehar due to lack of beds.

There are 11 SNCUs managed by State Government in the five Northern Districts (Darjeeling, Kalimpong, Jalpaiguri, Coochbehar and Alipurduar) of West Bengal. As per the data from Facility Based Newborn Care Cell of the State Health Department, West Bengal, 1951 premature babies (gestational age <34 weeks or birth weight ≤2000g) were admitted in the remaining 9 SNCUs, in 2019. Of these 139 babies had birth weight ≤1000g (Group A in our study), 496 babies had birth weight between >1000g-<1500g (Group B) and 1316 babies had birth weight between 1500g-≤2000g (Group C). Based on the incidence of ROP as revealed in this study, we estimate 312 babies to have developed ROP in 2019 of which 104 would have progressed to vision threatening ROP, requiring urgent intervention.In absence of any regular ROP screening program in these 9 SNCUs however, it is difficult to verify our conclusions.

This study, which is the first of its kind in the region, highlights the urgent need to develop a regular ROP screening programme covering all the SNCUs in North Bengal. It also highlights the urgent need to move away from a physical specialist dependent ROP screening program to a technology driven retinal camera based tele-screening program with the trained uploading of retinal images to be sent to the scarcely available retina specialist using tele-ROP platform and facilitating definitive interventions in specialised institutions with facility for interventions, including medical and laser managements.

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Original Article

Study of Serum Vitamin D Level in Patients Having Chronic Obstructive Pulmonary Disease

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Introduction : Chronic Obstructive Pulmonary Disease (COPD) is defined as a disease state characterised by airflow limitation that is not fully reversible. COPD includes both emphysema as well as chronic bronchitis. According to recent studies, there is a significant relation between Vitamin D levels and lung function.

Materials and Methods : A hospital based observational case control study of one year duration was conducted in the Department of Medicine, Silchar Medical College and Hospital with 100 COPD patients and 100 age and sex matched controls. A spirometry was performed on all patients, along with bronchodilator reversibility testing. A post-bronchodilator FEV_1/FVC of <0.7 confirmed the diagnosis of COPD as per the GOLD 2019 guidelines.Serum 25(OH) vitamin D was measured using auto-analyser ACCESS 2 immunoassay system.

Observations and Results : Mean age of patients in the cases and control groups were 60.4 and 60 years respectively Mean of serum vitamin D in cases (17.97ng/ml) was significantly lower than controls (24.28 ng/ml).The mean vitamin D levels in GOLD group 2,3 and 4 were 27.442 ng/ml, 18.890 ng/ml and 14.22 ng/ml respectively.

Conclusion : There is high prevalence of vitamin D insufficiency among COPD patients and more so amongst in patients with severe form of COPD. It is reasonable to conclude that deficiency of Vitamin D may be a factor in the development and progression of COPD and hence, replacement therapy of Vitamin D may be an effective public health intervention to improve vitamin D status in the population.

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Key words **: COPD, Vitamin, Case, Control, Patients.**

COPD includes emphysema, an *anatomically* defined condition characterized by destruction and enlargement of lung alveoli; and chronic bronchitis, *a clinically* defined condition with chronic cough and phlegm[1]. COPD is a preventable and treatable condition which is a disease of increasing public health importance around the world. Recent speculations suggest that COPD will rise from the sixth to the third most common cause of mortality world wide by 2020[2]. Vitamin D is a fat-soluble vitamin that regulates important number of body functions including calcium absorption, bone metabolism, neuromuscular function and immunity. Despite the classical role of vitamin D in skeletal health, new aspects of vitamin D have been discovered in tissues and organs other than bones. Vitamin D deficiency is defined as having serum levels of 25-hydroxyvitamin D of less than or equal to, 20 ng/ml. According to recent findings, the prevalence of vitamin D deficiency is between 33% and 77% among advanced COPD patients[3]. Lower levels of vitamin D in these patients may be explained by the reduction in cutaneous Vitamin D production by smoking and limited sunlight exposure. Other possible mechanisms include reduced Vitamin D production in liver and kidney and increased Vitamin D sequestration in adipose tissue.

Editor's Comment :

- It is concluded from the present study that low serum vitamin D is present in two third of the subjects with Chronic Obstructive Pulmonary Disease and more so amongst in patients with severe form of COPD.
- It is reasonable to conclude that deficiency of vitamin D may be a factor in the development and progression of COPD and hence, replacement therapy of vitamin D may be an effective public health intervention to improve vitamin D status in the population.

Aims of the Study :

(1) To assess the levels of serum Vitamin D in patients admitted with acute exacerbation of COPD.

(2) To correlate the values with severity of disease

Materials and Methods

This study will be conducted in the Department of Medicine Silchar Medical College and Hospital, Silchar, Assam.

Study Period : The study will be conducted among the indoor patients in the Department of medicine, Silchar Medical College over a period of one year from 1st June, 2018 to 31st May, 2019.

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Study Design : The present study will be a hospital based observational study.

Sample Size : All patients presenting with COPD in Medicine IPD and OPD, Department of Silchar Medical College over a period of one year from 1st June 2018 to 31st May 2019.

A detailed clinical history was taken and complete physical examination was done in all cases. A spirometry was performed on all patients, along with bronchodilator reversibility testing. A post-bronchodilator FEV_1/FVC of <0.7 confirmed the diagnosis of COPD as per the GOLD 2019 guidelines[2]. Severity of COPD was assessed using FEV_1% predicted values. The access 25-(OH) Vitamin D assay is a paramagnetic particle,chemiluminescent immunoassay for the quantitative determination of 25-hydroxyvitamin D levels in human serum using the Access 2 Beckman Immunoassay Systems.

The results for each parameter for discrete data are represented in numbers, percentages and average (mean, standard deviation) are represented for continuous data which are represented in Tables 1-3 and Figs 1-3.

Results

Out of the 100 cases of COPD and 100 controls, maximum number of subjects were seen in the age groups of 56-65 years (39/100 in case and 39/100 in control group). The next commonest age group was 66-75 years with 28% of the cases and 29% controls. The next commonest age group was 66-75 years with 28% of the cases and 29% controls. 26% of cases were between the ages of 46-55 years. The least number of subjects were seen between the ages of 36-45 years (7% cases and 7% controls). Mean age was 60.4 ± 8.7 years in COPD cases.

Out of 100 COPD patients, there were 40% patients with stage 4 GOLD severity, 32% patients with stage 3 GOLD severity and 28% patients with stage 2 GOLD severity.

The mean serum Vitamin D level was 19.9732±7.489 ng/ml among COPD cases and 24.84 ± 8.42ng/dl among controls. There was statistically significant difference in Mean (SD) of Serum Vitamin D levels between cases and controls (p value <0.05).

Table 1 — *Showing mean ±SD of serum Vitamin D levels in COPD cases and controls*

Subjects	Mean (SD) of Serum Vitamin D Level (ng/ml)	P value
Cases	19.973 ±7.489	<0.00001
Controls	24.98 ± 8.42	

Table 2 — Distribution of study groups based on serum Vitamin D levels

Serum Vitamin D levels(ng/ml)	Cases	Controls	P value
Deficiency (<20 ng/ml)	64(64%)	32 (32%)	0.0001
Insufficiency (20 to <30 ng/ml)	25(25%)	37(37%)	
Sufficiency (30-100 ng/ml)	11(11%)	31 (31%)	

Table 3 — *Comparison of Mean Vitamin D Level according to Gold Group among COPD Patients*

Gold Groups	No of Patients	Mean Vitamin D Level (ng/ml)	SD	P Value
Group 2	28	27.442	7.975	<0.00001
Group 3	32	18.890	4.448	
Group 4	40	15.555	4.717	

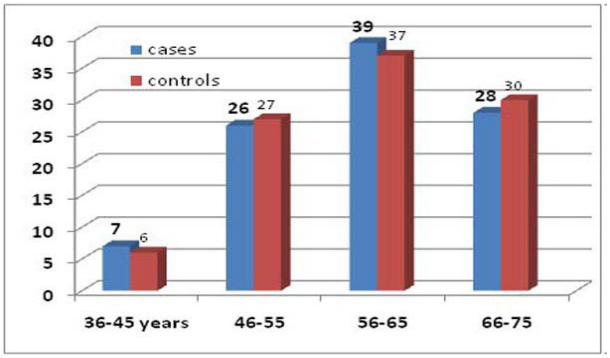

Fig 1 — Bar chart showing the Age distribution of the patients

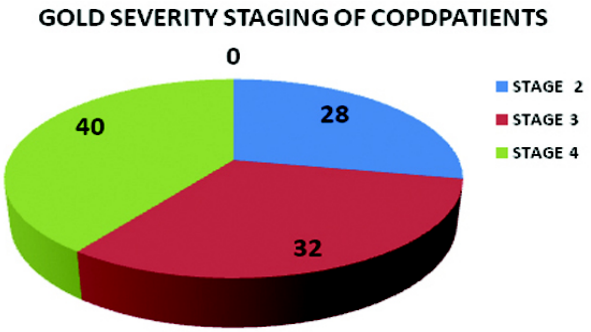

Fig 2 — Pie chart showing the GOLD staging of COPD patients

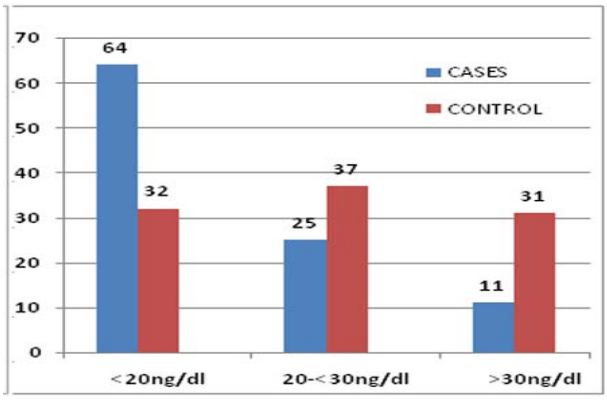

Fig 3 — Bar diagram showing distribution of study groups based on serum Vitamin D levels

The above table shows that 64% COPD cases were Vitamin D deficient (<20 ng/ml) in comparison to 32% subjects in control group. Vitamin D insufficiency was seen in 25 % cases and 37% controls whereas vitamin D sufficiency was seen in 11% COPD cases and 31% controls. The final result was also statistically significant (p value <0.05).

The above table shows that 28% of COPD patients were in GOLD group 2, 32% patients were in group 3 and 40% patients in GOLD group 4. The mean vitamin D levels in Gold group 2,3 and 4 were 27.442 ng/ml, 18.890 ng/ml and 15.555 ng/ml respectively and the results were statistically significant with p value <0.0001.

Discussion

Many similar studies have demonstrated significantly lower serum 25(OH) vitamin D level in people with COPD. Janssens *et al*[3] in Belgium in their study observed that Serum Vitamin D level was 19.9 ± 8.2 ng/ml in COPD cases and 24.6 ±8.7ng/ml in controls. It was observed that 51.9% of COPD patients were having vitamin D deficiency as compared to only 30.92%in the control group.

In the study by Duckers *et al*[4] in 2011, prevalence of vitamin D deficiency was observed in 80% of COPD patients compared to 20% in healthy control group. Mean vitamin D levels were significantly lower (mean 11.4±1.9 ng/ml) in COPD patients than in control groups (16.1±1.4 ng/ml).

Zhou *et al*[5] in their case control study in China in 2012 observed vitamin D deficiency in 94.3% of COPD cases and 84.19% of controls. Also, there was a significant difference in the mean of serum vitamin D levels between COPD cases and controls (12.86±4.3 in cases and 14.34±4.97 in controls, p value<0.0001). Sanket S *et al*[6] in his study in 2015 also reported similar results. In that study,mean concentration of 25-(OH) vitamin D was 22.24 ng/ml in COPD cases which was significantly low compared with control group whose mean concentration of 25-(OH) vitamin D was 26.25 ng/ml. in the study done by Kumar *et al*[7] where the mean serum vitamin D level was highest in GOLD group A patients (59.33± 15.51ng/ml) and lowest in GOLD group D patients (17.81± 8.74ng/ml). There was statistically highly significant negative correlation between GOLD group and mean serum vitamin D. In the study done by Gupta *et al*[8] where the mean serum vitamin D level was highest in patients with mild COPD (27.73± 2.72ng/ml) and lowest in patients with very severe disease (9.65± 4.57ng/ml) and the results were statistically significant.

Limitation : The study was a hospital based study with a small sample size conducted over a limited period of 1 year. So, to gather more detailed information regarding serum vitamin D status in COPD patients, we need a broader study covering a larger number of patients over a longer time period. Our study did not take into account few confounding factors like dietary habits and parathyroid hormone levels.

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Conflict of Interest : None

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Original Article

A Comparative Assessment of the Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in patients with Rheumatoid Arthritis in a Tertiary Care Hospital

Sandip Ghosh[1], Sangeeta Das Ghosh[2], Atanu Chandra[3], Jyotirmoy Pal[4]

Introduction : Rheumatoid arthritis is a chronic, systemic inflammatory autoimmune disease that affects a variety of tissues and most commonly attacks the joints. Autoantibodies such as anti-cyclic citrullinated peptide antibodies and rheumatoid factor are useful diagnostic tools.

Objective: As modern therapy of Rheumatoid Arthritis (RA) focuses on aggressive aggressive initiation of the Disease-Modifying Antirheumatic Drugs (DMARD) and biologics to limit the joint destruction, diagnostic tests with high specificity are preferred. Using a synthetic peptide design, a new serologic test (anti-cyclic citrullinated peptide or anti- CCP antibody) measured by Enzyme-Linked Immunosorbent Assay (ELISA) was designed to detect the presence of antibodies directed against citrullinated peptides.

Methods : Among the 200 cases with a history of polyarthritis included in the study, 133 individuals are clinically diagnosed with rheumatoid arthritis. The control group consisted of 67 patients with recent onset undifferentiated polyarthritis. Individual sensitivity and specificity of the aforementioned tests, as well as the combined specificity of the two tests and the three tests, were computed and the test results were compared to see whether there was any association.

Results : Among the 133 patients with Rheumatoid Arthritis, Anti-cyclic Citrullinated Peptide antibodies were found in 94 patients (70.7%), while rheumatoid factor antibodies were found in 61 patients (66%). Anti-cyclic citrullinated Peptide antibodies have sensitivity, specificity, positive predictive value and negative predictive value of 70.76%, 85.07%, 90%, and 59% respectively, for diagnosing Rheumatoid Arthritis. In case of Rheumatoid Factor, the values were 66.26%, 90.29%, 90% and 45% correspondingly. Using both RA and non-RA sera, the Anti-CCP ELISA established as an exceptionally specific (98%) and sensitive (88%) tool. At ideal cut-off levels, the Anti-CCP ELISA demonstrated a considerably greater specificity than the IgM Rheumatoid Factor (IgM-RF) ELISA (96 % for CCP *versus* 91% for IgM-RF).

Conclusion: The anti-CCP ELISA could be highly effective for diagnosing and treating recent onset Rheumatoid Arthritis. When this test is used in conjunction with the RF IgM ELISA and the Latex test, the combined specificity can reach up to 99%.

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Key words **: Anti-CCP Antibody, Rheumatoid Factor, Latex Agglutination Test, Enzyme Linked Immunosorbent Assay.**

Rheumatoid Arthritis (RA) is a chronic inflammatory disease which is mainly associated with a symmetric pattern of polyarthritis mainly involving the small joints of the upper extremities[1]. It is the commonest variety of chronic inflammatory arthritis and if not properly treated, may lead to deforming arthropathy. The estimated prevalence of RA is about 1% worldwide[2]. The recent management of RA is mainly focused on the early and aggressive initiation of the Disease-Modifying Antirheumatic Drugs (DMARD) and biologics to limit the joint destruction and also to prevent the development of the extra-articular complications. As most of such drugs are associated with several adverse reactions, therefore, proper use of specific diagnostic tests for RA is of utmost importance[3]. The diagnosis of this auto-immune

Editor's Comment :

- Rheumatoid arthritis is the commonest form of chronic arthritis and needs early diagnosis to prevent complications.
- Anti-CCP antibody measurement, whenever combined with rheumatoid factor, enhances the diagnostic accuracy.
- Anti-CCP antibody much more specific than RF and higher value of this antibody indicates more severe disease.

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disease is chiefly based on the inflammatory nature of the joint pain mainly involving the small joints of hands in a symmetric distribution with relative sparing of the distal inter-pharyngeal joints and elevation of the biochemical markers of inflammation combined with the presence of high titre of Rheumatoid Factor (RF).

Rheumatoid Factor is an Immunoglobulin-M (IgM) type of antibody directed against the Fc fraction of Immunoglobulin-G (IgG)[4]. Elevated levels of RF are seen in about 75-80% of the patients of RA, therefore, a negative result can not negate the possibility of RA. It is also found in 1-5% of the healthy population and in several other connective tissue diseases, such as Sjogren syndrome, systemic lupus erythematosus, mixed connective tissue disease, type-2 essential mixed cryoglobulinemia; and also in some chronic infective conditions like Hepatitis-B and C induced chronic liver disease and subacute bacterial endocarditis[5]. Furthermore, several studies are going on to differentiate "pathological" and "physiological" RF. This lack of sensitivity and specificity limits the usefulness of this serological marker of RA and necessitates the use of some other useful marker. Presence of serum anti-CCP antibodies has almost same or slightly higher sensitivity than RF, but the specificity is as high as 95%[6]. Therefore, its presence in early inflammatory arthritis helps to differentiate RA from the other chronic inflammatory conditions. According to recent data, Anti-CCP had an excellent specificity and a relatively high sensitivity for RA, especially for recent onset RA[7,8].

A hospital-based comparative study was conducted to determine the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor in patients with Rheumatoid Arthritis and the study results were also compared with a group of patients of undifferentiated arthritis.

Materials and Methods

Institutional ethics committee approval was obtained for this observational study. All patients were selected consecutively from outpatients being treated at the Calcutta National Medical College and Hospital. Serum samples were obtained from 133 patients with RA and 67 patients of Undifferentiated Polyarthritis (UPA) (157 women and 43 men, mean age: 56.7 years, range: 24–83 years, mean duration of the disease ± SD: 8.2 ± 10.5 years).

Approximately, 4-5 ml of venous blood was collected by aseptical methods from the patients of diagnosed RA and also from the control group. Serum was separated by appropriate technique (centrifugation) and vials were also marked carefully. Those samples were being stored at -80ºC until the assay. Presence of Anti-CCP antibodies was detected by Enzyme-Linked Immunoassay (ELISA), by using a commercial kit. Detection of Rheumatoid Factor (RF) was performed by Latex Agglutination slide test. A comparison between the results of those laboratory tests between study group and control group was performed by Chi- square test or the Fisher's exact test wherever applicable. Categorical variables are expressed as number and percentage of patients; they are compared across the groups by using Pearson's Chi-Square test for determining independence of Attributes. Continuous variables have been expressed as Mean ±Standard Deviation and they are compared across the 2 groups using the Mann-Whitney U test since the data does not follow normal distribution. Statistical software SPSS version 20 has been used for the complete analysis. An Alpha level of 5% has been taken, ie, if any p value is less than 0.05 it has been considered as significant.

Results

Presence of Anti-CCP antibodies were detected in 94 out of 133 patients with RA (70.7%) and RF was detected in 61 out of 133 patients with RA (46%). Of the 133 RA patients, 48 were Anti-CCP positive/RF positive, 46 were Anti-CCP positive/RF negative, 13 were anti-CCP negative/RF positive and 26 were anti-CCP negative/RF negative. In the group of 67 patients, of undifferentiated Polyarthritis (uPA) both Anti-CCP and IgM-RF tested was performed 6 were Anti-CCP positive/RF positive, 8 were anti-CCP positive/RF negative, 22 were Anti-CCP negative/RF positive and 31 were Anti-CCP negative/RF negative (Table 1).

Discussion

The clinical diagnosis of RA is largely based on the presence of chronic inflammatory Arthritis with suggestive laboratory and radiologic abnormalities. The 1987 classification criterion for RA proposed by the American College of Rheumatology (ACR) was revised in 2010 by a collaborative effort between the ACR and European League Against Rheumatism (EULAR)[9]. In this revised criteria proposed by the ACR-EULAR, there

Table 1 — *Test results at optimal cut-off values for the Anti-CCP and the IgM-RF ELISAs in patients with and without RA**

	No (%) of RA patients (n=133)	No (%) of undifferentiated Polyarthritis (uPA) patients (n=67)
CCP positive and		
RF Positive	48(36.09)	6(8.95)
RF Negative	46(34.6)	8(11.94)
CCP Negative and		
RF Positive	13(9.8)	22(32.83)
RF Negative	26(19.54)	31(46.26)

*The optimal cut-off value for the Anti–cyclic citrullinated peptide (Anti-CCP) enzyme-linked immunosorbent assay (ELISA) was 52 units; that for the IgM rheumatoid factor (IgM-RF) ELISA was 10 units. RA = rheumatoid arthritis.

is a point for positive Anti-citrullinated Peptide (anti-CCP) antibodies.[10] This diagnostic test is much more specific than the widely used RF. The use of both of these markers may add some incremental value in diagnosing RA, as some patients of RA may be positive for anti-CCP antibodies but negative for RF and vice versa. Therefore, Anti-CCP antibodies should not be an alternative to RF for the serological diagnosis. Moreover, Anti-CCP antibodies estimation is helpful for prognostication, as higher level of this antibody in patients with RA indicate worse outcome[11].

Our study results indicated that Anti-CCP positivity correlated with RF positivity. However, there are a significant proportion of patients in our study who were negative for RF but positive for Anti-CCP antibody and vice versa.

Saraux A *et al* conducted a study to compare the diagnostic values of serological markers such as Anti-keratin Antibody (AKA), Anti-perinuclear Factor (APF), and anti-CCP antibodies for determination of diagnostic value of anti-CCP when performed alone or combined with other markers; and to diagnose Rheumatoid Arthritis (RA) by using it[12]. They found that Anti-CCP (with a cut off value of 53 UI was 93% specific and 47% sensitive. On the other hand, the sensitivity of RF by Latex test was only 45%.

Another study conducted by Binesh F *et al* found the sensitivity of RF Latex test of only 46%, whereas the specificity was more than 90%[13]. Similar results were seen in another study conducted by Aflaky E *et al*[14]. They found the sensitivity of Anti-CCP, Citrullinated Protein Antibodies (CPA), and RF of 82, 83 and 61%, respectively; whereas specificities of those markers were 91, 79 and 90%, respectively.

Swedler W *et al* found that, the sensitivity and specificity of RF by ELISA was 91 and 75% respectively[15]. Slightly decreased sensitivity and higher specificity of RF IgM ELISA was observed in another study conducted by Bas S *et al*[16].

The sample size of both the disease and the control arm were small. Due to relatively smaller sample size, randomization could not be performed. Our study findings indicate that, combining RF with anti-CCP antibody may provide a much higher sensitivity without compromising specificity for the diagnosis of RA.

Conflict of interest : None of the researcher has any financial or other interest in the products that were used for this study.

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Original Article

Safety and Efficacy of Rituximab in Ankylosing Spondylitis — A One Year Prospective Clinical Study

Kripasindhu Gantait[1], Shinjan Patra[2], Rajdip Chowdhury[3]

Purpose of the study : Ankylosis Spondylitis (AS) has got very few therapeutic options limited to Non-steroidal Inflammatory Drugs (NSAID's) and biologics such as inhibitors of the tumour necrosis factor (TNF)-alpha; and additionally immunomodulators like methotrexate and sulfasalazine are therapeutic options in AS with predominantly peripheral joints involvement. Presence of abundant CD20+ cells in the histopathology specimens of AS was the main reason why we had chosen rituximab as the immunosuppressant in our study.

Study design : The modified New York Criteria was used as the diagnostic criteria for AS in our study. Patients fulfilling the criteria of AS with the disease duration of more than 10 years and those with evidence of active tuberculosis were excluded. We administered 2 doses of injection rituximab at 14 days interval (methyl-prednisolone was used as pre-medication in all cases) and patients were periodically followed-up till 48 weeks. We also monitored all the possible clinical and laboratory parameters for the assessment of its efficacy and also the possibility of any adverse drug reaction during the time-frame.

Results : Total fifteen patients (13 male and 2 female; 12 with predominantly axial involvement and 3 with predominantly peripheral involvement) were included in our study and all the parameters including the inflammatory ones had a significant improvement such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) which showed an improvement of more than 50% in comparison to the pre-treatment values among all the groups. No significant major side-effects are observed in all these patients.

Conclusions : Inhibitors of TNF-alpha, although a very efficient immunomodulating agent, has several adverse effects such as infusion-related adverse-reactions and reactivation of latent tuberculosis. Existing literature regarding the usage of rituximab as an immune-suppressant instead of TNF-alpha inhibitors are sparse and we are most probably studying it for the first time in such manner with a year-long observation. Rituximab in this study was found to have a great promise in terms of safety and efficacy.

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Key words **: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), CD20+ cells, Latent Tuberculosis, Tumour Necrosis Factor (TNF) alpha inhibitors.**

Ankylosing spondylitis (AS) is the prototype spondyloarthropathy and it has some unique characteristics that includes only a few therapeutic options. Axial skeleton is primarily involved in AS sometimes along with variable involvement of peripheral joints. It is also associated with some extra-articular manifestations like unilateral uveitis[1]. There is a markedly high association of AS with the histocompatibility antigen HLA- B27 and the global incidences are directly proportional to the prevalence of this histocompatibility antigen. Non-steroidal anti-inflammatory drugs (NSAID) are being used as the primary therapeutic option for a pretty long duration and it has shown some disease-modifying activity also[2]. Inhibitors of Tumour necrosis factor alpha (TNF-alpha) have revolutionized management of symptomatic AS in terms of halting the natural progression[3]. Additionally sulfasalazine and methotrexate may be of some useful value especially when peripheral arthritis is significant. Cartilage and bone surface is the primary site of inflammation and resultant degradation in AS[4]. Presence of abundant CD20+ cells in the histopathology specimens of AS was the main reason why we had chosen rituximab as the immuno-

Editor's Comment :

- Spondyloarthropathies have only a few therapeutic options, and the medications with proven benefit are mostly associated with various adverse effects.
- TNF-alpha inhibitors have several adverse effects such as infusion-related adverse-reactions and reactivation of latent tuberculosis.
- Rituximab may be a good therapeutic option in patients with AS, in terms of the efficacy and safety

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suppressant in our study[5]. Different adverse effects of TNF-alpha inhibitor therapy mainly reactivation of latent infections such as tuberculosis may be a valid reason to introduce a different class of drug for AS[6]. Only a few literature has highlighted about the efficacy, safety and tolerability of rituximab in AS. A few case reports have shown some kind of efficiency and control of the inflammation in patients treated with rituximab[7,8]. In this study we have not only monitored various clinical parameters of AS, but also we have assessed the global health scenario and working ability of this patient after treatment.

Materials and Methods

Our study area was Rheumatology Clinic and General Medicine Ward of Midnapore Medical College & Hospital. We included the patients of ankylosing spondylitis (AS) who were on conventional therapy {NSAID's and disease modifying anti rheumatoid drugs (DMARDs)} but inadequately responding with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) of more than 5. We excluded patients who underwent TNF-alpha inhibitor therapy for AS or patients suffering from serious co-morbid conditions like chronic liver disease, chronic renal disease, congestive cardiac failure etc. Immunocompromised patients due to any cause (leukemia, organ transplantation etc) were excluded from our study. Patients with active tuberculosis on anti-tubercular therapy or chronic hepatitis B or C were not included also. We didn't give rituximab in those AS patients with severe deformity or disease duration of more than 10 years. We got the ethical clearance for this study from the institutional ethics committee.

We had clinically examined the patients for assessing the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Metrology Index (BASMI) after obtaining written informed consent. We then did blood investigations-like complete hemogram, liver function tests, creatinine, HBsAg, Anti-HCV, HIV, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Roentgenogram of chest (postero-anterior view) was also obtained. Global assessment of the patient was made using the ASDAS-CRP scoring index (Ankylosing Spondylitis Disease Activity Score- C Reactive Protein) which takes account of clinical as well as biochemical values. This score correlates well with working capacity of an AS patient[9]. Magnetic Resonance Imaging (MRI) of the sacro-iliac (SI) joint was performed for assessing the presence of sacro-ilitis and also the extent of inflammation. We had put our patients on two subsequent infusions of rituximab (375 mg/m^2 each) at an interval of 14 days pre-medicated with 80 mg of methylprednisolone. Then we followed those patients up at week 4, 8, 16, 32, 48 and measured the ESR, CRP, BASFI, BASDAI, BASMI, and ASDAS-CRP score each time and put those scores in a tabular form. Flare may be defined as a worsening of 1.5 point of BASDAI score in comparison to the previous day score. More than 20% improvement in the disease activity as per the assessment of Spondyloarthritis international society criteria (ASAS20) was considered as having a significant response[10]. Fortunately, none of our study patients had any flare-up reactions, so we followed them up-to 48 weeks as per the standard protocol. We did the routine blood investigations in each follow-up to detect any systemic organ involvement, ruled out any systemic infections and assessed the vital parameters also. As we have successfully completed the follow-up of 15 patients (13 were male, 2 were female), we plan to extend our study to 50 total patients in upcoming times.

Results

The non-parametric Wilcoxon signed rank test was used to compare changes between baselines to after-treatment values. In these 15 patients, 12 patients suffered from axial-predominant AS, rest of them had peripheral-predominant AS. Study subjects in various age-groups were evenly distributed but we got peripheral-predominant AS in younger age group only. Subjects were mostly from rural areas and they lost their earnings after the disease onset. Apart from predominant sacro-iliac joint involvement, hip-joint was also involved frequently causing severe disability. Characteristic extra-articular features were associated with axial-predominant AS (Table 1).

The most significant finding from this observational study is the marked reduction of these indexes in the 13 male patients just after 4 weeks of two doses of rituximab (Table 2). All these scores have reduction of 66%, 56% and 66% respectively from pre-treatment values. Now for the females BASDAI, BASFI, BASMI scores reduced 81%, 59%, 56% respectively from pre-treatment values with an initial delay (Fig 1). Inflammatory markers decreased steadily and got undetectable from 16 weeks after treatment. The ASDAS-CRP score reduced to 62% and 74% respectively improving there working capacity and all of them got back to their jobs now. We have also compared the improvements in the clinical indexes between axial and peripheral predominant AS groups, where we didn't get any significant differences (Table 3).

Table 1 — *Demographical, socio-economical and basic clinical profile of all fifteen (15) subjects*

Features	Axial-predominant AS		Peripheral-predominant AS	
	(n=12)	(%)	(n=3)	(%)
Age (18-60) :				
18-25	5	41.6	3	100
25-40	5	41.6	0	0
40-60	2	16.7	0	0
Sex Distribution :				
Male	10	83.3	3	100
Female	2	16.7	0	0
Locality :				
Rural	11	91.7	3	100
Urban	1	8.3	0	0
Loss of paid jobs (2 Females were home-maker)	9 (out of 10)	90.0	3	100
Joint where the inflammatory activity is most prominent				
Sacro-iliac (SI)				
Hip-joint	10	83.3	NA*	
Knee-joint	2	16.7	NA*	
Ankle-joint	NA*		2	66.7
	NA*		1	33.3
Extra-articlular manifestations				
Unilateral uveitis				
Inflammatory Bowel				
Disease (both symptomatic	3	25.0	0	0
& asymptomatic)	1	8.3	0	0
Psoriatic skin changes				
Aortic insufficiency				
Renal Involvement	1	8.3	0	0
	1	8.3	0	0
	1	8.3	0	0

*NA- Not Applicable

Last but certainly not the least we have seen the reduction of sacro-ilitis on MRI SI joint of these patients after 16 weeks of rituximab treatment. MRI detected bone marrow edema in sub-chondral bone by showing us strong contrast enhancement, which is most specific sign of active sacro-ilitis in AS.

These fifteen patients didn't have any major side effects like infusion related toxicities or hypersensitivity reactions and there were no reactivation of any chronic infections. Broncho-constriction was aggravated in one patient with obstructive airway disease while rituximab infusion which was relieved on inhaled broncho-dilators and steroids. One patient had an episode

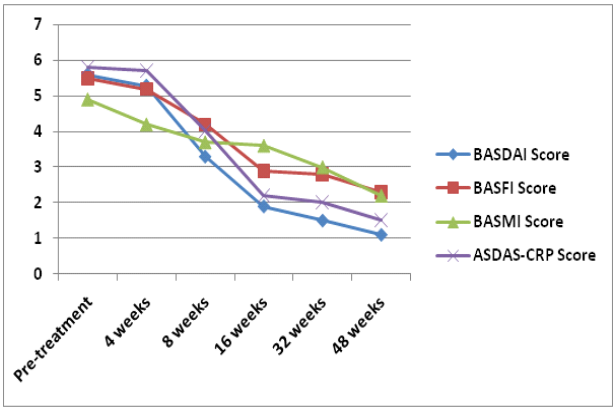

Fig 1 — Graphical representations of various AS indices values in 48 weeks of follow-up in 2 Female patients

of chills and rigor following rituximab infusion which was subsequently managed by anti-histaminics and it didn't repeat in subsequent dosing. After 48 weeks of follow-up they didn't complaint any specific problems except some epigastric discomfort which is probably attributed to prolonged use of NSAID's. Their blood investigations also didn't show any kind of abnormality in each follow-up visits.

Discussion

Some other forms of therapy, apart from NSAID's and TNF-alpha inhibitors, have been postulated in AS considering the diverse pathogenesis such as- Oadanacatib (a cathepsin K antagonist), Bevacizumab (antibody against vascular endothelial growth factor), Denosumab (a monoclonal antibody targeting RANKL)[11]. Song *et al* investigated the efficacy of rituximab in 20 AS patients who had inadequate response to TNF-alpha inhibitors, where at the end of 24 weeks rituximab was not effective in those patients[12]. Rodriquez administered rituximab to a

Table 2 — *Parameters of 13 Male patients after 48 weeks of follow-up*

Various Indices	Pre-treatment (Mean)	4 week (Mean)	8 week (Mean)	16 week (Mean)	32 week (Mean)	48 week (Mean)
BASDAI	6.7	3.9	3.7	2.8	2.7	2.3
BASFI	6.5	4.8	4.2	3.8	3.7	2.9
BASMI	4.5	3.75	3.5	2.5	1.5	1.5
ESR/CRP	82/56	54/26	30/18	28/08	22/Neg	20/Neg
Global assessment score (ASDAS-CRP)	5.2	4.2	3.8	2.6	2.4	2.0

Abbreviations :
AS- Ankylosing Spondylitis
BASDAI- Bath Ankylosing Spondylitis Disease Activity Index
BASFI- Bath Ankylosing Spondylitis Functional Index
BASMI- Bath Ankylosing Spondylitis Metrology Index
ASDAS-CRP- Ankylosing Spondylitis Disease Activity Score- C Reactive Protein
ESR- Erythrocyte Sedimentation Rate (measured in millimeter/hour)
Neg- Negative (below 6 mg/L)

patient with chronic hepatitis B and that patient had a very good response without any flaring up of the secondary infection[15]. According to the data from the French Rheumatology Society, the effect of rituximab was assessed retrospectively in 26 patients of spondyloarthropathy. Out of 26, 11 of them had a very good response, 8 of them were TNF-alpha naïve, 3 of them were non-responders[14]. Some studies have also showed that rituximab is a very good choice for those patients who could not have TNF-alpha inhibitors due to contra-indications. Still there is a huge lacuna of knowledge regarding this effective option. No study with the primary use of rituximab and follow-up of 1 year has showed the efficacy profile in patients of AS. As we have already discussed the pivotal role of CD20+ B cells in the pathogenesis of AS, rituximab is expected to play an important role in the treatment of both types of AS (with axial and peripheral involvement). This study on these 15 patients (including both male/female and axial/peripheral) with a 48 weeks of follow-up revealed a significant reduction in BASDAI, BASFI, BASMI, ASDAS-CRP scores, inflammatory markers and most importantly global working capacity improvement. The principal adverse effects and hazards of TNF-alpha inhibitor therapy such as the reactivation of latent tuberculosis were not noted in these cases. Another important point of our study is that we had given rituximab in weight dependent dosing (375 mg/m^2) in contrast to the previously used fixed-dose regimens (1000 mg) published in different literatures. That's how we have reduced the unnecessary extra cost of this medicine which is very important in a developing nation like India. As our follow-up of fifteen patients' up-to 48 weeks have been completed, we hope to extend our study to fifty patients to get a comprehensive picture of this therapy. Principal limitation of this study remains the small sample size. Also the improvements in terms of immunological mechanism haven't been studied that much in our study. But as per our data of these 15 patients we can think of rituximab as a safe and effective immuno-suppressant in a patient of AS without introduction of TNF-alpha blocker therapy.

Table 3 — *Parameters of improvement in Axial/Peripheral type of AS*

Axial predominant AS			Indexes	Peripheral predominant AS		
48 week	16 week	Pre-treatment		Pre-treatment	16 week	48 week
1.9	2.5	7.1	BASDAI	5.2	1.8	1.3
2.5	3.6	6.8	BASFI	5.2	3.2	2.1
2.1	2.7	4.8	BASMI	3.2	2.3	1.9
14/Neg	26/10	96/68	ESR/CRP	65/36	18/Neg	15/Neg
2.2	2.5	5.6	Global assessment score (ASDAS-CRP)	4.8	2.3	1.7

Abbreviations :
AS- Ankylosing Spondylitis
BASDAI- Bath Ankylosing Spondylitis Disease Activity Index
BASFI- Bath Ankylosing Spondylitis Functional Index
BASMI- Bath Ankylosing Spondylitis Metrology Index
ASDAS-CRP- Ankylosing Spondylitis Disease Activity Score- C Reactive Protein
ESR- Erythrocyte Sedimentation Rate (measured in millimeter/hour)
Neg- Negative (below 6 mg/L).

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Original Article

Use of Indomethacin in COVID-19 Patients — Experience from Two Medical Centres

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Background : Indomethacin is a widely used drug belonging to the class of the non-steroidal anti-inflammatory drug (NSAID), which has also a proven anti-viral effect. This academic study describes our experience in treating hospitalised symptomatic COVID-19 positive patients with it.

Materials and Methods : Patients with COVID-19 (detected by the real-time reverse transcription polymerase chain reaction) admitted to our department were provided the option of receiving Indomethacin 25mg (bid) or 75mg SR (sustained release), (OD) along with proton pump inhibitor, along with the standard care of treatment of the Indian Council of Medical Research (ICMR). Patients who did not agree to the Indomethacin option were offered standard care of treatment which included paracetamol. Development of hypoxia was considered as the endpoint. Time period to become afebrile and resolution of cough and myalgia; was considered as the secondary endpoint. Propensity Score Matching was used for the purpose of comparison between these two arms.

A separate group of patients with COVID-19 having severe disease, who were admitted with hypoxemia were given Indomethacin 75mg SR; admission to the intensive care unit or need for mechanical ventilation were considered as the endpoints.

Results : Twenty-eight of 72 patients in paracetamol arm developed hypoxia and required oxygen; whereas, only one patient out of 72 in the Indomethacin arm, developed hypoxia in the mild-moderate Covid patients. None of the patients in severe disease group treated with indomethacin needed mechanical ventilation. More rapid symptomatic relief was observed in indomethacin group than paracetamol group. Also, no systemic adversity including renal/liver functions observed.

Conclusion : In our experience Indomethacin is very effective and safe for treating COVID-19 patients.

[*J Indian Med Assoc* 2021; **119(7):** 42-6]

***Key words* : COVID-19, Indomethacin, Clinical recovery, Inflammation, Cytokine Storm.**

The WHO declared the COVID-19 outbreak as a pandemic on March 11th, 2020. It is caused by the Novel Coronavirus (SARS-CoV 2) and has claimed innumerable lives till date. No drug has been fully proved to be effective against this disease[1,2]. The anti-viral activity of Indomethacin was first reported in 2006[3]. Viral entry into host cells can be inhibited by down regulating the receptors ACE2 and TMPRSS2[4]. The other important factor is inhibiting Cathepsin L for fusion[5]. Ragav *et al*[6] showed that among the group of non-steroidal anti-inflammatory drugs, only Indomethacin has inhibitory property against Cathepsin L.

Editor's Comment :

- Indomethacin, a well-known anti-inflammatory drug; has additional antiviral action
- It produces rapid improvement of symptoms in COVID-19 patients.
- It can effectively substitute paracetamol in these patients, unless there is a contraindication like peptic ulcer or acute kidney injury.

Another important factor Nsp7, which acts as a cofactor of Nsp12 for synthesis of Ribonucleic Acid (RNA), has been discussed in detail by Gordan *et al*[1]. Inhibitory effect on RNA synthesis by this mechanism was also shown by Amici *et al*[3]. Indomethacin reduces IL-6, which is raised in Covid[7,8]. It has been used successfully used to prevent cytokine storm in patients having renal transplant on OKT3 therapy[9,10].

Amici *et al* has proposed effectiveness of Indomethacin in vitro against the Novel Coronavirus[3]. Direct evidence for its anti-viral activity against SARS-

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Cov-2, *in vitro*, *in cellulo* and in Corona-infected canine model are provided by Xu *et al*[11]. In a recent paper, Gordon *et al*[1] showed by retrospective data analysis that Indomethacin markedly decreases hospitalization. Two recent studies[12,13] have shown the effectiveness of Indomethacin in treating a small number of SARS – Cov – 2 patients with severe comorbidities.

Materials & Methods

This open-label study was conducted post ethics committee approval and consent from symptomatic RT-PCR positive Covid patients from two different Medical Colleges located in South and Central India. Effectiveness of Indomethacin (test drug; n=82) along with the SCT which included Hydroxychloroquine, Ivermectin, Azithromycin and vitamins was compared with its control (Paracetamol + SCT; n=109), obtained retrospectively after EC approval. A proton pump inhibitor was also added along with Indomethacin (25 mg bid or 75 mg SR od/5 days). Based on the WHO clinical progression score, hospitalized patients were categorized in to mild/moderate or severe. Mild and moderate patients were treated with Indomethacin (25 mg) in centre 1 and severe cases (n=22) were only treated in centre 2 with 75 mg –SR + SCT + Remdesivir. During admission 21 severe cases were administered supplementary oxygen (O_2); while one case required supplementary-O_2 subsequently. Based on the primary study endpoint (development of hypoxia) or physician discretion, patients were shifted to corticosteroid-regimen. Identical diagnostic tests were done for both the test- and control-drug received groups.

The investigations which were done at the time of admission : CT thorax, Liver Function Test (LFT), Renal Function Test (RFT), C-Reactive Protein (CRP) and D-Dimer. During the treatment or till clinical recovery, all the patients were closely monitored for oxygen saturation, temperature, respiratory symptoms and myalgia along the vitals. Symptomatic improvement was defined as the temperature below 99°F for two successive days and reduction of cough to score 2 on a 1 to 10 scale (1 – no complaint of cough, 2-3 – occasional cough, 4-6 – cough with the ability to do day to day activities, 7-8- persistent cough and 9-10 feeling much discomfort with the cough). Patients were discharged when a consistent oxygen saturation of more than 94% was noted. Except CT scan all other indicated tests were done at the time of patient discharge and their well-being was monitored via phone for additional 14 days.

Rigorous statistical analysis including Propensity Score Matching that mimics randomized controlled trial[14,15], was carried out using open source statistical software package R to match mild and moderate patients received test/control drug (72/arm) and to analyse the efficacy of Indomethacin *versus* Paracetamol.

Results

Propensity score based, on the covariates namely, age, gender, comorbidities (diabetes, hypertension), CT-score and CRP on admission, presence of dyspnea ensures that bias is removed in the recruitment to the two arms. Fig 1 gives the matching of the scores between the two arms and the comparison between the two arms in terms of the covariates. The figure shows that the propensity scores are matched well in

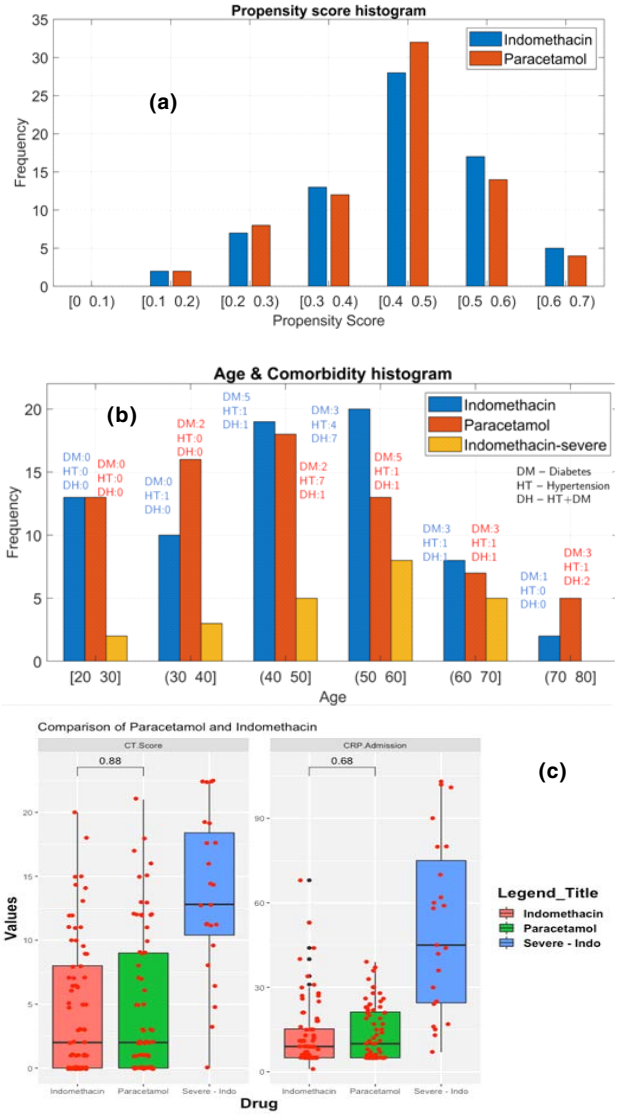

Fig 1 — (a) Propensity Score Matching between the SCT + Indomethacin received arm vs. SCT + Paracetamol received arm; (b) and (c) shows close match of the covariates at the time of admission

both the arms. Further the matching based on baseline characteristics show that both arms had patients with similar age and comorbidities, and the mean and distribution of CRP and CT-Score are similar between the patients. In other words, Fig 1 comprehensively demonstrates that the patients in both the arms are similar.

Efficacy of Indomethacin :

These results regarding the efficacy of indomethacin in improving fever, cough and myalgia are shown in Fig 2 (Fisher's Exact Test: p = 0.0005 for Afebrile, p = 1.7 x 10^{-13} for Cough and p = 0.0025 for Myalgia). Median (dark line) and interquartile ranges are also shown as a box. The recovery from fever, cough and cold in terms of median values is depicted in Table 1. The results are from a one-sample Wilcoxon test and IQR indicates Interquartile. Range. The Table clearly brings out the recovery in the Indomethacin arm of the study.

(a) No. of days to become Afebrile
(b) Days for Cough Reduction
(c) Days for Myalgia Reduction

Also, the temperature on admission or the CT score on admission had no relation to the patient recovery.

The two key questions in this study are – how many patients developed hypoxia and required steroid therapy? And number of patients stayed more than 14 days in the hospital?

In the paracetamol arm 5 patients with hypoxia and 67 patients without hypoxia were admitted and treated, while in Indomethacin arm 11 with hypoxia and 61 without hypoxia on admission was treated. Number of patients required supplementary oxygen was high in Paracetamol group than Indomethacin group (39% *versus* 1.3%) Table 2.

None in the Indomethacin group required a prolonged stay in the hospital. In the paracetamol arm 23 patients had a prolonged stay.

Twenty-one out of 22 patients in the severe category treated with Indomethacin 75 mg SR were discharged on or before 14 days and one patient, who had acute pancreatitis, was discharged after 17 days.

Safety profile of Indomethacin :

There has been many questions regarding the safety value of indomethacin sice its approval in 1965[16].

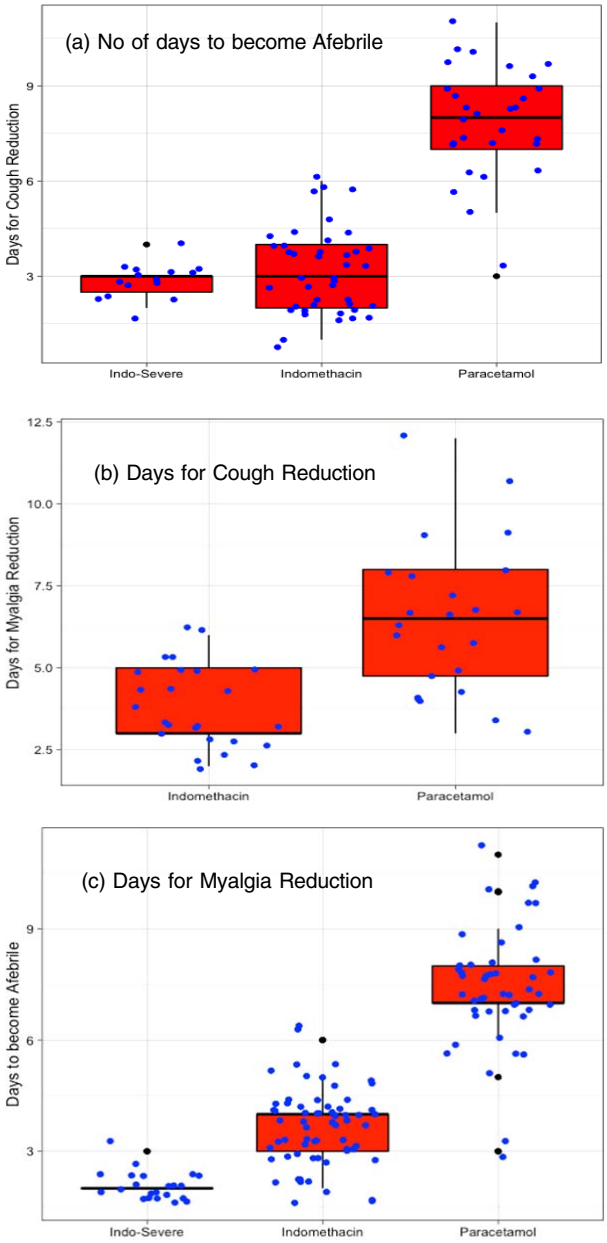

Fig 2 — Symptomatic relief of patients treated with Indomethacin or Paracetamol in combination with the SCT

Patients were tested for Serum Urea and Creatinine, SGOT and SGPT before and after the treatment and the results are given in Fig 3.

Table 1 — *Symptomatic relief due to various treatments*

Treatment	Days to become Afebrile			Days for Cough Reduction			Days for Myalgia Reduction		
	Median	95%CI	IQR	Median	95%CI	IQR	Median	95%CI	IQR
Indomethacin- Mild and Moderate	4	3.5,4.0	1	3	3.0,3.5	2	3	3.0,4.0	2
Paracetamol – Mild and Moderate	7	7.0,8.0	1	8	7.0,8.5	2	6.5	5.5,7.5	3.25
Indomethacin– Severe	2	2.0,2.0	0	3	2.5,3.0	0.5			

Discussion

The major objective of our study was to look whether the patients develop hypoxemia or not; out of total 11 patients who had symptoms of respiratory distress at the time of admission. In the Indomethacin arm, only 1 patient required oxygen, that too was given at low flow (2L/min) only for a couple of days for symptomatic relief. In contrast, all the patients who were in the paracetamol arm, who had symptoms of respiratory distress at the time of admission, needed supplementary oxygen. A significant proportion (34%) of patients, who did not have symptoms of respiratory distress at the time of admission, developed hypoxemia which necessitated administration of oxygen, while no one in the Indomethacin arm had developed hypoxemia. Supplementary oxygen was administered when peripheral oxygen saturation went below 94%. Patients in the paracetamol arm, even after a few days of treatment, deteriorated to hypoxemia. The odds ratio for the development of hypoxia when treated with Indomethacin, compared with Paracetamol was 0.02 (95% confidence interval of 0.003, 0.17).

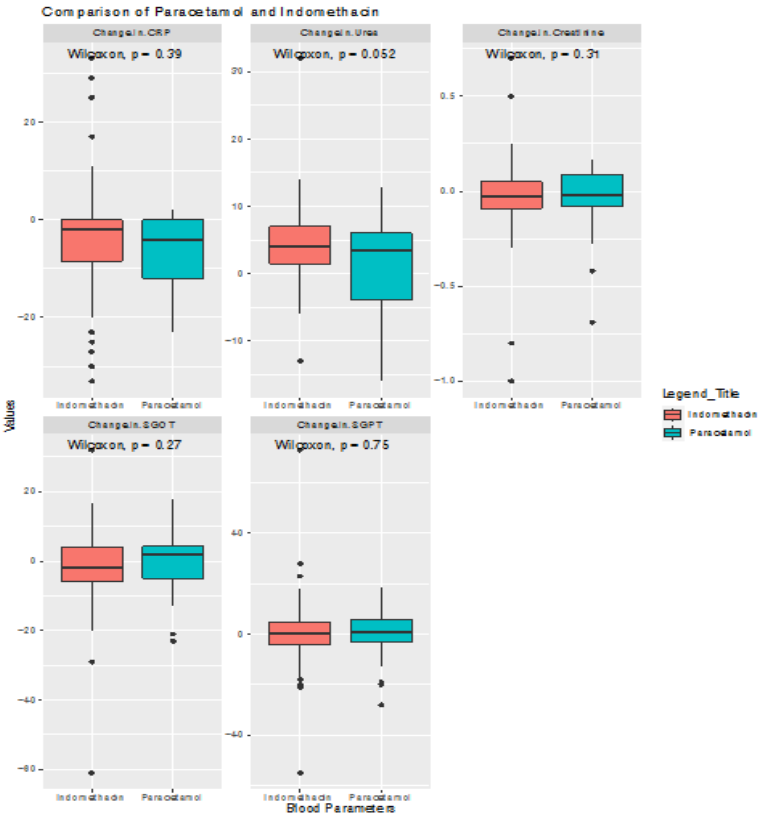

Fig 3 — Change of various blood parameters of the two set pf patients

Table 2 — *Patient requiring supplementary oxygen during treatment*

Outcome	Patients admitted with hypoxia		Patients admitted with no hypoxia	
Patient data	Paracetamol	Indomethacin	Paracetamol	Indomethacin
Patients admitted	5	11	67	61
Patients requiring supplementary oxygen during treatment	5	1	23	0

Our results showed that Indomethacin use is associated with a marked reduction in the duration and severity of symptoms. As depicted in Fig 2, a significant number of patients had symptomatic improvement even after two doses. Complete symptomatic recovery happens within 3-4 days in the Indomethacin arm, in contrast to 7-8 days in the paracetamol arm. Even in severe cases, the improvement in symptoms was rapid.

No study subjects had developed nausea or vomiting after administration of indomethacin, or gastro-intestinal bleeding in the form of hematemesis or melena. One patient who was admitted with acute gastroenteritis like symptoms (vomiting and loose motions) had persistent symptoms during the course of treatment. Though she developed mild hypoxia for a brief period of time, supplementary oxygen was not required.

There was no deterioration of renal- or liver-functions in the Indomethacin arm except in one patient with chronic kidney disease, whose creatinine went up by 0.5 mg% from 1.2 mg%.

Indomethacin use in 22 patients falling into the severe category was analysed separately. They received Indomethacin 75mg SR for a period of five days with Remdesivir. Though the patients had hypoxia at the time of admission, they had evidence of rapid relief of symptoms. However, there was a longer latency of recovery from hypoxia and all of them could be discharged by 14 days except the only patient who had pancreatitis, was discharged after 17 days. It is quite unlikely that Indomethacin caused the pancreatitis as it is under trial for the management of acute pancreatitis[17,18].

The anti-inflammatory efffect of Indomethacin is very

well-established[19]. SARS-Cov-2 is not a cytopahic virus, rather most of the complications of this disease is secondary to inflammation[19]. Indomethacin has both anti-viral and anti-inflammatory properties. It is quite unfortunate that early reports on this aspect cautioned the use of NSAIDs for COVID-19, insted of encouraging its use[20].

Conclusion

The administration of Indomethacin, along with the existing the standard protocol given by ICMR for the treatment of hospitalised patients with COVID-19, was significantly associated with decrease in the severity and duration of illness, without any significant adverse drug reaction. Further study is required to determine the benefit of Indomethacinalone for treatment of COVID-19.

Conflict of interest : The authors have no conflicts of interest to report.

Funding : This study was funded by Mr. Kris Gopala Krishnan, a IITM Alumnus; Chairman of Axilor Ventures & former executive Vice Chairman of Infosys.

Data Statement : This study has been conducted with the following approval. Data can be accessed with the consent of the Ethics Committee

(1) Approved 03/08/2020, Institutional Ethics Committee, Narayana Medical College (Nellore 524003, India; +91 (0)8008086119; dean{at}narayanamedicalcollege.com), ref: NMC/ Ethics/Project/006/2020 and Ref. XXXVI/ Ethics/001/ 11/2020

(2) Approved 10/10/2020, Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences (Sawangi (Meghe), Wardha - 442004, Maharashtra, India; +91 (0)7152 287701; icc.dmims{at}gmail.com), ref: DMIMS(DU)/IEC/2020-21/9034

(3) The trial has a registration number: ISRCTN 11970082

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Original Article

Balloon Mitral Valvuloplasty in Patients above 60 years age with Mitral Stenosis in Eastern India : A Prospective Analytic Study from IPGME&R and SSKM Hospital, Kolkata

Saroj Mandal[1], Debasmita Mandal[2], Suvendu Chatterjee[3], Kaushik Banerjee[4]

Introduction : This study is a Prospective Analytic Single-centre study performed at IPGMER & SSKM Hospital, Kolkata, West Bengal, India, to assess the safety and efficacy of Balloon Mitral Valvuloplasty in patients above 60 years age.

Methods : As per the protocol of our institution, after ethical clearance, elderly patients who were subjected to elective BMV from March, 2012 to November, 2016, were analysed with respect to mitral valve area, left atrial pressure and complications if any.

Results : We studied 76 patients of which 48 were female and 28 were male. Likes other series in our study female patients outnumbers male patients, The patients were of the age range of 60 to 75 years with the mean age of 64.5±4.0 years. Dyspnea on exertion was the most frequent symptom in all the patients. The plannimetric and pressure half time measurements of mitral valve area (MVA) increased from 0.76±0.15cm^2 to 1.8±1.0cm2, mean left atrial pressure decreased from 28.8±6.4 mmHg to 8.1±3.1mmHg. No major life threatening complications were noted and only 1 incidence of death was reported.

Conclusion : Our present study shows Percutaneous Transvenous Mitral Commissurotomy (PTMC) / Balloon Mitral Valvuloplasty (BMV) is a safe and easy to perform option in elderly patients with Severe Mitral Stenosis and provides excellent clinical and hemodynamic benefits in most without any major life threatening complications.

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***Key words* : BMV, PTMC, Rheumatic heart disease.**

Rheumatic Heart Disease (RHD) is the Chronic Rheumatic Heart Disease Is the most common cause of mitral valve stenosis, particularly in the developing countries[1]. In a recent Indian study, 41.5% of cases of chronic RHD in adults had mitral stenosis. Medical therapy is the mainstay of treatment in patients with mild to moderate mitral stenosis. On the other hand mitral valve intervention is required for those with symptoms and moderate to severe MS. Surgical closed mitral commissurotomy (CMC) was performed in 1940's for the treatment of severe mitral stenosis. In 1982 Ranji Inou, a Japanese Surgeon with the invention of the Inou Balloon, first developed the idea that an obstructive mitral valve could be dilated using a balloon with different compliance at two chambers, and since then the percutaneous approach of PTMC using Inoue or Accura balloons have become the therapy of choice for suitable valves and offers comparable results to open and closed surgical valvulotomy with a durable hemodynamic improvement.

Editor's Comment :

- Percutaneous Transvenous Mitral Commissurotomy an effective and well established procedure for management of patients with rheumatic mitral stenosis.
- PTMC procedure has revolutionized the management of rheumatic mitral stenosis.

Mitral stenosis usually presents in younger age group, but in India there are patients who present late with advanced rheumatic Mitral Stenosis and mitral stenosis presenting in elderly age group who are of more than 60 years of age is not uncommon in India and the clinical, pathological and hemodynamic profile at this age are to some extent different from those in younger age group. Elderly patients with Mitral Stenosis most commonly present with heart failure, they commonly have atrial fibrillation due to long standing elevated left atrial pressure and they may have associated left ventricular dysfunction due to multiple comorbidities like Diabetes and Hypertension and also associated Coronary Artery Disease. Elderly patients also tend to have more calcified, thickened and relatively immobile valves, often with significant subvalvular disease[3] and

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are usually traditionally considered unsuitable for balloon valvuloplasty. Efficacy of percutaneous ballon mitral valvuloplasty is presented in very few studies in world literature to support its role in elderly.

In an attempt to address this issue, this study was conducted aiming evaluation of the safety and efficacy of Percutaneus Miral valve Commissurotomy in patients above 60 years of age with mitral stenosis.

Materials and Methods

It is a Prospective Analytic study done at IPGMER and SSKM Hospital, Kolkata, West Bengal, India from March, 2012 to November, 2016. From March 2012 to November 2016 study 76 patients were included in our study which was less than 5% of our total number of PTMC done and these valves were not considered for PTMC using traditional Echocardiography scores. *****Elderly patients with symptomatic and severe mitral stenosis, defined by a valve area of 1.5 cm^2 or less with favorable mitral valve morphology were considered for PTMC.

Exclusion criteria : Patients who are unfit for the percutaneous mitral valvuloplasty procedures like who will need intervention of other valves like aortic valve and/or CABG. Patients with Wilkin's scoring, extensive mitral valve calcification and mitral annular calcification, more than moderate mitral regurgitation, persistence of left atrial clot even after 2 months of anticoagulation therapy, short life span with multiple co-morbidities. Patients needing surgical intervention for other indications, patients with valve calcifications or mitral regurgitation $\geq$grade 2/4, patients having left atrial thrombus not responding to oral anticoagulants or patients who had poor life expectancy due to other illnesses were excluded from the study[1].

The clinical profile and demographic variables which were considered for our present study were age, gender, functional status at admission, presence of atrial fibrillation, past history of mitral procedures and heart failure, history of penicillin prophylaxis, type of previous commissurotomy Routinely Echocardiography was done pre-operatively and postoperatively 24-48 hours after performed by an independent echocardiographer. Conventional methods like planimetry, Doppler pressure[1] half-time were used to assess the mitral valve area Echocardiography was done on a Siemense[1] Accuson 300 JPx machine with ap42 probe.

All data were collected and routinely compared pre and post procedure MVA by plannimetry and PHT pre and post procedure change in grading of Mitral regurgitation, left atrio-ventricular gradient across the mitral valve, LA Size and also other parameters assessed by Echocardiography.

Thepatients who left atrial clot documented by Transesophageal Echocardiography (TEE) treated adequately with oral anticoagulations for atleast 2 months and after 2 months of anticoagulations and a repeat TEE was performed TEE procedure was performed routinely in all patients who underwent PTMC procedure

Trans-esophageal echocardiography was performed in all elderly patients undergoing PTMC as part of the routine procedure as good number of patients had permanent atrial fibrillation; some had h/o paroxysmal AF or previous H/o embolic stroke. Patients with left atrial thrombus were PUT ON oral anticoagulant for at least two months, and PTMC was done if resolution of the left atrial thrombus was demonstrated by a repeat Trans-Esophageal echocardiography done usually after 2 months of initiation of anticoagulant therapy[1].

After a formal written informed consent all selected patients were subjected to PTMC using the usual trans-septal antegrade technique. Single balloon (with Inoue/ Acura balloon) technique was used in all patients. Balloon size was determined by using Hung's formula (Maximum balloon diameter (mm) = (patient's height (cm)/10) + 10). Successful PTMC was defined as i) 50% increase in mitral valve area (MVA) and or increase in mitral valve area (MVA) to $\geq$1.5 cm^2 post-procedure, iii) Significant fall in LA-pressure with a decrease in transmitral gradient to half of the initial value iv) no increase in the grade of MR. Suboptimal PTMC was defined as any increment in mitral valve area less than defined as successful[2].

Procedure related complications like operative mortality, patients needing mitral valve replacement (MVR), cardiac tamponade, embolic episodes, post-PTMC rise in the grade of mitral regurgitation were analyzed.

Statistical Analysis :

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 24.0. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests (Fig 1 & Tables 1-5).

Results

We studied 76 patients of which 48 were female and 28 were male. Like other different series we observed that female patients outnumber the male patients. All the patients in our series were symptomatic admission for PTMC procedure. Dyspnea

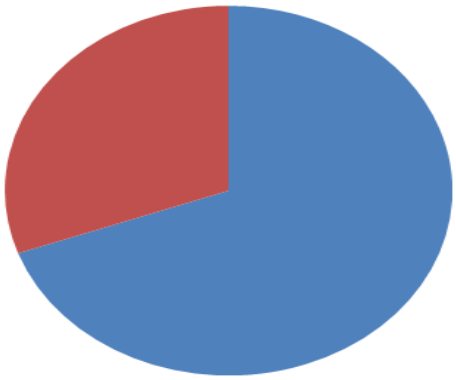

Fig 1 — Male and Female distribution of Elderly Mitral Stenosis patients

Table 1 — *Distribution of Elderly Mitral Stenosis patients according to NYHA class*

Variables	Frequency	Percentage
NYHA class-I	10	13.1
NYHA class-II	11	14.4
NYHA class-III	48	63.1
NYHA class-IV	7	9.2

Table 2 — *The Coronary angiographic, and echocardiographic abnormalities (Left Ventricular Dysfunction, Wilkins score) seen among the Elderly MS patients during the evaluation*

Variables	Frequency	Percentage
(a) Coronary Angiography		
Normal coronaries	48	63.1
Minnor CAD	23	30.2
Single or DVD	1	1.31
Coronary Embolism	2	2.62
Multivessel disease	2	2.62
(b) LV Dysfunction, LVEF 40%	11	14.4
(c) Wilkins score > 8	45	59.2

on exertion was the most frequent symptom in all the patients. 89.8% were in NYHA II to III class and rest of the patients was in NYHA class IV. 4 patients were denied surgical intervention due to their associated co-morbidities. All the patients used to get diuretic and those who had atrial fibriilation were receiving antiarrythmic medication and anticoagulation. Pre-PTMC routine echocardiography grade II MR with central jet in two patients and grade I MR in 32 patients. 3 patients presented with atrial fibrillation with fast ventricular rate which was adequately controlled before the procedure. Ten (20.4%) patients had moderate Pulmonary Arterial Hypertension (Pulmonary Artery Systolic Pressure >50 mmHg) and 9 (18.3%) patients had severe Pulmonary Arterial Hypertension (Pulmonary Artery Systolic Pressure >70 mmHg), Mean PASP was 55.1±24.7 mmHg. Coronary Angiography was done as a routine catheterization procedure prior to PTMC. One patient had severe triple vessel disease including critical left main coronary artery disease. 8 patients had severs LV dysfunction and 2 patients of severe left ventricular

Table 3 — *The echocardiographic variables Post- PTMC*

Variables	Pre PTMC	Post PTMC	p-value
MVA (cm^2)	0.76± 0.15	1.8±1.0	<0.001
Mean Left Atrial Pressure (mmHg)	28.8± 6.4	8.1± 3.1	<0.001
Left Atrio- ventricular PG (mmHg)	16.1± 4.1	4.2± 1.3	<0.001
PASP(mmHg)	60.0± 18.0	36.6 ± 7.4	<0.001

Table 4 — *Procedure related complications during PTMC*

Variables	Frequency	Percentage
Severe MR requiring MVR	1	1.3
Cardiac Tamponade	1	1.3
Mortality	1	1.3
Embolism	0	0.0
Moderate MR	2	2.6

Table 5 — *Success Rate of PTMC in Elderly Patients with MS*

Variables	Frequency	Percentage
Death	1	1.3
Severe MR	1	1.3
Moderate MR	2	2.6
Tamponade	1	1.3
Successful	71	93.4

dysfunction and had documented Ventricular Tachycardia and ICD was implanted. The mean mitral valve area increased from 0.76±0.15cm^2 to 1.8±1.0cm2, mean left atrial pressure decreased from 28.8±6.4mmHg to 8.1±3.1mmHg. No major life threatening complications were noted and only 1 incidence of death was reported. In one patient who presented with STEMI, mitral stenosis was diagnosed after hospital admission during routine work-up. Post procedure severe MR occurred in 1 patients and moderate MR in 2 patients. Optimum and desirable results observed in 71 (93.5%) patients compared to suboptimum results in 5 patients. Suboptimum results were achieved in 7 (14.25%) patients as assessed by mitral valve area (MVA<1.5cm^2) on Echocardiography. Post-procedure MR of more than moderate in 1(2%) patient, and moderate in 2 patients.

Though female predominance was observed in this series the difference was not very much significant likes other series. The patients were of the age range of 60 to 75 years with the mean age of 64.5±4.0 years. Dyspnea on exertion was the most frequent symptom in all the patients. Few patients present with left heart failure NYHA Class IV before the procedure, rest of them (89.8%) were in NYHA II to III (all the patients were symptomatic). 4 patients were denied surgical intervention due to their associated comorbidities. 3 patients presented with atrial fibrillation with fast ventricular rate which was adequately controlled before the procedure. All patients were taking diuretics in different doses to control the symptoms. Electrocardiography (ECG) findings were normal sinus

rhythm in 29 (38.7%) patients and atrial fibrillation in 47(61.84%) patients. Patients with atrial fibrillation were on anticoagulation. Left atrial size ranged from 4.2 cm to 10.2 cm with the mean of 5.8±2.8 cm. In almost all cases left atrial size were above the normal value (<4cm) and in more than 50% of cases left atrium was hugely dilated (>52cm). Mild MR was present in 32(42.1%) patients, moderate MR in two (2.6%) patient, and trace MR in 6 (12.2%) patients whereas rest 26.7% patients didn't have any MR. Mild to moderate AR was present in 16 patients. Ten (20.4%) patients had moderate PH (>50 mmHg PASP) and 9 (18.3%) patients had severe PH (>70 mmHg PASP), Mean PASP was 55.1±24.7 mmHg. Coronary Angiography was done as a routine catheterization procedure prior to PTMC. One patient had severe triple vessel disease including critical left main coronary artery disease. 8 patients had severs LV dysfunction and 2 patients of severe left ventricular dysfunction and had documented Ventricular Tachycardia and ICD was implanted. The mean mitral valve area increased from 0.76±0.15cm^2 to 1.8±1.0cm^2, mean left atrial pressure decreased from 28.8±6.4mmHg to 8.1±3.1mmHg. No major life threatening complications were noted, and only 1 incidence of death was reported. In one patient who presented with STEMI, mitral stenosis was diagnosed after hospital admission during routine work-up. Post procedure severe MR occurred in 1 patients and moderate MR in 2 patients. Successful results were observed in 71 (93.5%) patients compared to unsuccessful results in 5 patients. Unsuccessful results were due to suboptimal MVA <1.5 cm^2 in 7 (14.25) patients and postprocedure MR of more than moderate in 1(2%) patient, and moderate in 2 patients.

Discussions

Chronic Rheumatic Heart Disease most commonly complicated by and presented with Mitral Stenosis. PTMC procedure has revolutionized the therapy of rheumatic mitral stenosis and after the introduction of Inoue Balloon After the introduction of the PTMC procedure by Inoue *et al* in 1984 PTMC has become the most effective and popular therapeutic technique for 4^0 symptomatic patients with moderate to severe mitral stenosis . PTMC is recommended as a Class I indication for symptomatic patients of mitral stenosis as the immediate results of PTMC are similar to those of closed and open surgical mitral commissurotomy. But in elderly patients mitral stenosis valve morphology gets deformed with high Wilkin's score and with other related complication like low LVEF, and they become less suitable for PTMC. In our present study MS is predominantly presents in females. The mean mitral valve area usually got doubled post PTMC procedure, with a 60% to 70% reduction in left atrioventricular gradient. Our success rate was upto 93.5% which is comparable to other studies done in children with juvenile MS and adults.

Different literature showed that increase in severity of Mitral Regurgitation during Percutaneous Mitral Valvulopasty (PTMC) procedure inwith patients needing the of Mitral Valve Replacement in few. The development of severe MR in our series was extremely low and only one patient developed severe MR. Overall success in this aspect may be related to our patients having suitable mitral valve morphology and also using the strategy of stepwise up-titration of PTMC Balloon.

A similar low volume study with 13 elderly patients with MS done in Japan by Tsuchioka Y *et al* revealed percutaneous procedure in comparison to surgical procedure has the advantages of low complication rate and short hospital stay in elderly patients. Using injection Heparin during the periprocedural period the incidence of embolic episode during the procedure of percutaneous mitral commisurotomy is 0.3% and 3% In our study we didn't experience a single embolic episode during the PTMC procedure or postprocedure. These remarkable results may be related to our protocol of routine pre-procedure Trans-esophageal Echocardiography

Conclusion

PTMC is an effective and well established procedure for management of patients[4] with rheumatic mitral stenosis in young and adult. Our present study shows percutaneous transvenous mitral commissurotomy is safe and easy to perform even in elderly patients with MS and provides excellent clinical and hemodynamic benefit in most of the elderly MS patients.

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Conflict of Interest : None

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Review Article

CRISPR-cas Methods : Culminating in Crescendo of the COVID-19 Pandemic to FELUDA Test

Samashaptak[1], Pratnoja Das[1], Sukanti Bhattacharyya[2], Anindita Banerjee[3]

COVID-19 pandemic is a universal crisis at this very moment. Since 31st December, 2019 and as of 30th November 2020, 63,187,035 cases of COVID-19 have been reported including 1,467,284 deaths. While Nucleic acid amplification-based methods; particularly real-time RT-PCR remains the gold standard for the diagnosis of COVID-19, various other diagnostic strategies are on trial to find rapid as well as sensitive and feasible testing technique. One such technique using CRISPR-cas based gene editing method is aimed at developing cheap, easy-to-use and fast SARS-CoV-2 detection kit for early diagnosis of COVID-19. CRISPR-cas methods for precise genome editing took its shape in modern form through the works of Dr. Jennifer Doudna at the University of California-Berkeley and Dr. Emmanuelle Charpentier at Umeå University in Umeå, Sweden and used for gene therapy, gene regulation, medical diagnostics and therapeutics. For this contribution to revolutionized genome editing, both of them had the honour to share the Nobel Prize in chemistry this year.

This review article tries to capture this colourful history in a single frame starting from the sequencing of 1.7 kbp *E coli* DNA fragments in the late 1980s by Y Ishino and detection of the first CRISPR sequence of *E coli* in 1987, subsequently unveiling the mysteries of in veritable adaptive immune system of prokaryotes against bacteriophages and plasmids, to the development of cheap and easy-to-use SARS-CoV-2 detection kit for early diagnosis of COVID-19.

This article also reviews various applications right from food processing to drug processing, diagnosis of infectious and non-infectious diseases, therapies for genetic abnormalities and even cancer through novel pathways of gene editing and regulation. Use of it as an antimicrobial and antiviral agent has also been elaborated. Finally, it has been discussed how this novel technique has been utilised for designing FELUDA, a CRISPR-cas9 based detection of COVID-19.

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Key words **: CRISPR-cas, Gene Editing, COVID-19, SARS-CoV-2, Corona, Detectr, Sherlock, FELUDA.**

Dr Jennifer Anne Doudna at the University of California-Berkeley and Dr. Emmanuelle Marie Charpentier at Umeå University in Umeå, Sweden worked across the Atlantic Ocean to elucidate the mechanism behind the CRISPR-*cas9* system in bacterial immunity and innovate adapting this system for precise genome editing. For the seminal contributions, both of them had the honour to share the Nobel Prize in Chemistry this year. This year, 2020, is a nightmare to all of us for the devastating pandemic waves submersing our lives at stake. Using these CRISPR-*cas* methods, many scientists, at different research institutes, independently developed cheap and easy-to-use SARS-CoV-2 detection kit for early diagnosis of pre-symptomatic and early symptomatic cases of COVID-19 to minimise chances of transmission in the community and to initiate adequate treatment ab initio to mitigate further complications. Thus, this year signifies a great coincidence of recognition to the contributors, as well as relief to the mankind from the threat of the pandemic, only through these CRISPR-*cas* techniques.

In short, CRISPR-*cas* systems, evolved in bacteria as an inheritable adaptive immune mechanism to confer protection against invading bacteriophages and plasmids

Editor's Comment :

- Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR) — short palindromic repeats in gene sequences — was accidentally found to be involved in an inheritable adaptive immune mechanism in bacteria to confer protection against invading bacteriophages and plasmids through the activities of two main molecule types— a *cas* nuclease to cleave dsDNA and a gRNA to target specific viral DNA sequences.
- Through extensive work across the globe CRISPR-cas systems have been recognised as a very effective novel pathways of gene editing and regulation. Since then, there have been myriads of application right from food processing to drug processing, diagnosis of infectious, including recent COVID-19 and non-infectious diseases, including genetic disorders, therapies for genetic abnormalities and even cancer. Many more newer applications are ushering day by day at every nook and corner of the scientific world.

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through the activities of two main molecule types— a *cas* nuclease to cleave dsDNA and a gRNA to target specific viral DNA sequences, have myriads of application right from food processing to drug processing, diagnosis of infectious and non-infectious diseases, therapies for genetic abnormalities and even cancer through novel pathways of gene editing and regulation.

Aims and Objectives

To review the origin and development of CRISPR-*cas* system.

To study emerging applications of CRISPR-*cas* in basic and applied research.

To understand the integration of these know-how into daily practice.

A Puzzling Sequence from Bacteria Challenges the Early Sequencing Methodology

In 1987, Y Ishino *et al* in Osaka University sequenced 1.7 kbp *E coli* DNA fragments spanning over the *iap* (isozyme of alkaline phosphatase) gene region, and found that the same sequence appearing several times in different clones, downstream of the translation termination codon of the *iap* gene by conventional M13 dideoxy sequencing and autoradiography. Similar sequence were detected in other *E. coli* strains (C600 and Ymel), members of *Enterobacteriaceae*, *S. dysenteriae*, *S. enterica typhimurium* by Southern blot hybridization analysis; and also in *Actinobacteria*, e.g. *M. tuberculosis*. About fifteen years later, such repetitive sequence has been termed CRISPR.

Discovery of CRISPR in Archaea

Francisco J. M. Mojica, Cesar Díez Villaseñor, Elena Soria of Universidad de Alicante and Guadalupe Juez of Universidad Miguel Hernández, in January 2000, demonstrated transcription of genomic regions having the repeated sequences in extremely halophilic archaea, *H. mediterranei* and suggested regulation of gene expression in conversion of B to Z form of DNA, which is barely valid for bacteria;

Observations of such repeated sequences interspersed with variable sequences in various bacterial (H. Influenza, S Cerevisiae,-*Methanocaldo-coccusjannaschii*) and archaeal genomes (*H. mediterranei*, *H. Volcanii)* by many scientists across the globe led them to name such sequences differently as Short Regularly Spaced Repeats (SRSR), Spacers Interspersed Direct Repeats (SPIDRs), and Large Cluster of Tandem Repeats (LCTRs) – two groups of LCTR sequences were observed in hyperthermophilic archaea, *P. abyssi* and *P. horikoshii*, with presumed role in chromosome partitioning; but scrambling of numerous such repeats in the genome of *P. furiosus* frowned at this assumption. [Zivanovic *et al*., 2002]

Mojica *et al* (2000) pioneered with the concept of functional relationship among these repeated sequences in the genome of various bacteria and archaea and coined the term CRISPR, through correspondence with Ruud Jansen; but Jansen et al. first used the term Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR) in print version in 2002.

Comparative genomic studies focussed at the common characteristics of CRISPR as their intergenic locations, multiple short direct repeat sequences with meagre variation and nonconserved interspersed sequences and a common leader sequence of hundreds of bps at one side of the repeat clusters. CRISPR sequences have been demonstrated in archaeal genomes and 50% of bacterial genomes but none in eukaryotic genome till date.

Identification of *cas* Genes

At the onset of new millennium, four conserved genes were found to be present regularly juxtaposed to the CRISPR regions and were termed as CRISPR-associated genes (*cas 1, 2, 3 and 4*). *cas 3* was having the seven motifs peculiar to the superfamily 2 helicases and *cas 4* was found to be working as a part of RecBCD complex for the terminal resection of the double strand breaks to initiate homologous recombination, related to RecB exonucleases. Thus *cas 3* and *cas 4* were thought to be operational in DNA repair and recombination, transcriptional regulation and chromosome segregation. But, *cas 1* and *cas 2* were not identified with similarity to functional domains of any known proteins. [Jansen *et al*, 2002] Simultaneously, Makarova *et al.* (2006) independently and systematically analysed the conserved genes in all available prokaryotic genome and found multiple clusters of genes corresponding to *cas* genes (encoding putative DNA polymerase, helicase, RecB-like nuclease) in genomes of hyperthermophilic archaea and in two hyperthermophilic bacteria, *Aquifexaeolicus* and *Thermotoga maritima* but not in mesophilic and moderate thermophilic archaea and bacteria, predicting these proteins to be a part of some 'mysterious' ill-defined DNA repair mechanism in thermophilic organisms.

Discovery of CRISPR Function

Independent breakthrough observations—by Francisko Mojica in Alicante and Christine Pourcel in Orsay—of interspersed or spacer regions between repeat sequences homologous to sequences of bacteriophages, prophages and plasmids and background knowledge of immunity against phages and plasmids by the host strains harbouring the homologous spacer sequences in the CRISPR led to the proposition of CRISPR-mediated biological defence system similar to eukaryotic RNAi system from the entry of foreign mobile genetic elements. Both Mojica *et al* and Pourcel *et al,* in 2005, independently

suggested some triggering mechanism by CRISPRs to form a memory of previous genetic aggressions. In 2005 itself, Bolotin *et al* confirmed these observations by further finding of a correlation between number of spacer of phage origin and degree of resistance to phage infection and suggested possible production of anti-sense RNA using CRISPR.

Seminal works over decades by... Makarova et al. (2006), Barrangou *et al* (2007), Marraffini et al. (2008), Brouns et al. of van der Oost's group (2008), Moineau et al. (2010), Siksnys' group (2011), Andersson and Banfield (2018) have elucidated many intricate details of CRISPR-*cas* systems and reinforced our good understanding of their precise details[1-4].

Cas1 and *cas2* are the conserved protein in most of the CRISPR-*cas* system, representing the adaptation module for insertion of new spacer in the CRISPR arrays. During expression stage mature crRNAs are processed by type-specific *cas* endonucleases from pre-crRNA which are transcribed from CRISPR locus. The crRNAs are bound by effector *cas* endonucleases and recruited to and sequentially cleave the target DNA or RNA, during the interference stage. In contrary to the adaptation module, *cas* enzyme are variable from one CRISPR-*cas* type to another, while involved in the above two stages but with participation of the same enzymes.

Diversity and Classification of CRISPR-*cas*

CRISPR contents and distribution varies strikingly even in closely related strains, eg, *M tuberculosis* has CRISPR but not *M. leprae*. Contrarily, *E coli* and *M avium*, as well as *Methanothermobacto-rthermautotrophicus* and *Archaeoglobusfulgidus*, although being phylogenetically distant, contain nearly identical CRISPR sequences. There are 1 to 18 CRISPR arrays in one genome and 2 to 374 repeat units in one CRISPR array.

According to the latest (09 May, 2017) CRISPR database[5], CRISPR were identified in 87% (202) of 232 analysed archaeal species and 45% (3059) of 6782 analysed bacterial species. But peculiarly, CRISPR-*cas* system as found to be much less (around 10%) prevalent in wild microbial communities in a survey of 1724 draft genomes, might be due to lack of CRISPR-*cas* systems across majority of uncultured bacterial lineages[6].

Shmakov *et al*, in 2017, classified CRISPR-*cas* system in two distinct classes: Class 1 (nearly 90% of all identified loci) — included types I, III and IV, widespread in bacteria and archaea, including all hyperthermophiles and working with multisubunit effector complexes having unevenly stoichiometric 4 to 7 *cas* proteins and Class 2 (remaining 10%) — included types II, V and VI, almost exclusively spread in bacteria and working with a single multi-domain effector protein.

Unit signature proteins, like *cas3*, *cas9* and *cas10* distinguish type I, type II and type III respectively. Architecturally similar and evolutionarily related CRISPR-associated complex for antiviral defence (Cascade) and Csm/ Cmr complexes are multimeric effector complexes of type I and type III systems respectively[7-9]. Functionally, nonspecified type IV systems contain no adaptation module with *cas1* and *cas2* nucleases[10]. Effector modules of subtype III-B systems utilizing spacers made by type I systems stood for the modularity of CRISPR-*cas* systems. Albeit being devoid of identifiable CRISPR loci in many of the genome encoding type IV systems, it— alike subtype III-B— utilizes available crRNAs from different CRISPR arrays[10]. Lastly, additional signature genes and characteristic gene arrangements classify each type into multiple subtypes: I-A, B, C, D, E, F, U; III-A, B, C, D(of Class 1); II-A, B, C; V-A, B, C, D, E, U; IV- A, B, C (of Class 2)[8,11].

Class 2 Systems are Suitable for Genome Editing Technology

A new generation of genome editing technology attractively utilised simple architecture of effector complexes of Class 2 CRISPR-*cas* systems, which contain multiple distinct effectors like *cas9* in type II, *cas12a*(Cpf1) and *cas12b*(C2c1) in type V, *cas13a*(C2c2) and *cas13b*(C2c3) in type IV[8,11]. *cas9*, a crRNA-dependent endonuclease with two distinct nuclease domains, RuvC and HNH—respectively responsible for cleavage of nontarget (displaced/ non-complementary) and target (complementary) DNA strands in the crRNA target DNA complex—was the most commonly and best studied multidomain effector protein

Cas12a (Cpf1) present in several bacterial and archaeal genomes is a prototype of type V effectors containing two RuvC like nuclease domains but not HNH domain; although a second nuclease domain with a unique fold being functionally analogous to HNH domain of *cas9* has been reported recently[11,12]. *cas12a*, as a single RNA-guided nuclease, unlike *cas9* activity, is independent of a tracrRNA (*trans*-activating crRNA); and has different cleavage pattern and protospacer adjacent motif (PAM) recognition[13]. Like *cas9*, type V effectors also require a tracrRNA for the targeted activity. The group of Emmanuelle Charpentier, in 2011, besides finding *cas9* containing CRISP-*cas* system while sequencing small RNA of *S. pyogenes*, also discovered a second small RNA as tracrRNA, which guided *cas9* to its target by forming a duplex with crRNA. Unlike most of the functionally specified CRISPR-*cas* system reposted to target DNA, only multi-component type III-A and III-B systems also target RNA[14]. On the contrary, type IV effectors, *cas3a* and *cas3b*, containing a pair of higher eukaryote and

prokaryote nucleotide-binding (HEPN) domains instead of RuvC-like nuclease domains, specifically target RNA to mediate RNA interference[15]. Novel Class 2 effectors presumably paved CRISPR systems through a newer avenue to genome engineering technology[16].

Applications of CRISPR-*cas*

1. CRISPR typing: CRISPRs were initially used for diagnostic and epidemiological typing of *M. tuberculosis* and subsequently *Yersinia pestis, Salmonella sp., Corynebacterium diptheriae* using the property of heterogeneity of CRISPRs among isogenic isolates. An initial PCR-based method was improvised into spoliogotyping, a hybridization-based method, suitable for routine use and High Throughput (HTP) genotyping.

2. In dairy industry: Danisco (DuPont) first demonstrated immune function of CRISPR-*cas* and utilized that to yield phage resistant *S. thermophilus* in improved cheese production. [Barrangou *et al*., 2007]

3. Genetic tools for eukaryote cells: Development of the type II system, particularly by combining crRNA and tracrRNA into a sgRNA (single guide RNA) [Jinek *et al*, 2012] and deploying *cas9* system—unlike target-degrading *cas3*—produces a single DSB in the DNA as an important gene editing tool for genetic alteration in either of the two ways, (a) NHEJ and (b) HDR.

a. Non-Homologous End Joining (NHEJ), which joins the cut end but may cripple the gene product by deleting a few bases or may inactivate it by frameshift mutation.

b. Homology Directed Repair (HDR), which uses another piece of DNA with homology to the target to repair the damaged allele by inserting that DNA element either through recombination, insertion, deletion or changing sequence. [Cong *et al*, 2013; Mali *et al*, 2013]

Instead of previous complicated approaches using ZFNs (Zinc Finger Nucleases) and TALENs (Transcription Activator-like Effector Nucleases), [Mali *et al*, 2013] *cas9* is very simple to retarget and concurrently to modify several targets. Albeit the necessity of a PAM juxtaposed to the target *cas9* within a short spell of time has become a very popular tool for genome editing in studying eukaryotes, from yeast to humans. [Terns *et al*., 2014] *cas9*-based methods have also been applied in genetic screening, [Wang *et al*., 2014] and programmable RNA recognition and cleavage. [O'Connell *et al*., 2014] Strategies for prediction and prevention [Tsai *et al*., 2015] of off-target effects (interactions with unintended targets) by *cas9* are being developed, using an artificial CRISPR-*cas* nuclease RFN (RNA-guided FokI nuclease)[17].

4. Diagnostic uses:

a. CRISPR-based diagnosis of viruses—Methods based on the CRISPR-*cas12a* (DETECTR) and CRISPR-*cas13a* (SHERLOCK) families are the most widely explored arena for CRISPR-based diagnostic system.

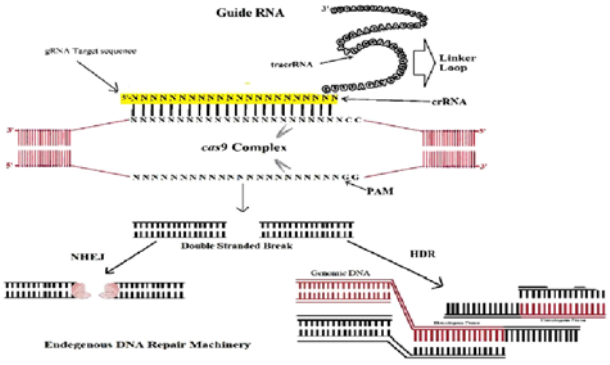

Fig 1: Schematic representation of gene editing by CRISPR-Cas9 complex.

The guide RNA recruits the complex at the desired site on the genome; the Cas9 protein cuts at the PAM site and then one of the two DNA double strand break repair mechanisms repair the break leading to gene editing.
Non-homologous end joining (NHEJ) is a pathway that repairs double-strand breaks in DNA, where the break ends are directly ligated without the need for a homologous template, in contrast to homology directed repair, which requires a homologous sequence to guide repair.
Homology directed repair (HDR), a naturally occurring nucleic acid repair system, can be used to modify genomes in many organisms, including humans, which is initiated by the presence of double strand breaks (DSBs) in DNA.

i. DETECTR—A guide RNA primarily leads the type V*cas12a* enzyme to a ds sequence of DNA in a specified viral genome; then *cas12a* enzyme indiscriminately cleaves a quencher-molecule-bound ss DNA molecule and a reporter fluorophore, emitting a fluorescent signal from the fluorophore and quencher. This highly sensitive DETECTR method can detect a single molecule of viral particle, within a µl of sample[18,19]. DETECTR may detect any viral particle but most significantly used for diagnosis of HPV (Human Papilloma Virus)[20].

ii. SHERLOCK—Using crRNA targets via type IV *cas13a* enzyme, a target RNA-bound targeting molecule along with an attached fluorophore are cleaved indiscriminately and emit fluorescent signal in presence of specific sequences of viral nucleic acid through simplified and more specific SHERLOCKv2 protocol. The sensitivity can be enhanced over three times by supplementing *cas13* enzyme with CRISPR-associated *Csm6* enzyme. SHERLOCK protocol can be optimized for diagnosis of HIV (Human Immunodeficiency Virus). To enhance amplification and detection of viral material, recombinase polymerase amplification (RPA) may be united with either of SHERLOCK and DETECTR[19-22].

Heating unextracted diagnostic samples to obliterate nucleases (HUDSON) protocol escalates the efficiency of SHERLOCK procedure to detect genetic material from urine, saliva, blood and its isolates. Conserved region within the genetic material of these viruses can be recognized using universal-flavivirus RPA and viral species-specific crRNAs. SHERLOCK technique with HUDSON protocols can detect any virus but flaviviruses like Zika, Dengue, West Nile and Yellow fever viruses have mostly been detected[21,22].

Presence of SARS-CoV-2 in the specimen can be detected by identifying both of the N and E gene variants of the virus by DETECTR method[23], and both of the S and Orflab gene sequences by SHERLOCK method[24].

b. CRISPR-based diagnosis of bacteria—FLASHit is a software tool to design a *cas9* enzyme set to target a total of 3624 bacterial genetic sequences associated with microbial resistance including drug-resistant *S aureus* culture isolates. MRSA (Multidrug-Resistant *S Aureus*) and vancomycin-resistant *E faecium*. FLASHit is based on FLASH (Finding Low Abundance Sequences by Hybridization), using *cas9* enzyme recombination along with multiplex guide RNAs for precise identification of pathogen by eliminating background sequences and the *cas9* system to cleave target sequences into fragments ideal for next generation sequencing. [Quan *et al*, 2019]

FLASH method can rapidly detect Mtb using RPA and *cas12a*-optimized enzyme. [Ai *et al*, 2019] With a 99.06% sensitivity rate, FLASH method can detect hybrid strain of STEC O104:H4.

c. CRISPR-based diagnosis of non-infectious diseases—CRISPR method effectively sought for specific oncogenes related to almost all cancer cells in the body and could successfully identify synergistic gene reaction behind drug-resistance for better understanding molecular pathophysiology of cancer and identifying new precision therapy biomarkers. SHERLOCK method using *cas13* enzymes thus helps in early and precise diagnosis vis-à-vis prevention of many cancer. [Khambhati *et al*, 2019; Tian *et al*, 2019]

5. Commercially available CRISPR-based diagnostic tools: Three CRISPR-based diagnostic systems or instruments are available commercially

a. DETECTR Diagnostic Tests—Manufactured by Mammoth Biosciences, using CRISPR type V and programmable for a wide array of viral, bacterial, infections and also cancer diagnosis. Like SHERLOCK method, DETECTR method has been proposed for detecting SARS-CoV-2.

b. SHERLOCK Diagnostic Tests—Manufactured by SHERLOCK, using CRISPR type IV and presented a cheap and faster method using paper strip, lateral flow, read-out assays for Zika and Dengue virus. Boosted with HUDSON protocol, the SHERLOCK method bypasses purification and dilution steps for rapid diagnosis of viral infections and in field studies.

c. CRISPR-*cas9* Products for Gene Editing—Manufactured by Sigma-Aldrich, using type II CRISPR-*cas9* systems for gene editing and diagnosis.

6. Therapeutic uses: CRISPR-*cas* systems have immense potential at therapeutic level, like

a. As an antimicrobial agent—Specifically to target antibiotic-resistant and/or highly virulent strains of bacteria. [Bikard *et al*, 2014; Citorik *et al*, 2014]

b. As Gene therapy—

i. By repairing the *cftr* gene in cultured cells from cystic fibrosis patients. [Schwank *et al*, 2013]

ii. By altering DNA in mouse germ cell lines to cure dominant cataract disorder and Duchenne muscular dystrophy. [Wu *et al*, 2013; Long *et al*, 2014]

iii. By genetic alteration in adult mice to cure hereditary tyrosinemia. [Yin *et al*, 2014]

A short version of *cas9* delivered through adeno-associated virus facilitate its use in somatic gene therapy. [Ran *et al*, 2015]

c. As an antiviral agent—Potential for treatment of viral infections, like HIV [Hu *et al*, 2014; Ye *et al*, 2014] and Hepatitis B [Zhen *et al*, 2015]

7. In disease modelling: Precise genetic modification through Gene editing in embryos of primates, close to human, allows development of disease model. This approach might be used to change DNA in human embryos to stop noncomplex hereditary diseases but not for complex trait, due to strong ethical issues. [Baltimore *et al*., 2015] *cas9*-mediated genome editing also accelerated the generation of transgenic models to expand biological research beyond traditional genetically tractable animal model organisms. This could be useful to develop novel transgenic models, [Wang *et al*., 2013] to engineer isogenic ES and iPS cell disease models with specific mutations introduced or corrected, respectively, or in vivo and ex vivo gene correction.[Schwank *et al*., 2013; Wu *et al*., 2013]

8. Gene regulation: Programmable gene regulation utilising CRISPR-*cas* systems have been developed for

a. Gene silencing by interfering with RNA-polymerase binding or elongation by using both Cascade and a nuclease-deficient *cas9* mutant (d*cas9*)[25,26].

b. Achieving transcriptional activation or repression by fusing d*cas9* with a transcriptional activation domain or repressor. [Cheng *et al*, Farzadfard *et al.* and Gilbert *et al*., 2013]

c. Achieving strong induction by adding multiple activity domains[27].

9. Genome-wide application of this system helps in identifying the genes behind treatment resistance of melanoma cancer cells.

10. Functional genomic screening: Unbiased genome-wide functional screen, through genome editing with *cas9* parrallely over many targets helps to identify genes behind a particular phenotype, eg, robust negative and positive selection screens in human cells by presenting loss-of-function mutations into early constitutive coding exons of a different gene in each cell. *Cas9*-mediated pooled sgRNA screens are more sensitive and consistent than older RNAi approach to

target almost any DNA sequence significantly obviating off-target effects and limitation of partial knockdown.[Wang *et al*. and Shalem *et al.* 2014]

11. Transcription modulation: CRISPRi (CRISPR-based interference) performs much better in prokaryotic genomes than eukaryotic cells and this repressive function of CRISPRi is further enhanced by linking d*cas9* to transcriptional repressor domains, eg, KRAB or SID effectors, to promote epigenetic silencing; even adding helper functional domain yields only partial transcriptional knockdown. Being fused with VP16/VP64 or p65 activation domains, *cas9* turns into a synthetic transcriptional activator[27].

12. Epigenetic Control: Instead of having achievement in locus-specific targeting of epigenetic modifying enzymes in a small number of proof-of-concept studies with the use of zinc finger proteins and TAL effectors as before, *cas9* epigenetic effectors (epi*cas9*s) can artificially add or remove specific epigenetic marks at specific loci more flexibly to find causal effects of epigenetic modifications in shaping the regulatory networks of the genome. Possible off-target effects or cross-talk between effector domains and endogenous epigenetic complexes should be cautiously specified and mitigated by harnessing prokaryotic epigenetic enzymes[27].

13. Live imaging of cellular genome: Alternative to DNA-FISH, a powerful live imaging technique using fluorescence-tagged cas9 labelling of specific DNA loci was developed recently. [Chen *et al*, 2013]

14. Inducible regulation of *cas9* activity: Bilobed structure of *cas9* can be split into two units and be reassembled under control via small molecule induction facilitating systemic control in patients or animal models or via light inducible heterodimeric domains, eg, CIB1 or CRY2 or chemically inducible analogues, eg, ABI and PYL, to construct inducible TALEs (Transcription Activator-like Effectors)[27].

Detecting SARS-CoV-2 by CRISPR-*cas* Method[28]

As have already described the detection of SARS-CoV-2 by CRISPR-*cas*-based SHERLOCK and DETECTR methods, the latter is more commonly used in RT-LAMP using *cas12*. COVID-19, due to SARS-CoV-2, is a devastating pandemic, jeopardizing economic foothold of many nations, social existence of the population thereof, financial independence of most of the citizens across the globe, is a real menace to the fruitful existence of mankind. Many pre-symptomatic carriers are the real threat to others for its transmission. Fast and cheap tool to detect the presence of the specific virus in any susceptible person, at anytime and anywhere, is the need of the hour.

DETECTR RT-LAMP/*cas12* (improvised) is like 'the magic lamp' in the present scenario. It is claimed to be equally effective as the gold standard diagnostic tool for SARS-CoV-2, i.e.qRT-PCR. Although qRT-PCR is a quantitative assay, it is a complicated procedure at altered temperature over a prolonged time with less temporal turnover. Whereas, qualitative assessment at normal temperature in a simpler way makes RT-LAMP a handy tool even for the novice, particularly when it is supplemented with microfluidic- or SPR (surface plusmon resonance) -based detection system, it becomes a transportable rapid test that can be applied at the patient's site.

Charles Chiu of the University of California-San Francisco, in collaboration with San Francisco-based biotech Mammoth Biosciences tried to develop a CRISPR-based diagnostic test for SARS-CoV-2 detection with their previous experience of developing similar test for Lyme disease. Chiu et al. developed SARS-CoV-2 DETECTR, for SARS-CoV-2 DNA endonuclease-targeted CRISPR trans reporter and published its mode of action in *Nature Biotechnology* on April 16, 2020. After RNA extraction, it uses Loop Mediated Amplification (LAMP) at isothermic condition avoiding expensive thermocyclers for cycling temperatures as in PCR study. On July 9, 2020, Emergency Use Authorization (EUA) was allowed by USFDA only for the use at the UC-SF's clinical lab. On May 20, 2020, Mammoth Biosciences allied with Glaxo SmithKline Consumer Healthcare to develop DETECTR into a handheld disposable instrument at a very cheap rate.

On August 27, 2020, *PLOS Pathogens* published a paper describing a test by a group of China-based researchers, named 'CRISPR-COVID', for RNA extraction, followed by cleavage of single stranded reporter using *cas13a* and having florescence in presence of SARS-CoV-2 RNA.

On August 31, 2020, researchers published in PNAS about a LAMP-based SARS-CoV-2 detection tool utilizing microfluidic cartridge and smartphone-based reader, avoiding laboratory-grade infrastructure and resources for diagnosis at the collection-point, like schools, sports arenas, old age homes, etc. On the other hand, Max Wilson of University of California-Santa Barbara developed a COVID-19 test that uses CRISPR for detection and PCR for amplification but avoided LAMP considering it to be less sensitive.

On May 6, 2020, COVID-19 test kit based on CRISPR using SHERLOCK platform by Dhanda et al. at Sherlock Biosciences was granted EUA and started distribution in the USA in collaboration with Integrated DNA Technologies. Wyss Institute at Harvard University developed INSPECTR (Internal Splint-pairing Expression Cassette Translation Reaction) through hybridization of a sample like saliva to cryosynthetic DNA complementary to SARS-CoV-2 RNA. In presence of viral RNA, a reporter protein is activated and can be

observed without any instrument, making it perfect for domiciliary use.

FELUDA : The Indigenous SARS-CoV-2 detection Kit[29]

On October 11, 2020, Dr. Harsh Vardhan, the then Union Health Minister of India, stated with high optimism that in the following couple of weeks the FELUDA paper strip test for SARS-CoV-2 detection would be available. He also acclaimed 96% sensitivity and 98% specificity of the test, based on trials at the Council of Scientific & Industrial Research-Institute of Genomics and Integrative Biology (CSIR-IGIB).

FELUDA (FNcas9 Editor-Limited Uniform Detection Assay), also after the name of a popular Bengali fictional private investigator character in the thrillers by Satyajit Ray, utilises CRISPR-cas technology for the detection of genes specific to SARS-CoV-2 virus with the help of a protein called FNcas9 and a guide RNA (gRNA). Using a paper strip, binding of this gRNA-FNcas9 complex to the SARS-CoV-2 viral gene could be visualized by simple coloured line(s) within a spell of one to two minutes. Single line indicates negative and double lines indicate positive test. It got approval from the Drugs Controller General of India (DCGI).

FELUDA test is very less expensive, very less time consuming, very simple to operate and interpret by the lay person as claimed by Dr.Debojyoti Chakraborty, Senior Scientist at CSIR-IGIB. He is one of the authors of the pre-print shared on bioRxiv that describes the development of FELUDA.

In May, 2020, CSIR IGIB and TATA Sons signed a MoU for licensing the know how related to development of the kit.

Discussion

Accidental finding of short repeats in genome sequences in a palindromic pattern drew the curious attention of young researchers in late 1980s. Sincere attempt to isolate the gene for a single protein (*iap* enzyme) led the scientists to dive deep into the gubernaculums. A mysterious finding of inheritable adaptive immunity with memory, similar to vertebrates, overwhelmed the scholars. Probing deep into it, they discovered a very peculiar mechanism of prokaryotic adaptive immunity against bacteriophages and plasmids, utilising those short palindromic repeats in gene sequences, namely CRISPR. This is the beginning of a new journey through a novel path towards gene editing technology, gene regulation, gene therapy, so on and so forth.

Prokaryotic adaptive immunity, as well as gene editing technique with minimum off-target effects, are through the CRISPR-*cas9* systems, whereas *cas12a*, *cas13a* systems help in detection of several viral and bacterial infections. Cheap and fast detection of SARS-CoV-2 using DETECTR or SHERLOCK platform of CRISPR techniques, including the indigenous FELUDA Test Kit, is a boon to the threatened world population during this dreaded pandemic. Fermenting milk to produce cheese with the help of CRISPR-mediated phage-resistant lactose fermenting bacteria is a bonanza to the food processing industry. Getting into the genetic link behind drug resistance in antimicrobials and getting over of it are the contributions of this novel technology. Finding specific oncogene, vis-à-vis cancer, biomarker and suggesting gene therapy for cancer and also understanding the molecular pathophysiology of different monogenic and polygenic diseases through cell modelling and animal modelling, even at the level of embryo with the help of CRISPR technology are of immense help for the medical fraternity and mankind. The CRISPR revolutionized genome editing, identifying right enzymatic system in a very simpler way and this is as significant as the discovery of thermostable DNA polymerase in PCR evolution.

The great inquisitiveness of human mind brings forth the latest generation of genome engineering tools from the component of microbial anti-phage defence systems. Similarly and very likely, the unabated human wisdom will get to the bottom of the amusing biological variety of nature to refine genetic modification in a more competent and precise way.

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Case Report

Avascular Necrosis of Femur Neck in Young Adult Secondary to Indigenous Medicines — An Eye Opener for Clinicians

Parshika Panwar[1], Ravi Kant[2], Manjunath Totaganti[3], Rohit Raina[4]

Psoriasis is a chronic inflammatory disorder affecting the skin, its treatment is dependent on both topical and systemic therapy including glucocorticoids. The use of indigenous medicine is rampant in countries like India and at time they contain steroids in one or the other the forms. Hereby, we present a case of psoriasis in young adult consuming indigenous medicine for the control of psoriasis that resulted in diabetes mellitus, cushingoid features and avascular necrosis of bilateral hips. Probably it contained steroids, Physician should be aware of the warning signs when coming across the use of long term indigenous medicines.

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Key words **: Psoriasis, Indigenous medicine, Iatrogenic Cushing, Avascular necrosis of hip.**

Psoriasis is a dermatological chronic inflammatory disorder and treatment has been revolutionised by the use of glucocorticoid because of anti-inflammatory and anti-proliferative properties[1]. The practice of using indigenous herbal medicines for treatment is rampant in countries like India. Indigenous herbal medicines may contain one or other substances including steroids which may cause adverse effects on prolonged use[2]. The physician may be unaware of the use of alternative medicine herbal products by their patients. Possible adverse events of these products need to be highlighted so that both the physician and consumer should be cautious in their use. Here we present a case of indigenous herbal medicine abuse presented with complications in a resource limited country like India.

Case Report

A 28-year-old male presented to diabetic clinic for control of hyperglycemia which was for undergoing hip replacement surgery. This gentleman was suffering from bilateral hip pain for last 6 months. It progressed insidiously and was associated with limping. He had history of psoriasis diagnosed at the age of 16 years, for which he was taking indigenous oral medications and topical applications. Detailed history revealed that he developed diabetes mellitus four years back along with diminition in vision for two years after starting the indigenous medicines, for which he consulted an ophthalmologist and detected to have cataract in left eye that was treated by surgery. On examination he had facial erythema suggestive of acne rosacea, purplishstriae over the abdomen with width >1cm (Fig 1), cataract was detected in right eye, pseudophakia in left eye, musculoskeletal examination showed limping gait, apparent shortening and reduced range of motion in all directions at left hip joint. His blood chemistry revealed HbA1c 12.5 gm%, C-peptide was 2.41 ng/ml, postprandial blood glucose of 463 gm/dl. His x ray pelvis was done which showed osteosclerosis in right head of femur with chip fracture and discontinuity in shenton's line (Fig 2). Magnetic resonance imaging (MRI) of pelvis showed changes suggestive of collapse of right femoral head with architectural changes in acetabulum and avascular necrosis (AVN) of both femur head (Fig 3).

Editor's Comment :

- The use of steroid is quite common in herbal preparations.
- Clinicians need to be aware about the early adverse effects of steroids to prevent the advanced complications like osteoporosis and avascular necrosis of femur.

Discussion

Indigenous or traditional system of medicine has been

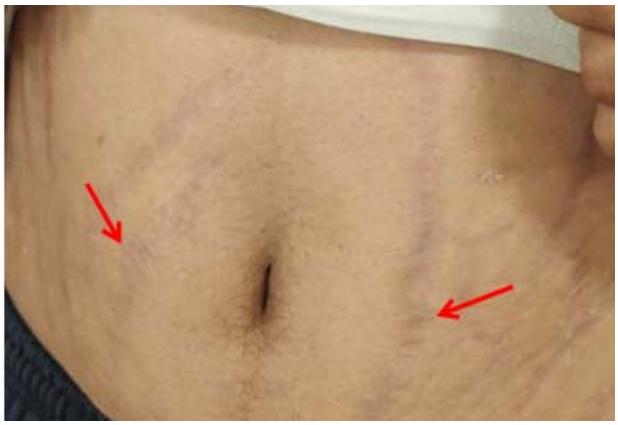

Fig 1 — Striae on anterior abdominal wall (red bold arrows)

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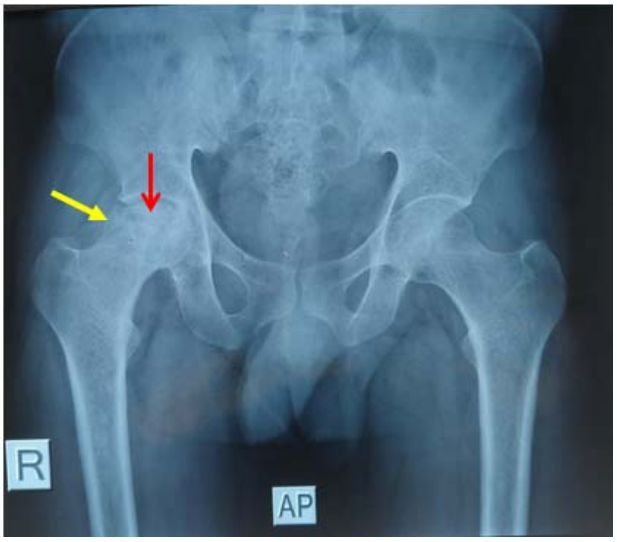

Fig 2 — Plane X-ray hip AP view showing osteonecrosis of right femoral head with collapse (red bold arrow) and chip fracture in right femoral head (yellow bold arrow)

widely practiced in Indian subcontinent and people have faith on these products as these are based on the natural products. The use of indigenous herbal preparations is common for psoriasis both in oral and topical form. These medicines are considered to be devoid of side effects by people and advertised similarly[3,4].

There are plenty of reports regarding steroidal contents and possible adulteration of these indigenous medicines[2]. Certain other constituents of these products like heavy metals and alkaloids may also lead to adverse events[5]. Steroids may result in systemic side effects including Cushing syndrome and Hypothalamic Pituitary Adrenal (HPA) axis suppression if used for prolonged period[1]. These are commonly seen in children secondary to use of topical steroids for diaper dermatitis and in adults secondary to use of steroids for Psoriasis[6]. Index case is also an adult with prolonged use of indigenous oral and topical preparations. The nature of product not been told by patient but he was describing it some kind of local application and 'churan' so constituents can't be commented but we believe it to be steroid adulteration. Avascular necrosis is a known consequence of overuse of steroids but not frequently reported in literature.

Avascular necrosis is a severe adverse outcome of glucocorticoid excess, commonly seen in age group of 30-50 years. Most common site of AVN is femoral head particularly its weight bearing anterolateral part, being risk of developing AVN of the femoral head is 0.3% with an incidence of one per one thousand patients per year[7].

AVN is characterised by necrosis of cellular component of bone secondary to impaired blood circulation. Various etiologies may lead to AVN including alcoholism, steroid excess, trauma, gaucher disease and sickle cell disease[8]. Mechanism of AVN due to steroid excess is imbalance between bone resorption and repair, vascular impairment and apoptosis. Steroids promote

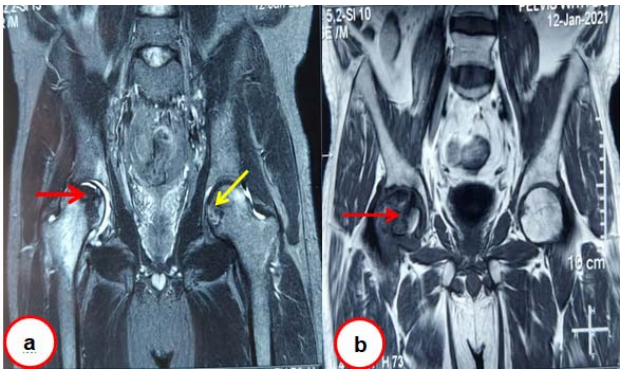

Fig 3 — MRI Images (a) STIR sequences showing destruction of right femoral head with collapse and osteoarthritic changes (red bold arrow), osteonecrosis of left femoral head (yellow bold arrow) (b) T1 weighted images showing osteonecrosis of right femoral head (red bold arrow)

adipogenesis in bone marrow precursor cells due to upregulation of transcription factor like PPAR-y and down regulation of RUN-X2 thus affecting osteoblast differentiation. Steroids also affect the vascular supply of bone by affecting angiogenesis process due to decreased VEGF, fat emboli and direct compression of arteries due to increased intraosseous pressure from adipogenesis, occlusion of vasculature due to thrombi and finally due to hypertension leading to epiphyseal artery constriction and damage[8,9].

Patient may not notice any pain in early stages of avascular necrosis but with worsening of condition severity of pain increases causing functional impairment[8]. As in our index case, symptoms were mild initially which worsen with time affecting his daily activities.

Early radiological changes are patchy subchondrallucency and sclerosis. Advanced stages show subchondral fracture crescent with articular surface collapse and secondary osteoarthritis. X-ray findings in our case were pointing towards AVN so further confirmation with MRI was done which was showing collapse of right femoral head with arthritic changes in acetabulum and AVN of left femoral head. MRI is more sensitive in catching disease in early stages.MRI allows sequential evaluation of asymptomatic lesions that are undetectable on plain radiographs and facilitates initiation of therapeutic measures early[10].

Treatment of AVN depends upon the stage and extent of AVN, early options include conservative measures restricted weight bearing, continuous use of a wheelchair, bisphosphonates and vitamin D[11]. Before collapse of joint and osteoarthritis treatment is preferably with core decompression along with bone graft and rotational osteotomy to divert weight bearing. If more than two-thirds of the weight bearing articular surface area is involved, it may lead to collapse of femoral head. After collapse of joint without osteoarthritis, hemiarthrolplasty is recommended and after osteoarthritis with collapse, total hip replacement is the method of treatment[11,12].

Conclusion

Indigenous medicines may be effective in some medical conditions but not devoid of serious adverse events which may be due to inherent constituents or adulteration with steroids or heavy metals. Clinician should be aware and vigilant about the use of indigenous products by patients. Early diagnosis and identification of complication is required for best treatment outcomes particularly in adults.

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Case Report

Atypical Hemolytic Uremic Syndrome in Snake Bite : An Often Missed Entity

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Snake envenomation is an important and common cause of Acute Kidney Injury(AKI) in India. AKI can occur following bites from snakes belonging to various families, due to multiple mechanisms. Hemolytic Uremic Syndrome (HUS) is an unusual cause of AKI following snake envenomation. We are reporting the case of a patient who developed HUS following envenomation by an unknown snake, presumed to be vasculotoxic. The patient presented with oligouria within 12 hours of the bite and having grossly deranged KFT, was promptly started on hemodialysis with improvement being noticed after 4 episodes of hemodialysis. The patient finally improved with hemodialysis and supportive treatment alone, obviating the need for plasma exchange, which is generally the standard treatment modality for HUS, making the case rare. HUS complicating snake bite has been reported earlier but recovery with hemodialysis is not common.

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Key words **: Schistocyte, Hemolytic, Uremic, Syndrome, Hemodialysis, Snake bite.**

Snakebites are a major occupational hazard in tropical countries like India, particularly in the rural areas. There are more than 5.4 million snake bites per year worldwide with 81,000-138,000 deaths all over the world[1], and an average of 58,000 deaths per year in India[2]. Snake envenomation is an important occupational injury, which affect a multitude of workers including farmers, fishermen, herders and plantation workers.The case in discussion is about a farmer who was bitten by an unknown snake while working on the field and developed rapidly progressing renal failure and was ultimately diagnosed to be a case of atypical HUS. It has been noted that there are spikes of snakebites during rainy seasons and during harvesting seasons and the incident in our case too happened during the mid monsoon.

There are roughly 236 species of snakes in India of which 13 are venomous[3]. There are four families of poisonous snakes: Elapidae, Viperidae, Hydrophidae and Colubridae. Most often the clinical effects of venom of vipers are vasculotoxic whereas that of Elapids are neurotoxic and hydrophids or sea snakes are mostly myotoxic. In India viper bites are most common and incidence of AKI following Russell's viper and E carinatus bites is 13-32%[4-6]. Although the snake in our case was not identified by the patient, in all likelihood, it was a viper, considering the gross nephrotoxicity and vasculotoxicity the venom was seen to induce.

Editor's Comment :

- Atypical hemolytic uremic syndrome often complicates snake bite which needs plasma exchange coupled with hemodialysis
- Awareness of this condition is important while managing a case of acute kidney injury in snake bite for therapeutic decision

The complications of snakebite can be local or systemic and our case displays predominantly a systemic manifestation with minor local changes. Local changes are part of an acute inflammation process causing local edema, pain, ecchymosis, blisters, bleeding, bruises, lymphangitis, lymph node enlargement, skin necrosis and may lead on to infection and cellulitis. Systemic complications include manifestations of hemotoxicity, neurotoxicity, rhabdomyolysis and Acute Kidney Injury (AKI)[4].

AKI, one of the most common and important manifestations of snake envenomation, particularly vasculotoxic envenomations, in turn, occurs by numerous mechanisms such as hemodynamic disturbances, direct tubular toxicity, hemoglobinuria, myoglobinuria, coagulopathy and thrombotic microangiopathy[4]. Our case was diagnosed to be a case of HUS, one of Thrombotic microangiopathy, which recovered with multiple episodes of Hemodialysis alone and there was no need of plasma exchange or any targeted therapy like Eculizumab. It is extremely uncommon for a case of Atypical HUS with such severe kidney injury to recover without any specific intervention, thereby necessitating a case report.

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Case Report

A 60-year-old male, farmer by profession, hailing from a rural area in West Bengal, was bitten by an unknown snake on the right leg. The patient presented to our Tertiary Care hospital, 4 days after the bite, with complaints of swelling of the right foot along with decreased urine output and generalised swelling of the whole body. According to the patient and his relatives, within 3 to 4 hours of the bite, the patient's right leg and foot became swollen. He was taken to a district hospital, where he was not given any Anti snake venom due to uncertainty of bite by poisonous snake. Over the 24 hours after the bite, the urine was dark brown and the urine volume was less than 100 ml in 24 hours with progressively increasing generalised body swelling. He remained anuric over the next few days and on the fourth day after the bite, he was referred to our tertiary care referral centre in view of renal dysfunction. He also complained of mild pain at the bite site. There was no history of fever, bleeding at bite site or any distant site, breathing difficulty, perioral tingling or blurring of vision. There was no history suggestive of neurological involvement. There was no history of intake of any nephrotoxic drugs.The patient was not known to be diabetic, hypertensive, smoker or to have any other comorbidities.

Table 1 — *Lab values at the time of presentation and at the time of discharge*

Parameters	At Presentation	At Discharge
Urea (mg/dL)	292	79
Creatinine (mg/dL)	10.8	2.9
USG KUB	RK: 10.5 cm X 5 cm, CMD maintained LK: 11.2 cm X 5.5 cm, CMD maintained	RK: 10.7 cm x 5.1 cm, CMD maintained, Cortical echo mildly raised. LK: 11.5 cm X 5.3 cm, CMD maintained, cortical echo mildly raised.
Urine R/E, M/E	Protein +, RBC 4-5, Pus cells 2-3/HPF.	Protein +, Pus cells: 1 -2/HPF, epithelial cells: 3-4/HPF, RBC: 1-2/HPF.
Hemoglobin (g/dL)	7.1	8.2
WBC (/mm3)	12800	5400
Platelet Count (/mm3)	90000	2,20,000
LDH (U/L)	814	290
Total Bilirubin (mg/dL)	2.0	0.6
Indirect Bilirubin (mg/dL)	1.6	-
SGPT / SGOT / ALP (U/L)	19/21/65	12/10/83

The patient presented on day 4 of bite. At presentation, patient was conscious, alert and oriented. His blood pressure was 134/ 84 mm Hg and the pulse rate was 92 beats/min, temperature 98.2 F, and respiratory rate was 18/min. Physical examination revealed moderate pallor, bilateral pitting pedal edema. Jaundice was absent. Examination of the cardiovascular, respiratory, gastrointestinal and central nervous system was unremarkable. The right leg and foot were swollen more than left side without any redness or other signs of local inflammation. There was no active bleeding from the fang mark site. There were no signs and symptoms suggestive of neurotoxic bite. There was no weakness in any of the extremities and no respiratory muscle weakness (single breath count was 35).

His investigation reports are given in Table 1. He was diagnosed to have acute kidney injury AKIN Stage-3 and was found to have evidence of Thrombotic Microangiopathy (TMA). His peripheral blood smear showed >3 schistocytes per high power field (Fig 1). He did not have clinical or laboratory features of sepsis. His urine routine examination had few pus cells and RBC in high power fields. Corrected Reticulocyte count 2.1 The coagulation tests such as Prothrombin Time (PT) & activated Partial Thromboplastin Time (aPTT) were normal. Serum haptoglobulin level was low and d-Dimer and FDP levels were normal. His serum vitamin B12, folate and iron studies were normal. Direct Coombs test was negative. The ultrasound and MRI of both the kidneys did not reveal any significant abnormality. In view of evident Microangiopathic Hemolysis with thrombocytopenia and normal coagulation profile associated with Acute Kidney Injury, a diagnosis of snake bite induced Atypical HUS was entertained[3]. Unfortunately, we could not measure ADAMTS-13 levels due to logistic reasons. A renal biopsy to confirm HUS and other causes of AKI could not be performed.

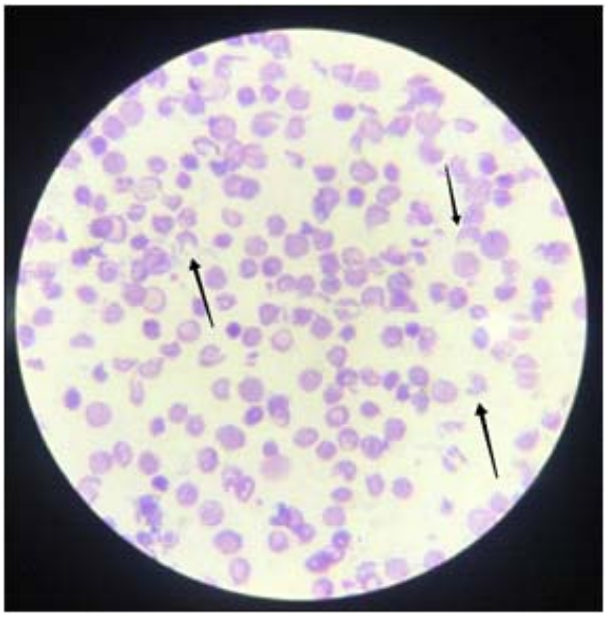

Fig 1 — Peripheral blood smear of the patient showings chistocytes (shown by arrows)

So in view of AKI, hemodialysis was started and considering HUS, he was planned for treatment with

therapeutic plasma exchange. While undergoing hemodialysis, he improved in terms of urine output, platelet count and renal function. He received 9 sessions of hemodialysis during his hospital stay. By 26 days after admission his renal function recovered with good urine output. He was discharged with a serum creatinine level of 2.9 mg/dl at the time of discharge and was expected to reduce further to near normal value. He was asked to follow up in nephrology OPD.

Discussion

Acute kidney injury following snake envenomation can occur due to multiple mechanisms. Hypotension and circulatory collapse occurs due to numerous mechanisms such as vasodilatation, increased capillary permeability, bleeding due to coagulation abnormalities and myocardial depression, ultimately culminating into ischemic AKI. Intravascular hemolysis due to phospholipase A2 and direct lytic factor (found only in elapid venom), present in snake venom can release myotoxins and cause hemoglobinuria and myoglobinuria leading to AKI. Direct action of toxin enzymes, especially phospholipases and metalloproteases can also cause renal injury. A number of proinflammatory cytokines and mediators such as Tumor Necrotic Factor (TNF), interleukins 1,6,10, interferon-Y and nitric oxide are released following exposure to toxin enzymes.

Venom Induced Consumptive Coagulation (VICC) is a very well known consequence of viper snake bites. It is caused by activation of coagulation pathway, mediated by the procoagulant effect of snake venom (eg, Factor X in Russell's viper venom,thrombin like enzymes in many vipers and prothrombin activators in Echisspp).VICC is characterized by rapid onset coagulopathy within hours after the snake bite with elevated D-dimer levels, prolonged prothrombin time and low fibrinogen levels which at times is associated with thrombocytopenia[7,8]. This resolves within 24 to 48 hours. It is not associated with systemic microthrombi and end organ failure. This is in contrast to Disseminated Intravascular Coagulation (DIC) which appears much later, its pathogenesis involves tissue factor/factor VIIa pathway with depression of fibrinolysis leading to reduced fibrin removal and is characterized by evidence of systemic microthrombi, bleeding from multiple sites and end organ failure.

Acquired/ Atypical HUS has been described as a complication of snake envenomation. HUS is characterized bypresence of thrombocytopenia, micro angiopathic hemolytic anemia and renal dysfunction with normal prothrombin time and activated partial thromboplastin time. HUS comes under constellation of Thrombotic Microangiopathy (TMA) which also includes Thrombotic Thrombocytopenic Purpura (TTP), which in addition of above features also has neurological involvement and fever. The exact mechanism of TMA following envenomation is unclear but it has been proposed that a toxin in the venom may initiate TMA by inducing endothelial damage[9]. Snake venom or its vascular endothelial toxins may act as Von Willebrand factor activators or vascular endothelial growth factor–type factors and initiate TMA by inducing endothelial damage. The role of ADAMTS-13, a Von-Willebrand factor-cleaving protease in snake bite is unclear and requires further investigation[10]. Microangiopathic haemolytic anemia is suggested by drop in hemoglobin, high Lactate Dehydrogenase (LDH), reduced serum haptoglobin levels, indirect hyperbilirubinemia and presence of schistocytes in peripheral blood smear. TMA/ HUS is also seen in other conditions such as viral and bacterial infections, toxins, pregnancy, HELLP syndrome, bone marrow transplantation, drugs (mitomycin, cyclosporin A, ticlopidine) therapy and cancer. The diagnosis of HUS is mainly indirect as there are no definite tests to prove and renal biopsy is not always feasible.

Our patient had evidence of Thrombotic Micro-Angiopathy in the absence of disseminated intravascular coagulation (DIC)as PT & aPTT were within normal limits,serum haptoglobulin level was low and d- Dimer and FDP levels were normal without malignanthy pertension and without any use of drugs or other causes known to cause HUS. The secondary causes of HUS were ruled out in our case. Table 1shows trend in creatinine, hemoglobin, platelet count and other investigations and laboratory parameters of our patient at the time of admission and discharge. We could not do the renal biopsy and ADAMTS-13 levels due to logistic reasons. There was no hypotension, rhabdomyolysis or clinical and laboratory evidence of sepsis inour patient. So, the diagnosis of AKI secondary to snake envenomation induced Acquired/ Atypical HUS was made. We treated our patient with renal replacement therapy/ hemodialysis. Following treatment his renal function recovered with stable levels of creatinine of around 2.9 mg/dl and urine output also improved to normal daily amount.

TMA is rarely reported as a cause of snake bite related AKI. In a study by Isbister *et al*[11] in 2007, 13% of cases with brown snake envenomation were found to have features of TMA, suggesting that TMA could have been overlooked in most of the previous studies. This could be explained by the coexistence of VICC in most cases which makes the diagnosis of TMA challenging, with clinicians erroneously attributing MAHA, thrombo-cytopenia, and renal injury to disseminated intravascular coagulation (DIC)[9]. So the presence of HUS in snake envenomation should besought as timely intervention earlyinterventions in the form of hemodialysis and therapeutic plasma exchange can aid in recoveryof renal function as well as increase the survival probability.

Our patient recovered with hemodialysis alone, without any plasma exchange therapy. Some published cases of snakebite-associated TMA with acute kidney injury (AKI) have reported successful treatment with plasmapheresis. Other published cases of snakebite-associated TMA with AKI report that the renal end organ damage resolves with renal replacement therapy alone[12].

However, the mechanism for which is not known and various in-vitro studies may point to its pathogenesis. The potential role of plasmapheresis in the treatment of snake bite associated TMA treatment is also unknown. Any association with respect to the pathophysiology or long- term outcomes of snakebite TMA with either a HUS or TTP is not established. When the existing gap in the knowledge of mechanism and pathophysiology of snakebite induced a HUS will be known, then we would have some clear idea of what the preferable option for snakebite associated a HUS would be.

Conclusion

Atypical HUS should always be kept in mind whenever a patient presents with the triad of haemolytic anemia, thrombocytopenia and AKI following a snake bite; while it may be rare, it is albeit a distinct entity and plasma exchange in addition to hemodialysis, is often life saving if the diagnosis is made early.The paucity of case reports, particularly from India, pertaining to this rare systemic manifestation of snake bite, that too one where the patient completely recovered without any use of AVS, should make this case an interesting and informative addition to existing literature.

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Case Report

Middle Colic Artery Pseudoaneurysms in Acute Necrotising Pancreatitis

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Acute pancreatitis is notorious for causing pseudo-aneurysms of the surrounding vessels, which though rare are associated with a high mortality if they rupture. Such pseudo-aneurysms commonly most commonly involve the splenic artery which lies just behind the pancreas. A high index of suspicion for such lesions and a timely intervention helps decrease the mortality associated with these lesions. We report a very rare case of multiple pseudo-aneurysms in the middle colic artery, following acute necrotising pancreatitis managed conservatively with endovascular coiling.

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Key words **: Acute pancreatitis, Pseudo-aneurysms.**

Pancreatitis is an acute inflammation of the pancreas, which is situated retroperitoneal in location and is surrounded by many major vessels of the abdomen. These vessels may be involved in the inflammatory process of the pancreas and lead to either pseudo-aneurysm or a thrombosis.

Pancreatitis leads to pseudo aneurysm in 2-5% of cases, mostly involving the splenic (50-60%) and gastroduodenal (15-20%), pancreaticoduodenal (5-10%) arteries[1]. We report a case of multiple Middle colic artery pseudo-aneurysm in a patient due to acute necrotising pancreatitis successfully managed by coiling of the pseudo-aneurysm.

Editor's Comment :

- Pseudo-aneurysms adjacent to pseudocyst or pancreatic necrosis form due to enzymatic breakdown of small arterial wall from contact with pancreatic secretions.
- Pseudo aneurysm of middle colic artery is a very rare entity. Almost all are symptomatic and display greater risk of rupturing than true aneurysms. Angiographic Coil embolisation being the preferred technique of treatment for Middle Colic artery Pseudo-aneurysm with a success rate of almost 90 %.

Case Report

A 34 year gentleman presented with acute epigastric pain with radiation to the back. He had history of binge alcohol which he was having for past 8-10 years with daily.

On examination patient had tachycardia of 140 with a blood pressure of 140/90 mm Hg. Abdominal examination revealed tenderness in the epigastric region with no evidence of mass or abdominal distention. Lab investigations revealed Haemoglobin of 10.4mg%, White blood cell count - 13000/mm^3, platelets - 2.6 lakh/mm^3. Liver enzymes not elevated. Serum lipase was 1000U/L.

Ultrasonography of abdomen showed mild hepato-splenomegaly with gallbladder sludge, pancreas was obscured. Contrast enhanced computerised tomography (CECT) scan showed a well defined iso to hyper dense lesion with surrounding peripheral hypodensity of 4.6* 4.1cm in the body of pancreas showing progressive enhancement with a suspicious foci of arterial hyper enhancement seen in inferior wall of the lesion with no demonstrable arterial feeder. The lesion was enhancing in venous phase and was suspected to be a venous varix. This lesion was displacing the pancreas anteriorly. There were also bilateral large sub diaphragmatic collections communicating with collection in the body of pancreas.

Angiography was done with a 5Fr 8cm trans-femoral catheter which revealed multiple aneurysms along a left branch of middle colic artery largest being 4cm in proximal region with other 3-4 small aneurysms distally. The aneurysm had a narrow neck delaying its filling in arterial phase and filling up in venous phase, which was seen well as enhancement in venous phase of the CECT. No active extravasation of the contrast was seen. Using a 2.7Fr Progreat micro-catheter selective cannulation of middle colic artery performed and 200 polyvinyl alcohol (PVA) particles installed to embolise the distal aneurysm. Various coils were deployed to occlude the aneurysm by distal to proximal technique. Check angiogram revealed no flow into aneurysm. Patient was started on nasojejunal enteral feeds and discharged. Patient was totally asymptomatic at 6 weeks followup (Figs 1&2).

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Discussion

Very few cases of middle colic artery pseudo-aneurysms have been documented.

Arteries have 3 layers namely endothelium, muscularis and adventitia and a true aneurysm has all these three layers. Visceral artery aneurysms are extremely rare, and most of them have been reported after spontaneous haemorrhage. Only 3% of all reported splanchnic artery aneurysms have been located in the jejunal, ileal, or colic arteries[1]. The aetiology of these aneurysms is poorly known: most appear to be due to congenital or acquired medial defects - atherosclerosis, connective tissue disorders, vasculitis, rarely post traumatic.

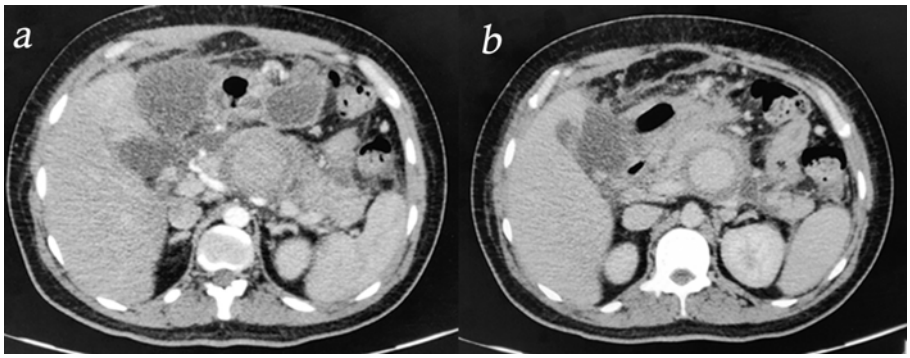

Fig 1 — Body of Pancreas (a) Arterial phase CT with a well defined lesion just behind pancreas (b) Venous phase CT of the same lesion with enhancement

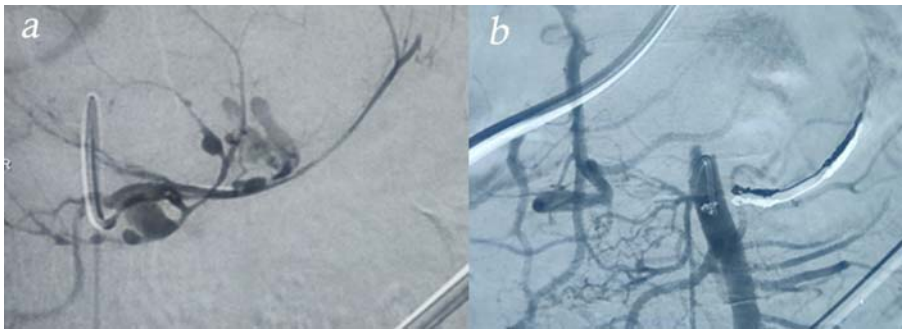

Fig 2 — Totally asymptomatic at 6 weeks followup (a) Angiography showing aneurysms along the branches of middle colic artery (b) Post coiling angiography

False aneurysms or pseudo-aneurysms are defined as missing complete arterial walls lined by adventitia or perivascular tissue and arises from the weakening of the arterial wall caused by trauma, including surgical trauma. Pseudo-aneurysms adjacent to pseudocyst or pancreatic necrosis form due to enzymatic breakdown of small arterial wall from contact with pancreatic secretions. Depending on the extent of spread of pancreatic inflammation, such pseudo aneurysms form in any vessel surrounding the pancreas and have been documented in splenic artery, gastroduodenal and pancreaticoduodenal arteries. The middle colic vessel arises from the superior mesenteric artery just below the pancreas and passes between the two layers of transverse mesocolon and rare to get isolated involved due to pancreatic necrosis.

Extensive literature search documented only 5 cases of single pseudo aneurysm of middle colic artery, however, our patient had multiple such pseudo-aneurysms in only middle colic artery which is not found in literature[2-4].

Almost all cases of pseudo-aneurysms hence are symptomatic and display greater risk of rupturing than true aneurysms. These generally present with abdominal pain in 62% patients, however, they have a tendency to bleed into pancreatic pseudocyst or the gastrointestinal tract in approximately 30% of patients and present with hypovolemic shock, fall in haemoglobin, melena. Though a CECT can clinch the diagnosis, however angiographic Coil embolisation being the preferred technique with a success rate of almost 90 % in higher centres[5].

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Case Report

A Case of Sturge Weber Disease with Portal Hypertension

Debasmita Biswas[1], Manjari Saha[2], Abhinav Das[3]

Sturge Weber Syndrome is a rare congenital disorder which is characterized by presence of angiomas that involves the face most commonly over the distribution of ophthalmic and maxillary division of the trigeminal nerve and the leptomeninges leading to central nervous system malformation. The disease is characterized by port wine nevus over the face, focal seizures and glaucoma. The disease results from the malformation of the cerebral vasculature which leads to hypoxia in the cortex causing neurological damage.

Portal venous malformations and portal cavernoma are quite rare features of Sturge Weber Syndrome which can lead development of portal hypertension and its complications like gastrointestinal bleeding and hypersplenism.

Here, we shall discuss about a case of a female young mentally retarded patient who presented to us with seizure and anemia

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Key words **: Congenital, Malformations, Portal hypertension**

Sturge Weber Syndrome belongs to congenital neurocutaneous syndromes characterized by abnormal growth of ectodermal tissue, producing distinct skin lesions and malformations and tumours of the nervous system[1]. The hallmark of the disease is a characteristic port wine nevus over the face supplied by the ophthalmic and maxillary divisions of the trigeminal nerve. The facial nevus is usually unilateral[2], rarely bilateral[3]. Associated neurological findings include seizures, intellectual sub normality, visual loss due to glaucoma, focal signs like hemiparesis and sometimes soft tissue hypertrophy[4]. Association with intestinal hemorrhage is also possible[5].

Portal hypertension is an extremely rare condition associated with the syndrome. A Spanish report presented a case of recurrent hematemesis in a patient of Sturge Weber Syndrome from fundal varices as a result of portal hypertension[6].

Case Report

A 16-year-old non diabetic non hypertensive female presented to us with complaints of recurrent episodes of jerky movements suggestive of seizure since 2 years of age, poor scholastic performance less social interactions, failure to attain menarche and generalized weakness for 6 months. The episodes of seizures began when she was 2 years old. Initially one episode occurred every 2-3 months. The abnormal movements used to start in the left side of the body but later used to involve the whole body with loss of consciousness. The seizures became more frequent subsequently and she started having seizures even on medications. There was also complaint of less social interactions. Her scholastic performance was poor from early childhood and stopped going to school after a couple of years. She could not read and write. There were also complaints of reduced feeding for past 5 to 6 months with feeling of generalized weakness and two episodes of passing black coloured stool. No history of vomiting, pain abdomen, jaundice. She did not start menstruating. History of a reddish patch over the right forehead and upper eyelid was there. Apart from the seizure and features suggestive of mental retardation, no significant past history was present. She has 3 other sisters in her family. They are all healthy without any mental retardations or seizures.

Editor's Comment :

- As portal hypertension is a rare manifestation of a rarer disease it may be overlooked.
- High index of suspicion is needed to intervene early.

On examination, the patient was alert, conscious and did not interact much. She could talk a few sentences and could recognize her sister. She was poorly nourished. On facial examination apart from temporal hollowness and malar prominence a distinct maroon colored patch was seen over her right forehead, eyebrows upper and lower eyelids extending up to the nasal side of right face. Significant pallor was found but her vitals were normal. Apart from the higher mental function derangements as described above no other neurological abnormalities were found. On abdominal examination, spleen was palpable, non-tender about 5 cm below the left costal margin. It was firm in consistency, had a smooth surface with well-defined margin. Liver was non palpable and no other abnormalities were found.

After history and clinical examination, we kept a working diagnosis of Sturge Webber Syndrome. But we were also dealing with the issues of anemia,

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splenomegaly and primary amenorrhea.

On complete blood count we found significant decline in all the cell lines with hypochromia and microcytosis. The reticulocyte count was normal. We found hypoalbuminemia in biochemical investigation. Abdominal ultrasonography suggested an enlarged spleen (17cm).

These findings were suggestive of pancytopenia possibly due to hypersplenism and portal hypertension was kept as a possibility of hypersplenism.

An iron profile showed low serum iron and transferr in saturation. An HPLC ruled out Thalassemia and vitamin B12 and folate levels were within normal limit. Further we did a bone marrow aspiration cytology which suggested reactive marrow with erythroid hyperplasia. MP/MPDA and an rK39 ruled out any remote possibilities of Kala Azar and Malaria. Meanwhile, on NCCT brain dense gyral pattern of calcification was seen in the right occipito-temporal cortex with dilatation of sulci – a clearly suggesting Sturge – Weber Syndrome. As per advice of endocrinologist serum FSH/LH were done and the results were normal. At this point, we assumed that the primary amenorrhea was probably a consequence of pancytopenia. Keeping portal hypertension in mind we did Prothrombin time which came to be slightly deranged, ie, 4 secs over the control with an INR of 1.38. An MRI brain was done for the primary disease and apart from atrophy in the right occipeto-temporal region a curious finding of signal change (hyperintensity inT1) was noticed in Basal Ganglia region bilaterally which further suggested liver involvement. Due to all these suggestions, we promptly did an upper G.I. endoscopy where Grade II esophageal varices were found. We also did a CECT abdomen which showed only splenomegaly but did not comment on portal vein. CECT whole abdomen showed a normal uterus and ovaries, thus ruled out any structural cause of amenorrhea. For proper visualization of the portal veins we finally went for an MR portovenography and expert opinion of a radiologist was sought. The imaging was suggestive of portal vein dilation with possibility of portal cavernoma. According to the radiologist, the dilation had probably extended deeper inside the hepatic parenchyma. The patient was treated conservatively, antiepileptics were given to control her seizures and was discharged in a hemodynamically stable state.She was advised to attend gastro medicine and gastro surgery for opinion regarding management of portal cavernoma and was asked to follow up in our department (Figs 1-4).

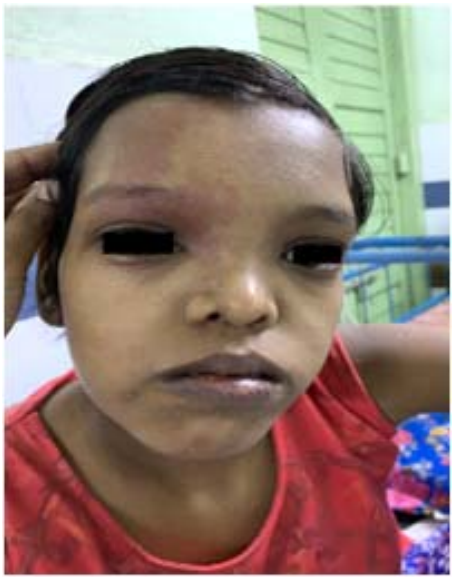

Fig 1 — Patient

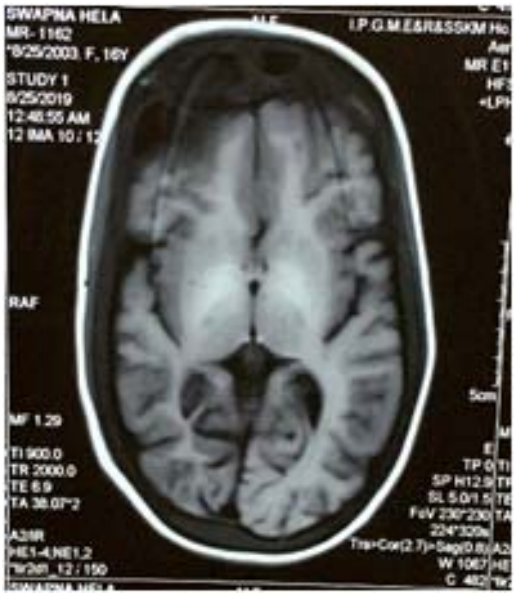

Fig 2 — MRI showing Atrophy and Basal Ganglia Hyperintensity

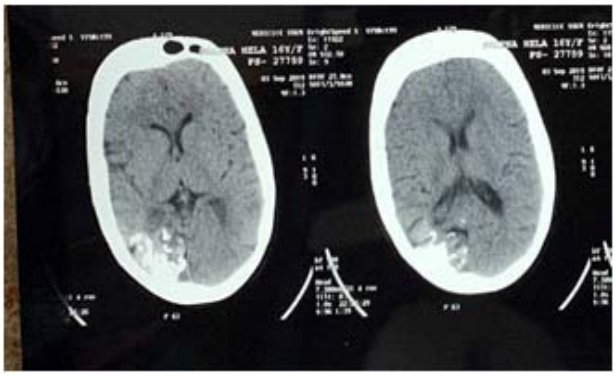

Fig 3 — GyriformCalcification in Right Occipeto-temporal Region

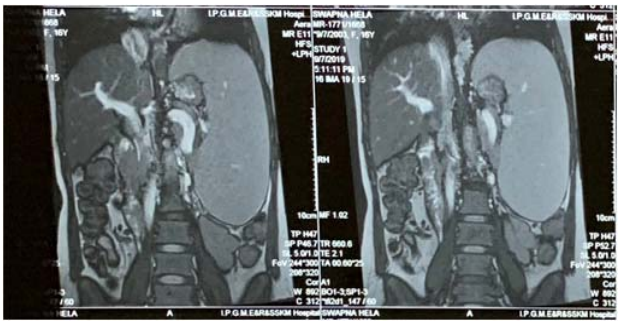

Fig 4 — MR Portovenography Showing Dilated Portal Vein

Investigations

Complete Blood Counts :

	5/9/19	2/9/19	28/8/19	24/8/19	26/8/19	23/8/19
Hb%	4.5	4.4	4.9	4.9	4.9	5.1
PCV	18.1	17.6	19.9	20.6	19.9	20.5
MCV	67.3	64.7	66	68.9	67.5	
MCH	16.7	16.2	16.4	16.4	16.6	
MCHC	24.9	25.0	24.6	23.8	24.6	
RDW		19.4	19.7		19.8	
RBC		2.7	2.9		2.95	
WBC	2000	1000	1700	1400	1300	1800
Neutrophil	55	48	50	39	49	42
Lymphocytes	40	42	43	53	47	46
Eosinophils	03	08	04	03	03	08
Monocytes	02	02	03	05	01	04
Basophils	00	00	00	00	00	00
Platelets	80000	150000	64000	90000	55000	76000

Biochemical Parameters :

	4/9/19	2/9/19	28/8/19	24/8/19
T BIL	0.6	1.05	0.8	1.6
D BIL		0.6		0.6
T Protein	6.1	6.24	4.6	6.5
ALB/GLOB	3.2/2.9	3.43/2.81	2.5/2.1	3.0/3.5
AlkPO4	76	85	82	86
ALT/AST	24/29	12.5/33.7	18/28	11/28
UR/CR	27/0.7	12.6/0.71	13/0.4	25/0.7
Na/K	137/3.2	139/3.5	139/3.4	145/3.7
Ca				7.2
iPO4				1.8
RBS				78

Iron Profile: (2/9/19)
Serum Fe : 28 Percentage Saturation: 7.7
TIBC : 364 Prothrombin Time : (28/8/19)
PT : 16 sec INR: 1.38 Control : 12.2sec

Discussion

Sturge Weber Syndrome (SWS) also known as encephalotrigeminal Angiomatosis is a rare congenital disorder that occurs due to malformations of the cerebral blood vessels located in the pia mater, most commonly over the occipital region. The disease is commonly unilateral and rarely bilateral. It affects males and females equally. The incidence is 1 in 50000 live births[5].

SWS is caused by the persistence of embryonic blood vessels within the developing cephalic part of neuroectoderm beyond 9 weeks of gestation. These persisting immature vessels cause hypoxia, ischemia, venous occlusion, infarction and calcification which result in neurological dysfunctions like seizures, hemiplegia and mental retardation. The skin is affected because of the persistence of the vessels which may be associated lesions in the choroidal vessels of the eye.

Portal hypertension is a rare feature of SWS which is associated with portal venous malformation and hence the diagnosis is often missed.

Therefore, early clinical suspicion with proper imaging is necessary for the diagnosis of this rare condition in patients with Sturge Weber Syndrome.

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Case Discussion in Medicne

Locally Advanced Gastric Cancer

Samir Bhattacharyya[1], Arnab Gupta[2], Saradindu Ghosh[3]

Gastric cancer is the third most common cause of cancer-related death in the world. Most cases present in advanced stage in non-endemic areas due to a lack of screening programmes. Multidisciplinary treatment (surgery along with chemotherapy with or without radiotherapy in adjuvant or neoadjuvant schedule) forms the basis of present-day management protocol for advanced non-metastatic gastric cancer. Here we discuss the diagnosis, staging work-up and treatment of locally advanced non-metastatic gastric cancer in a 55 years old patient treated with perioperative chemotherapy and surgery. Important trials that have a bearing in the management are also presented.

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***Key words* : Gastric cancer, staging, multidisciplinary management.**

55 years, male patient presented with chief complaints of pain upper abdomen for last 1 year (usually after meals) and difficulty in swallowing solid food for last 5 months with occasional nausea but no vomiting. He complained of significant weight loss (more than 8-10 kg) over last 4-5 months. His stool colour was generally normal but occasionally blackish but not tarry. Patient was a smoker (~20 bidis a day) and an occasional drinker. There is no known co-morbidities eg. diabetes, hypertension, obstructive respiratory disease. He underwent appendectomy at age of 17 years. He had no other past history of major illnesses or hospitalisation. No significant family history relating to his symptoms.

On examination : ECOG-1 (Eastern Cooperative Oncology Group); no anaemia or oedema of the lower limbs, vitals within normal limits. Abdomen was soft, non-tender with no organomegaly or palpable lump. No ascites was detected. No significant lymph nodes were palpable in node-bearing areas.

Provisional diagnosis : Considering the patient's age, presenting symptoms (eg, dysphagia, weight loss, pain abdomen and occasional black stool) and a history of smoking and alcohol intake, esophageal/proximal stomach malignancy is the likely diagnosis. Differential diagnoses are enumerated below.

Differential diagnosis :

Benign Diseases —

Esophagitis
Gastritis
Peptic Ulcer Disease
Esophageal Varices

Malignant Diseases —

Gastric or esophageal carcinoma
MALT (Mucosa Associated Lymphoid Tissue) lymphoma of the stomach
Primary gastric lymphoma (non-MALT type)
Gastro-intestinal Stromal Tumor (GIST)

Editor's Comment :

- Stomach cancer is the 5th most common cancer worldwide and third most common cause of cancer-related death.
- In early stages, when it is at the most curable state, gastric cancer has very few symptoms. Hence, clinicians should be aware to the possibility of gastric cancer, especially if risk factors are present.
- Upper GI endoscopy is diagnostic.
- CECT is the most useful imaging modality, though PET-CT has added value in non-mucinous or non-diffuse gastric cancer.
- Perioperative chemotherapy improves outcome in locally advanced gastric cancer.
- Margin free resection with an adequate lymphadenectomy (more than 15 lymph nodes) is the surgical goal.

Work-up :

Components —

Thorough history and physical examination, laboratory testing, diagnostic imaging and invasive tests (eg, endoscopy).

Risk assessment for gastric cancer —

Environmental factors: H pylori, smoking, alcohol

Family history suggestive of some hereditary cancers (eg, hereditary diffuse gastric cancer, Lynch Syndrome II, BRCA2 mutation and familial polyposis coli).

Physical findings :

Early stages : few significant physical findings

Localy advanced or metastatic :

Palpable upper abdominal mass (from a large primary or liver secondaries)

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Lower abdominal mass (from omental or ovarian mass – Krukenberg's tumor)

Gross ascites

Enlarged left supraclavicular node (Virchow's node)

Periumbilical nodule (Sister Mary Joseph node)

Pelvic deposits on rectal examination (Blummer's shelf)

Jaundice (from obstruction of extrahepatic biliary tract or extensive liver metastasis)

Paraneoplastic syndromes associated with gastric cancer :

- Acanthosis Nigricans
- Thrombophlebitis
- Circinate erythema
- Dermatomyositis
- Pemphigoid
- Seborrheic keratosis

Where malignancy is suspected, the aims of the workup are :

Establishment of diagnosis

Clinical staging (ie extent of disease as can be determined by clinical and radiological investigations)

Fitness of the patient regarding the proposed line of treatment.

Diagnosis: Upper gastrointestinal endoscopy is the modality of choice. It is highly accurate (~98%). Apart from tissue diagnosis, the procedure helps in determining the extent of the disease, any obstruction, and in early cases, endoscopic ultrasound can be utilised to consider suitability for endoscopic resection.

Case capsule continued :

Upper GI endoscopy (Fig 1)

Cricopharynx/Esophagus: normal

Proximal body of stomach: edematous mucosa with large ulcer with clean base

Normal antrum and pylorus

Duodenal bulb: normal

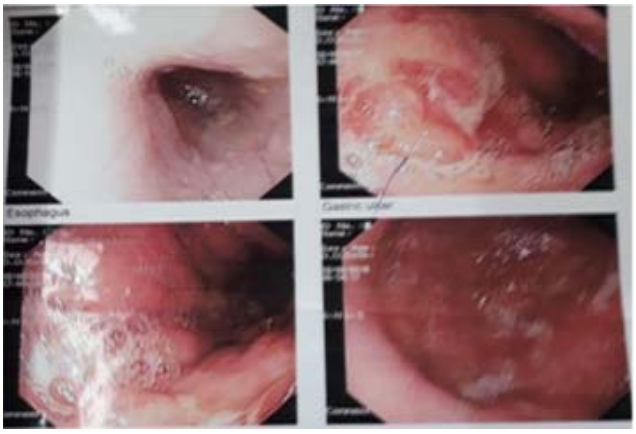

Fig 1 — Upper GI endoscopy

Biopsy report (Fig 2, H&E, x 100) : Mucin secreting adenocarcinoma

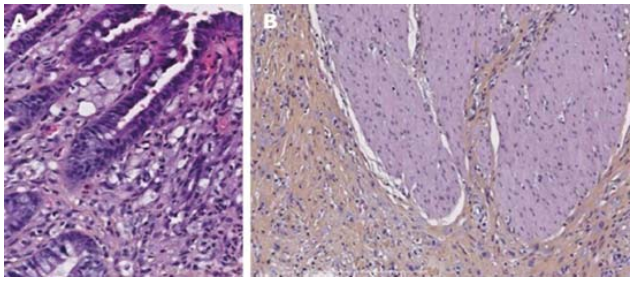

Fig 2 — Biopsy report

Clinical staging :

Evaluates

Local extent of the tumor

Resectability

Lymph node involvement

Presence of metastasis

Imaging modalities :

Contrast Enhanced Computerized Tomography (CECT) scan: most commonly used

Magnetic Resonance Imaging (MRI) : accuracy similar to CECT

Positron emission tomography-CT scan (PET-CT)

Endoscopic Ultrasound (EUS) : useful in early cases

- Value of imaging limited in detecting peritoneal deposits or small (<1 cm) liver metastases
- PET-CT : very useful in staging in advanced gastric cancer, especially in detecting distant metastases.
- Mucin secreting gastric cancer is not PET-avid, hence PET-CT does not have any added benefit over CECT in these cases.

Case capsule continued : A CECT of chest, abdomen and pelvis was performed, which showed a large proximal stomach mass with a significant lymph nodal involvement, staged as cT3N+M0 (Fig 3 & 4)

Role of diagnostic laparoscopic staging :

Currently recommended in cT3 or above, and/or cN+ M0

Detects small metastases (<0.5 cm) of the peritoneum and liver, leading to upstaging in up to 40% patients

Laparoscopic peritoneal lavage, if positive for malignancy is considered as M1 and as such not operable upfront

Case capsule continued : The patient underwent diagnostic laparoscopy. No peritoneal, liver or other organ deposits were detected and the peritoneal lavage did not show the presence of malignant cells.

Multidisciplinary approach to gastric cancer: Surgery is the cornerstone of treatment of localised gastric cancer. However, multidisciplinary approach leads to a better

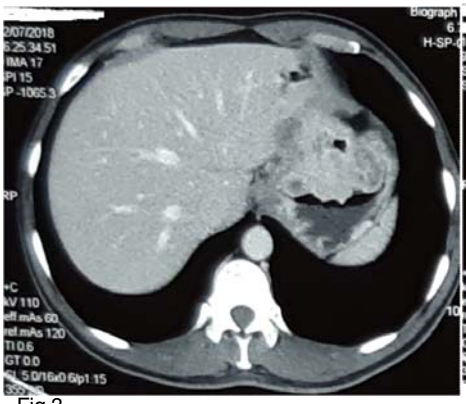

Fig 3

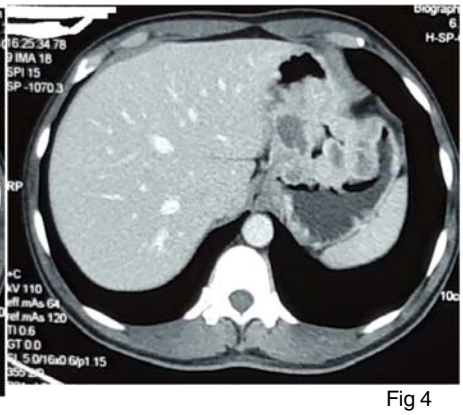

Fig 4

Fig 3 & 4 — Pre-chemotherapy CECT of abdomen

outcome. Standard multidisciplinary approaches are:

(1) Adjuvant Chemoradiotherapy (CRT): based on Intergroup INT-0116 trial that showed a better over all survival, as well as recurrence free survival with adjuvant chemoradiation[1]. It formed the basis of acceptance of adjuvant CRT in the US and Canada. However, questions were raised regarding the quality of surgery in this trial. The South Korean ARTIST trial did not show any added benefit of adjuvant CRT over adjuvant chemotherapy alone[2].

(2) Adjuvant chemotherapy: Mostly used in the East. This approach is based mainly on two trials. First of these is the Japanese ACTS-GC trial that showed that S1 monotherapy for 1 year after surgery led to a better over-all survival with a low incidence of side effects[3]. The second one is the South Korean CLASSIC trial that reported a significant lowering of the relative risk of relapse with adjuvant capecitabine/ oxaliplatin regime administered for 6 months postoperatively[4].

(3) Perioperative chemotherapy: Used in Europe and the rest of the world. The approach is based on several trials. The first one is the British Medical Research Council's landmark MAGIC Trial that showed that perioperative chemotherapy with Epirubicin, Cisplatin and 5-fluorouracil (ECF) achieved a better over-all and progression free survival compared to surgery alone[5]. Another pivotal study recently reported is the German FLOT4-AIO Phase 3 trial that compared perioperative ECF (Epirubicin, Cisplatin and 5-Fluorouracil or ECX (EC + capecitabine) to another perioperative systemic therapy regimen, FLOT (5-Fluorouracil, Leucovorin, Oxaliplatin and docetaxel). The FLOT arm showed improved progression free and overall survival, along with more frequent pathologic complete response (ie. no viable tumour cell in resected specimen). This regimen however has a significant side effect profile compared to ECF/ ECX regime[6].

Case capsule continued : The case was discussed in the multidisciplinary tumour board and the patient received perioperative 3 cycles of ECX regime. After 3 cycles he was restaged with CECT chest, abdomen and pelvis that showed a good response (Fig 5 & 6).

Case capsule continued: the patient underwent a total gastrectomy with Roux-en-Y reconstruction. The biopsy report revealed mucus secreting adenocarcinoma, diffuse type, ypT2N2 status with 3/26 nodes positive, no LVI/PNI, both proximal & distal margins free. Patient received a further 3 cycles of the same chemotherapy regimen. On completion, he was put on follow up. He remains disease free at the last follow up at 4 years.

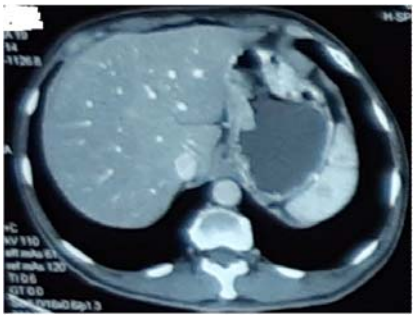

Fig 5 — Postchemotherapy CECT scan

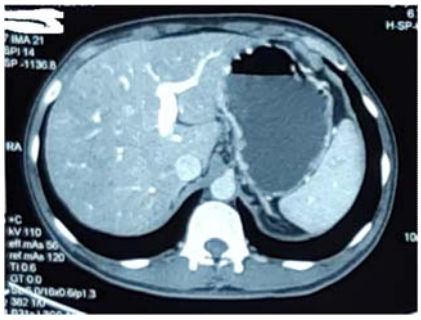

Fig 6

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Voice of the Expert

The Technology must be Accessible and Affordable to All

(1) Now being a Robotic Surgeon, Do you feel Robotic Surgery is at all necessary ?

Certain operations cannot be done by conventional approach. Enhanced tissue detection particularly cancerous tissue and newer instruments for better manoeuverability has essential for safe surgery. Robotic assisted surgery mean performing surgery using computer-technology enhanced device not by any kind of humanoid robots. Computer Programmed instruments with multiple joints like wrist provides wide range of instrument movements. Well controlled tip movements, Enhancing dextrity improves precision in surgery. Also enhances surgical skill otherwise limited in laparoscopy. Physiological tremor filtering effect improves safety. 3 D real time HD vision provides wider operating field.

(2) How technology improves Patient care?

Robotic assisted surgery allows perfect tissue identification enabling accurate surgery.

1. Improves radicality in cancer clearance.

2. Improved tissue detection reduces functional disability. 3 D vision and robotic assisted surgery prevents presacral nerves injury avoids urinary retention and sexual dysfunction during rectal cancer resection. During esophageal cancer surgery recurrent laryngeal nerve injury is minimised and loss of voice and need for tracheostomy are prevented.

3. Delicate reconstruction such as urethral and biliary reconstruction effectively can be done perfectly using robotic system.

(3) 'Machine' or 'Man behind the machine' which is more important ?

Robotic assisted surgery is purely depends on surgeon's skill and expertise. Surgical planning and execution of operative techniques purely by surgeon. Quality of surgery and type of surgery entirely left to surgeon skill and expertise. Robotic surgery only enhances surgical skill and precision in surgery. In oncological surgical procedures functional and oncological outcome are equally important.

(4) What is your opinion about necessity of clinical examination? Is technology can take all?

Clinical examination is very essential. Based on clinical diagnosis specific investigations are done and planning of treatment is worked out. Making the patient understand the disease is very important to achieve the maximum outcome by making confident.

Prof. C. Palanivelu
Gastrosurgeon
MS, MCh, DNB, FACS, FRCS(Hon) Ed.
Chairman, GEM Hospital and Research Centre
Dr BC Roy National Awardee

(5) Though Laparoscopy is gold standard and has changed surgical care, this is not accessible to all Indians. How we can achieve it?

Laparoscopy revolutionised the surgical approach. Laparoscopy has become the standard of care for many surgical diseases. Laparoscopy facility is available only in limited centres. Even in centres laparoscopy available due to lack of training many surgeons are not utilising laparoscopic surgery. Due to low socioeconomic strata also many are not affordable to get laparoscopic surgery in private institutions because of the higher cost. Cost effective methods have been developed, need structured training to surgeons both in basics and advanced laparoscopic procedures. Similar to other countries we need public insurance scheme.

(6) To adopt any technology do you feel it requires a structured training pathway ?

Laparoscopic surgery depends on endoscopic view totally different from conventional surgery. Newer instruments and advanced energy devices are available. Structured training enhances the safety of the procedures, reduces complications and thereby reduces overall treatment cost.

(7) Basic Laparoscopic Surgery can be trained in Medical Colleges during PG Course. Will you have any suggestions to implement this?

Basic laparoscopic surgery can be effectively

implemented in MS training. all teaching institutions should have surgical skill lab. Step by step training in acquiring surgical skill should be part of the teaching curriculum. Laparoscopic surgery can be learnt using simulation models and working on animate tissue. Basic laparoscopic surgery has become the standard of care, lot of apprtunites available for postgraduate trainees to assist and finally may be made to perform basic procedures before completing residency.

(8) Newer technology means escalation of cost of healthcare. How we can make technology affordable for all?

Capital cost and maintenance cost may add to cost of healthcare. But saved by reduced hospital stay, reduced medicine and more number of patients may be treated in the limited number of hospital beds. Enhanced utilisation of equipments and advanced devices save overall cost. Patients getting of faster recovery and resume routine work early. Newer technology reduces operation theatre time significantly saving overall operation cost.

(9) What are the upcoming technology that will change the present healthcare?

a) Firefly technology using ICG aids in cancer tissue detection and enhances complete clearance there by reduces recurrence and improves survival.

b) Single port surgery is becoming popular. By reducing ports pain is less and incision related problems are minimised.

c) 3D print - pre operative print helps in surgical planning of tumor excision.

d) Augmented Reality provides real-time imaging during surgery. Augmented Reality in Robotic surgery using Tile-pro to visualise the intraoperative anatomy overlapping the 3D models aids Navigational surgery.

e) AI -Artificial Intelligence.

f) Remote surgery- operating from distance using Robotic surgery.

g) Intelligent Knife - recognising cancerous tissue during surgery.

AIM of surgery :

Future surgery is with new technology enhanced power, high precision in surgery and without functional disability.

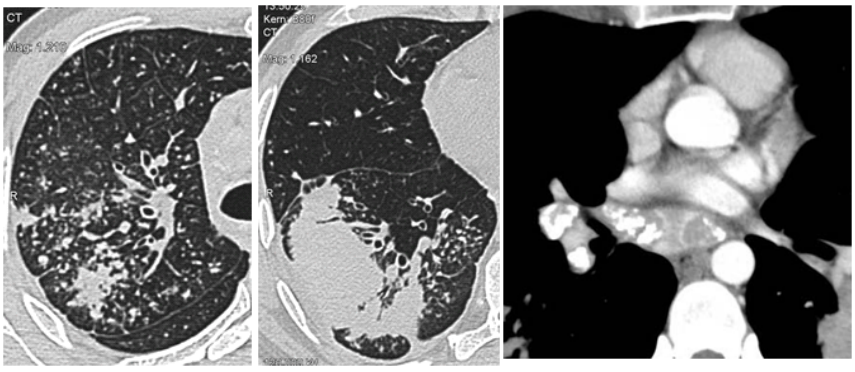
Prof. C. Palanivelu, thank you for the valuable insight into New Technology of Robotic Surgery

Image in Medicine

Bhoomi Angirish[1], Bhavin Jankharia[2]

Quiz 1

CT scan images of the chest of a 51-year-old man with gradually progressive breathlessness



Questions :

(1) What is the diagnosis?
(2) What are the imaging features of silicosis?
(3) What are the differential diagnosis of egg-shell pattern of calcification of lymph nodes ?

Answers :

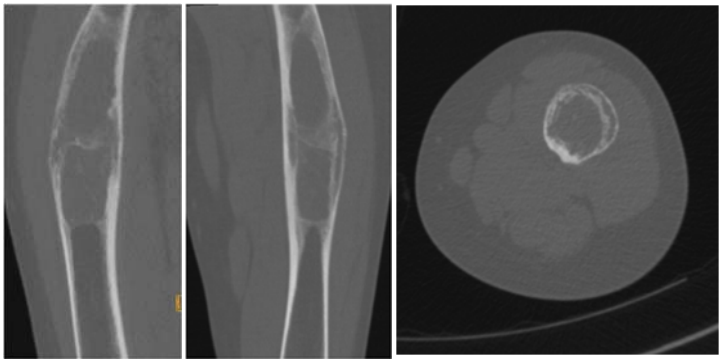
(1) Multiple centrilobular nodules and confluent dense opacities are seen, predominantly in central distribution in lungs with enlarged mediastinal and hilar lymph nodes showing egg shell pattern of calcification. Findings are in favour silicosis.

(2) Numerous bilateral centrilobular nodules and consolidation are seen in both the lungs, with calcification of nodules. There is predominant upper lobe involvement and perihilar distribution. Calcified hilar and mediastinal lymph nodes are also seen.

(3) Egg-shell pattern of lymph node calcification is seen in silicosis, sarcoidosis, coal workers' pneumoconiosis, treated lymphoma. Other rare causes are amyloidosis, histoplasmosis.

Quiz 2

A 12-year-old girl presented with painless swelling involving mid thigh since 6 months.



Questions:

(1) What is the diagnosis?
(2) What are the common locations of this lesion?
(3) What are the common associations of fibrous dysplasia?

Answers :

(1) There is bony expansion, remodelling and ground glass matrix involving diaphysis of femur. These imaging findings favour diagnosis of fibrous dysplasia (FD), which was confirmed on biopsy. Fibrous dysplasia is a non-neoplastic tumour like process with replacement of normal bone with fibrous stroma and immature bone.

(2) FD can present as monostotic form (involving only one bone) which is more common or polyostotic form (involving multiple bones). FD usually involves ribs, proximal femur, tibia, craniofacial bones. Polyostotic form is usually unilateral and monomelic.

(3) FD is associated with

(A) McCune-Albirght syndrome – which presents as polyostotic FD with endocrinopathy.

(B) Maxabraud syndrome –FD with soft tissue myxomas.

Department of Radiology, Picture This by Jankharia, Mumbai, Maharashtra 400004

[1]MD, DNB (Radiology)

[2]MD, DMRD (Radiology)

Student's Corner

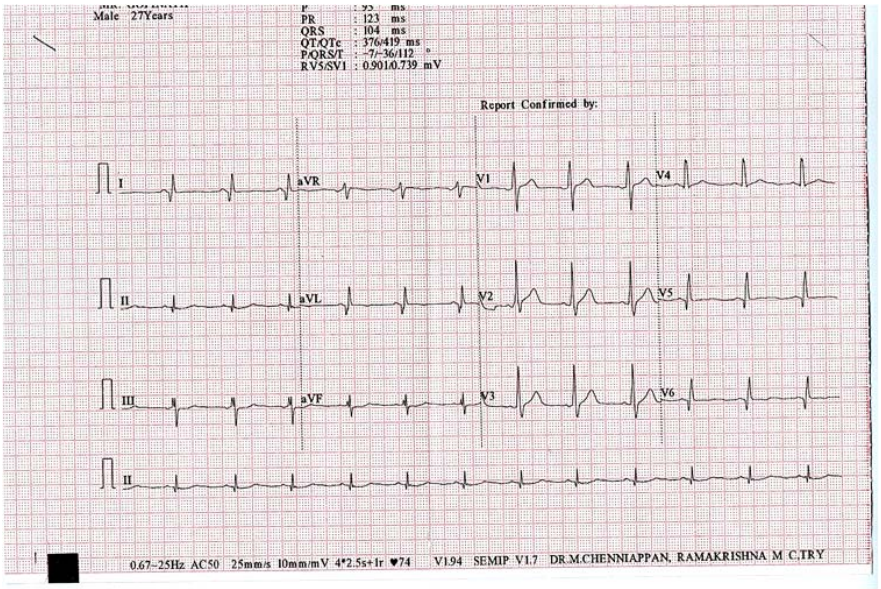
Become a Sherlock Holmes in ECG

M Chenniappan[1]

Series 7 :

"Muscle in Dystress"

This is the routine ecg of 27-year-old male with no risk factors



Questions :

(1) What are the ECG changes?

(2) What is the differential diagnosis for this ECG ?

(3) Why is the clue?

(1) What are the ECG changes ?

The ECG shows Tall R wave in V1. There is deep and Broad Q waves in anterolateral and high lateral leads. There is left ward Axis. There is fractured QRS in L II, L III, avF

(2) What is the differential diagnosis ? (See table)

As the QRS is narrow, the causes of Tall R wave with wide QRS are excluded. Coming to Tall R waves with narrow QRS this is not RV Hypertrophy as the axis is not right and there is no right atrial enlargement. This is not dextrocardia as the P and QRS are upright in L I; Patient is not cyanosed and there is no ecg signs of single ventricle. The ECG is recorded properly.

It is unlikely to be Hypertrophic cardiomyopathy (HCM) as the QRS voltage is normal. Usually the septal Q in lateral leads is deep and narrow in HCM. Here the Q in lateral leads and broad and deep. So, the DD boils down to lateral and posterior wall MI (PWMI) Vs Deschene Muscular Dystrophy (DMD). Here the PWMI is not the possibility as the age of the patient and risk factors are against. So, this ECG is likely to be due to DMD. In DMD, the tall R in V1 and deep broad Q waves are due to fibrosis of postero lateral wall. The fractured QRS in inferior leads is suggestive of LV dysfunction.

(3) Why is the clue?

"Muscle in dystress" is given to indicate it is a myopathy. The intentional wrong spelling of "dystress" instead of distress is a clue towards muscular 'dystrophy'. The ecg signs of DMD are sinus tachycardia, QT interval abnormalities, supraventricular and ventricular arrhythmias as well as conduction disturbances. In addition to arrhythmias, autonomic nervous system abnormalities may lead on to sudden cardiac death.

SUMMARY

TALL "R" IN V1

NARROW QRS	WIDE QRS
POST WALL MI	RBBB
RVH	WPW –TYPE A
HCM -ASH	AIVR / V-TACH
DEXTROCARDIA	BRUGADA
WRONG LEAD PLACEMENT	EPICARDIAL PACING FROM LV
SINGLE VENTRICLE	ASD
DUSCHENE MUSC.DYSTROPHY	

[1]Adjunct Professor, Dr MGR Medical University, Tamilnadu; Senior consultant cardiologist, Tamilnadu; Ramakrishna Medical Centre, Apollo Speciality Hospital, Trichy

Mediquiz - 07 / 2021

Paediatrics

Dr Nilanjan Ghosh,
Assistant Professor, Department off Pediatric Medicine, RG Kar Medical College, Kolkata

(1) A mother of a 2-week-fullterm baby noted doll's-eye movement of her baby eyes. Of the following, The MOST appropriate next action is to

A. reassures her by informing her that, this is a normal reaction
B. refer the baby to an ophthalmologist
C. takes a detailed history of perinatal period
D. order brain ultrasound
E. order brain MRI

(2) A mother to a 4-year-old child who has pauses and repetitions of initial sounds visited outpatients department. Of the following, the MOST appropriate advice is

A. this is a normal phenomenon affecting about 5% of preschool children
B. there is no need for action as 80% of affected children recover by their own
C. tries to reduce pressures associated with speaking
D. I'll refer him to ENT specialist for further evaluation
E. I'll refer him to speech therapist

(3) A 7-year-old boy presented with recurrent eye blinking behavior and recurrent extension of extremities, mother describe the movement as sudden, rapid, and repetitive movements, it was present in the last 9 months. Of the following, the MOST appropriate diagnosis is

A. Tourette's disorder
B. persistent motor tic disorder
C. provisional tic disorder
D. post-viral encephalitis
E. Sydenham chorea

(4) A mother to a well 2-year-old girl with thumb sucking behavior, she is worried that the behavior may continue or may cause dental problem. Of the following, the BEST response is to

A. reassurance to mother
B. leave the behavior as the complications usually started after 5 years
C. ignore thumb sucking and encouraging a substituted behavior
D. use of bitter ointments will resolve the problem early
E. asses the social status of the family

(5) You are evaluating a 5-year-old child with breath holding spells, the history given includes pallor with abnormal limb movement lasted for 5 minutes followed by sleep. All the following are true responses/advices EXCEPT

A. reassurance, behavioral instruction to parents and follow up
B. order MRI brain
C. order an ECG
D. order an EEG
E. neurological consultation

(6) A concerned young parents asking about frequent lying behavior experienced by their 3-year-old girl. The following advices are true EXCEPT

A. it is a method of playing with the language
B. it is a part of their magical thinking
C. it indicates a potential for future lying behavior
D. it is an approach to avoid unwanted confrontation with adults
E. it is a way to describe things as they wish

(7) The MOST consistent statement of structural MRI brain finding of autistic spectrum disorders (ASD) is

A. diffuse brain atrophy
B. increase brain size
C. focal fibrosis
D. white matter degenerative changes
E. gray matter degenerative changes

(8) You are assessing an eight-year-old male child with attention-deficit/hyperactivity disorder (ADHD). Of the following, the LEAST useful test/ investigation is

A. thyroid function test
B. lead level
C. EEG
D. Blood film
E. polysomnography

(9) Regarding breast engorgement; All the following are true EXCEPT

A. usually happens in the first stage of lactogenesis
B. poor breast feeding technique can cause engorgement
C. breastfeeding immediately at signs of infant hunger will eventually prevent this
D. to reduce engorgement, breasts should be softened prior to infant feeding with a combination of hot compresses and expression of milk
E. between feedings, cold compresses applied, and oral nonsteroidal anti-inflammatory medications administered

(10) Regarding Glasgow Coma scale in pediatrics, all the following are true EXCEPT

A. in modified type it uses 15 score points
B. it has 3 components
C. valid as a prognostic scoring system
D. score less than 8 require aggressive management
E. verbal response component has 5 possible points

(11) Of the following, the mandatory test/study for all patients presenting for the first time with syncope is

A. ECG
B. EEG
C. echocardiography
D. holter monitoring
E. complete blood coun

(12) Soft areas in the occipital region suggest the irregular calcification and wormian bone formation usually associated with the following conditions EXCEPT

A. osteogenesis imperfecta
B. craniosynostosis
C. cleidocranial dysostosis
D. cretinism
E. Down syndrome

(13) One of the following drugs may cause pyloric stenosis if administered to a premature infant

A. intravenous vitamin E
B. indomethacin
C. enteric gentamicin
D. prostaglandins
E. dexamethasone

(14) The best "Rescue " medication in the treatment of acute asthma symptom is

A. oral SABA
B. inhaled SABA
C. oral corticosteroid
D. inhaled ipratropium
E. inhaled corticosteroid

(15) Delayed eruption of the primary teeth can be due to the following EXCEPT

A. familial
B. hypopituitarism
C. hyperthyroidism
D. cleidocranial dysplasia
E. trisomy 21

(Answer Page 93)

Special Article

Challenges in Management of Surgical Site Infections — Lessons Learnt

Y Muthuswaraiah[1], Amjad Mallik[2], Anshu Agarwal[3], Pravin Kesarkar[4], Srivatsan V[5], Umesh Shah[6], Krishna Chaitanya Veligandla[7]

Surgical site infections develop due to contamination of the surgical site with microorganisms.Depending on the extent of wound and bacterial load at the time of surgery, the surgical wounds are classified into different types.Antibiotics,wound protectors, antibacterial sutures and silver containing antiseptic topical agents and wound dressing have effective roles in preventing these infections.The application of NPWT in surgical infections helps in reducing postoperative wound complications. [*J Indian Med Assoc* 2021; **119(7):** 80-2]

Key words **: Antibacterial sutures, NPWT, Contamination,Surgical site infection SSI,Wound protectors.**

Surgical site infections (SSIs) develop as a result of contamination of the surgical site with microorganisms. The source is mostly patient's flora when integrity of the skin or wall of a hollow viscus is violated. Sometimes, the source may be exogenous when a break in the surgical sterile technique occurs that includes contamination from the surgical team, implants, equipment, gloves or surrounding environment. Gram positive cocci account for the half of the infections and *Staphylococcus aureus* is the most common organism. Hospital acquired MRSA is associated with nosocomial infections and affects immunocompromised patients. Gram negative bacilli account for the one-third of the SSIs and *E. coli, pseudomonas aeruginosa* and Enterobacter spp are the most common organisms causing these infections. The risk of infection is related to the specific surgical procedure performed (Table 1).

What are the types of surgical site wounds?[3]

The risk of SSIs is directly proportional to the extent of wound contamination. Depending on the extent of wound and bacterial load at the time of surgery, CDC classified the surgical wounds as clean, clean-contaminated, contaminated and dirty or infected wounds.These different types of wounds affect outcome of the surgery and post-surgery morbidity (Table 2).

Is there any role of prophylactic antibiotic in surgery? What should be the timing and choice of antibiotic?

Antibiotic levels should be maximum at the time of the induction of surgery. Prophylactic antibiotics are more commonly used in patients with immuno-compromised conditions and rheumatic heart disease. The timing of administration should be 0-60 minutes prior to induction or at the time of incision of the surgery. Choice of antibiotics entirely depends on the spectrum of the organisms which are likely to be encountered and also on the type of surgery.

Role of surgical intervention in SSI :

Wound protectors and antibacterial sutures seem to have effective roles to prevent SSI in intra-abdominal infections[4]. The application of NPWT in preventing SSI can be useful in reducing postoperative wound complications. It is important to pursue normothermia with the available resources in the intraoperative period to decrease SSI rate.

Iodine impregnated adhesive drapes probably make no difference to SSI risk compared with non-adhesive drapes[5]. There is probably no difference in SSI risk when antibiotics are given in the short term compared to the long term during colorectal surgery. One comparison showed that adhesive drapes increase the SSI risk compared with no drapes.

What are the new advancements in wound care products? Any use in post surgically high-risk patients?

For early treatment management of burn wounds, there are 3000 products and 30 different methods available in the market. The most commonly used are

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Received on : 25/06/2021
Accepted on : 06/07/2021

Table 1 — *SSI categorization as per CDC*[1,2]

SSI classification	Features
Superficial incisional SSI	• Infection occurs in <30 days after surgery • Usually involves skin and subcutaneous tissue • Symptoms include pain, local edema, erythema or purulent discharge
Deep incisional SSI	• Infection occurs in <30 days after surgery. • Soft tissue is involved • If surgery with implant, then deep incisional SSI occurs in <1 year after surgery involving deep soft tissues. • Symptoms include fever, pain, tenderness, leading to wound dehiscence or purulent discharge.
Organ space SSI	• Infection occurs in <30 days after surgery in patients without implant and in <1 year after surgery in patients with implant. • It involves any part of the operation opened or manipulated. • It may need rehospitalisation or IV antibiotic therapy.
Superficial or partly deep incisional SSIs	• Easy to manage on oral antibiotics • May not require hospitalization or intervention.

Table 2 — *Types of surgical site wounds*

Wound type	Characteristics
Clean wounds	• Uninfected operative, primarily closed wound without inflammation • Respiratory, alimentary, genital or uninfected urinary tracts are not entered. • The infection rate is around 1-3%.
Clean-contaminated wounds	• Operative wounds in which the respiratory, alimentary, genital or urinary tracts are entered. • Involves operations of the biliary tract, appendix, vagina and oropharynx. • Infection rate is around 5-8%.
Contaminated wounds	• Open, fresh, accidental wounds. • Operations with major breaks in sterile technique or gross spillage from the gastrointestinal tract • Infection rate is around 20-25%.
Dirty or infected wounds	• Old traumatic wounds with retained devitalized tissue, existing clinical infection or perforated viscera with pus in operative wound. • Open supportive wound and severe inflammation. • Infection rate is around 30-40%.

Silver Sulphadiazine such as Silver nitrate, Povidone Iodine and Nano Crystal Silver. Melonin sheet is a newer low adherent, absorbent dressing used for management of burns. Outer hydrophobic non-woven material is designed to resist fluid strike through, reduce bacterial access, provide structural integrity and resist external fluid.

Nanocrystalline silver releases sufficient and sustained levels of silver that are proven to be antimicrobial at 70-100 ppm. It remains effective for three days, increasing comfort and convenience for patient, especially for pediatric patients. The topical application of ionic silver kills pathogens and helps protect the wound from becoming contaminated or infected. Since the ionic silver destroys bacteria, viruses and mold, the wound does not get infected thus reducing the need for phagocytosis, which reduces the immune cascade. There are different forms

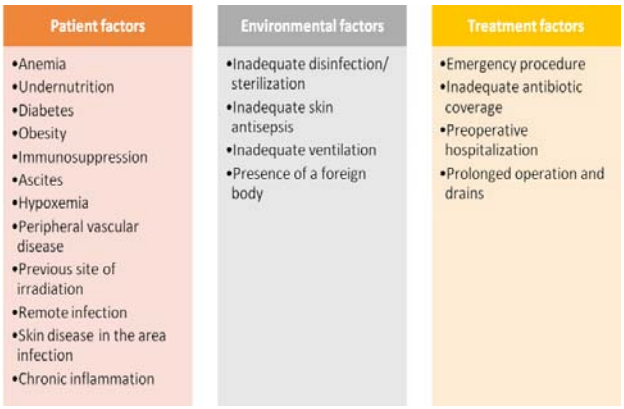


Fig 1 — Risk factors for surgical site wound infection

of Nanocrystal silver dressing materials available in the market. These include paper-based, net-based, foam-based and pet type. Among these, Silver Sulphate

absorbent foam dressing is the best form used for management of a wide range of acute and chronic wounds.

Presenter emphasized on the use of other newer techniques such as meek micro grafting and flexi seal-perineal burn care. Till today, flexi seal-perineal burn care is not used in India and he pinpointed the need of adopting this technology in India.

Role of NPWT for SSI?

In NPWT, the combined effect of macrostrain and microstrain promotes wound healing.

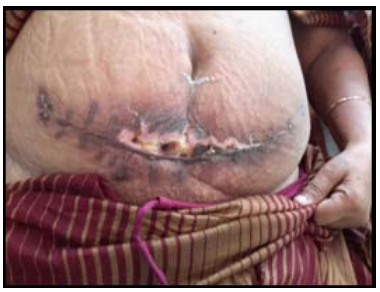
NPWT contraindications include:

- Foam dressings of the VAC therapy system directly in contact with exposed blood vessels, anastomotic sites, organs, or nerves.
- Malignancy in the wound
- Non-enteric and unexplored fistulas
- Necrotic tissue with eschar present
- Sensitivity to silver
- Untreated osteomyelitis

Case studies of surgical site infection:

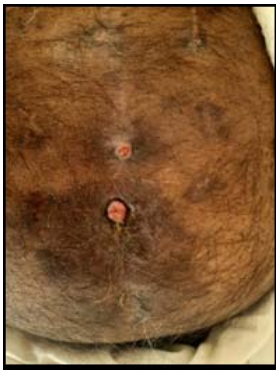
SSI after left modified radical mastectomy

Surgical site infection after incisional hernia repair with mesh



Sinus after right inguinal hernioplasty

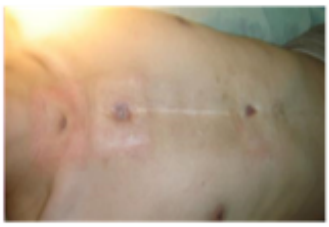
Sinus over abdomen after emergency laparotomy

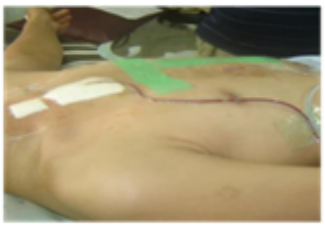


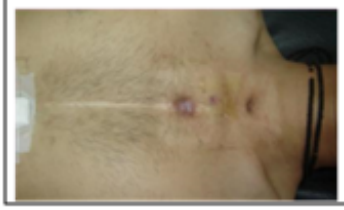
Which is the best postoperative dressing?

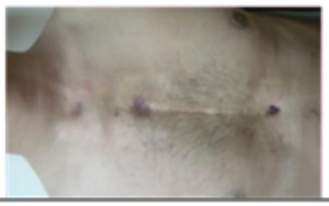
It depends on the surgeon's preference. According to the presenters, the silicon-based impregnated Nanocrystalline Silver dressings are the best one.

Chronic sinus wound post cardiac surgery: NPWT was applied after debridement and complete healing of the wound occurred in two sittings of 5-day therapy.









Summary :

The risk of SSIs is directly proportional to the extent of wound contamination. Different types of wounds affect outcome of the surgery and postsurgery morbidity. Wound protectors and antibacterial sutures can prevent SSIs in intra-abdominal infections. Silver nitrate, Povidone Iodine, and Nano crystal silver are commonly used for early treatment of burn wounds. Silver Sulphate absorbent foam dressing is the best dressing for management of a wide range of acute and chronic wounds.

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Special Article

[We are publishing this Special Article to commemotare World Hepatitis Day on 28th July]

Operational Guidelines of NVHCP for management of Hepatitis B

Ajoy Chakraborty[1], Pallav Bhattacharya[2], Nandini Chatterjee[3]

Viral Hepatitis is a global public health problem with deaths comparable to that caused by tuberculosis or HIV. Infection can be caused by the five known hepatitis viruses- A,B,C,D and E.While, Hepatitis B and C are transmitted by exchange of blood and other body fluids other virus like Hepatitis A and E are transmitted mainly byfaeco oral route.

Viral hepatitis is increasingly being recognized as a public health problem in India.National Viral Hepatitis Control Program aims to combat hepatitis and achieve country wide elimination of Hepatitis C by 2030. This program also aims to achieve significant reduction in the infected population, morbidity and mortality associated with Hepatitis B and C like cirrhosis and hepatocellular carcinoma (liver cancer). NVHCP also aims to reduce the risk, morbidity and mortality due to Hepatitis A and E.

This article describes the scope of this program with special reference to the management of Hepatitis B.

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Key words **: Viral Hepatitis, Hepatitis B, NVHCP, Hepatitis B Immunization.**

Viral hepatitis is a global public health problem of epidemic proportions that caused 1.34 million deaths in 2015 and is comparable to deaths caused by tuberculosis and higher than those caused by HIV. Infection can be caused by the five known hepatitis viruses – A, B, C, D and E (HAV, HBV, HCV, HDV and HEV). Many of these infections are preventable. Hepatitis B and C are responsible for 96% of overall hepatitis mortality.

World Hepatitis Day - 28th July

Hepatitis B and C are transmitted by unsafe injection practices& through contaminated syringes and needles, infected blood and blood products, sexual transmission, from infected mother to child. Globally, in 2015, an estimated 257 million people were living with chronic HBV infection, and 71 million people with chronic HCV infection.

Viral hepatitis is increasingly being recognized as a public health problem in India. In the general population, Hepatitis B surface Antigen (HBsAg) positivity ranges from 1.1% to 12.2%, with an average prevalence of 3-4% In India, approximately 40 million people are chronically infected with Hepatitis B Chronic HBV infection accounts for 40% of Hepato-cellular Carcinoma (HCC) and 20-30% cases of cirrhosis in India.

The Government of India is a signatory to the resolution 69.22 endorsed in the WHO Global Health Sector Strategy on Viral Hepatitis 2016-2021 at 69th WHA towards ending viral hepatitis by 2030. Thus was introduced the National Viral Hepatitis Control Program (NVHCP).

This article describes the scope of this program with special reference to the management of Hepatitis B.

Editor's Comment :

- Hepatitis B Immunization, safety of blood products and promotion of behavioral change regarding use of condom, single use of needles are important keys in prevention of transmission of Hepatitis B.
- Acute and Chronic Hepatitis B may be determined by the assessment of Serological Markers.
- Management Hepatitis B depends upon the serological markers of HBV infection, measurement of HBV DNA levelsand assessment of severity of liver disease by -Liver enzymes, Non-invasive tests (NITs) such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), FIB-4, transient elastography (FibroScan) or Liver biopsy.
- A Chronic HBsAg positive patient with cirrhosis clinically or APRI >2 or FIB4 >3.25 is to be treated with Tenofovir disoproxil fumarate (TDF) 300 mg once daily or Entecavir (adult with compensated 0.5 mg liver disease and lamivudine naive) once daily or Entecavir (adult with decompensated 1 mg liver disease) once daily or Tenofovir alafenamide 25 mg fumarate (TAF) once daily.
- Monitoring of treatment is done bymonitoring for disease progression and treatment response in persons with CHB prior to, during and posttreatment;looking for tenofovir or entecavir side-effects and monitoring for hepatocellular carcinoma.

NVHCP

Aim :

(1) To combat hepatitis and achieve country wide elimination of Hepatitis C by 2030

(2) To achieve significant reduction in the infected population, morbidity and mortality associated with

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Hepatitis B and C viz, cirrhosis and hepatocellular carcinoma (liver cancer)

(3) To reduce the risk, morbidity and mortality due to Hepatitis A and E.

Key objectives :

(1) To enhance community awareness on hepatitis

(2) To provide early diagnosis and management of viral hepatitis at all levels of healthcare

(3) To develop standard diagnostic and treatment protocols for management

(4) To strengthen the existing infrastructure facilities and human resource

(5) To develop linkages with the existing National programmes

(6) To develop a web-based "Viral Hepatitis Information and Management System" to maintain a registry of persons affected with viral hepatitis and its sequelae.

Components :

The key components include:

1. Preventive component :

2. Diagnosis and Treatment :

3. Monitoring and Evaluation, Surveillance and Research

4. Training and capacity Building

Program management :

The NVHCP is coordinated by the units at the centre and the states.

(1) National Viral Hepatitis management unit (NVHMU)

(2) State Viral Hepatitis management unit (SVHMU)

(3) District Viral Hepatitis management unit (DVHMU)

Firstly key populations or high-risk groups (HRGs) under the National Viral Hepatitis Control Program have been designated. All key and bridge population groups under the NACP for HIV infections are specially vulnerable to viral hepatitis infections too .

High Risk Groups are :

(1) Recipients of multiple blood/blood products transfusion (especially before implementation of hepatitis C testing at a large scale in India; ie, before 2001)

(2) Patients on haemodialysis

(3) People Who Inject Drugs

(4) Male having sex with male (MSM)

(5) Female sex workers

(6) Sexual partners of infected people

(7) Prisoners, migrant workers and truck drivers

(8) Close first degree relatives and family members: mother, siblings, spouse and children, of persons affected with viral hepatitis.

India's target for Hepatitis B immunization

Sl No	Country Targets (to be provided by UIP)	Baseline 2019-20 (2016-17)	
1.	Coverage of Birth Dose of Hepatitis B (All deliveries)		90%
2.	Coverage with three doses of Hepatitis B vaccine in infants (B3).		95%
3.	Routine Hepatitis B vaccination among health-care workers.	N/A	Will be made Available

Safety of blood and blood products :

- Strengthening of blood safety is necessary as HBV and HCV can be transmitted through contaminated blood and blood products.
- it is compulsory to screen every unit of blood for HBV and HCV along with other transfusion transmitted infections (TTIs) before transfusion, in all licensed blood banks.
- Screening for HCV was made mandatory and introduced in 2001 across blood banks in India.

Harm reduction in key populations :

- To provide a package of prevention services including behavioural change communication, condom promotion, prevention and management of sexually transmitted infections (STI), community mobilization and enabling environment, and linkages to HIV testing, care, support & treatment to high risk groups .
- Needle syringe exchange program and opioid substitution therapy are to be provided
- Since the mode of transmission of Hepatitis B and Hepatitis C are largely similar to HIV/AIDS, NVHMU and SVHMU will coordinate with NACP for including prevention/management of hepatitis B and C in the package of prevention services for the key and bridge population.

Injection safety and infection control :

Targets WHO Regional Action Plan for Viral Hepatitis in South-East Asia : 2016-2021

By 2020, 50% of all injections are administered with safety engineered devices.

- Inadequate implementation of bio-medical waste management rules results in sharps injuries and increased risk of infections.
- States need to identify CBOs/NGOs and incentivise them for training on prevention of community barbers for HBV and HCV infections

National program for Surveillance of Viral Hepatitis :

- The initiative will undertake surveillance of acute, chronic hepatitis as well as their sequel over the next three years thereby estimating the disease burden for Hepatitis B and C in the country.

Diagnosis and Management of Viral Hepatitis with focus on treatment of Hepatitis B & C

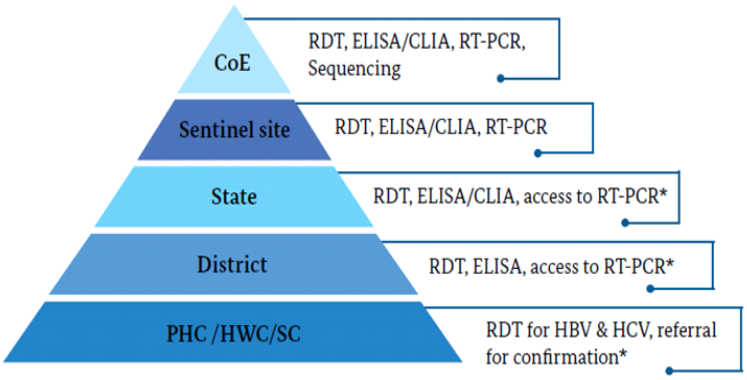
- The various components of service delivery under this head will include:

(a) Laboratory services; (b) Treatment services

Treatment Sites

- The services under this program will be delivered through the designated treatment sites that are located within an existing health facility, such as district hospitals and state medical colleges.
- There will be a few sites that will be labelled as Model Hepatitis Treatment centres (MTC) which will act as places for referral and mentoring of the other treatment centres (TC).

Network of Laboratories under the National Viral Hepatitis Control Program



**If samples are to be transported, they need to be collected, packaged and transported within six hours ofcollection under suitable environmental conditions.*

Clinical presentation :

Acute Hepatitis B :

Approximately 70 percent of patients with acute HBV infection have subclinical or anicteric hepatitis, while 30 percent develop icteric hepatitis. The disease may be more severe in patients co-infected with other hepatitis viruses or with underlying liver disease.

Fulminant hepatitis B is unusual, occurring in approximately 0.1 to 0.5 percent of patients; it is believed to be due to massive immune-mediated lysis of infected hepatocytes.

The rate of progression from acute to chronic hepatitis B in immunocompetent persons is determined primarily by the age at infection. The rate is approximately 90 percent for a perinatally acquired infection, 20 to 50 percent for infections between the age of one and five years, and less than 5 percent for an adult-acquired infection.

Chronic Hepatitis B (CHB)

A history of acute hepatitis is elicited in only a small percentage of patients with chronic HBV infection. In low or intermediate prevalence areas, approximately 30 to 50 percent of patients with chronic HBV infection have a past history of acute hepatitis; such a history is lacking in the remaining patients in these areas and in the majority of patients in high prevalence areas (predominantly perinatal infection).

Many patients with chronic HBV are asymptomatic (unless they have decompensated cirrhosis or have extrahepatic manifestations), while others have nonspecific symptoms such as fatigue. Some patients experience exacerbations of the infection which may be asymptomatic, mimic acute hepatitis, or manifest as hepatic failure.

Laboratory Investigations & Diagnosis :

Laboratory testing during the acute phase reveals elevations in the concentration of alanine and aspartate aminotransferase levels (ALT and AST); values up to 1000 to 2000 international units/L are typically seen during the acute phase with ALT being higher than AST. The serum bilirubin concentration may be normal in patients with anicteric hepatitis. The prothrombin time is the best indicator of prognosis. In patients who recover, the normalization of serum aminotransferases usually occurs within one to four months. A persistent elevation of serum ALT for more than six months

HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Interpretation
+	–	IgM	+	–	Acute hepatitis B , high infectivity *
+	-	IgG	+	-	Chronic hepatitis B , high infectivity *
+	-	IgG	-	+	1. Late acute or chronic hepatitis B, low infectivity 2. HBe Ag negative("precore- mutant") hepatitis B (chronic or rarely acute)
+	+	+	IgM	+/-	1. HBsAg of one subtype and heterotypic anti-HBs (common) 2. Process of seroconversion from HBsAg to anti-HBs (rare)
-	–	IgM	+/-	+/-	1. Acute Hepatitis B* 2. Anti-HBc "window"
-	-	IgG	-	+/-	1. Low level Hepatitis B carrier 2. Hepatitis B in remote past
-	+	IgG	-	+/-	Recovery from Hepatitis B
-	+	-	-	-	1. Immunization with HBsAg(after vaccination) 2. Hepatitis B in the remote past 3. False Positive

*IgM Anti-HBc may reappear during acute reactivation of chronic Hepatitis B

indicates a progression to chronic hepatitis.

Assessment and Staging of HBV Chronic infection:

Routine assessment of HBsAg-positive persons is needed to guide management and indicate the need for treatment. This generally includes assessment of:

1. Serological markers of HBV infection ;
2. Measurement of HBV DNA levels; and
3. Assessing severity of liver disease by - a. Liver enzymes b. Non-invasive tests (NITs) such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), FIB-4, transient elastography (FibroScan). c. Liver biopsy, if available

Serological markers of HBV infection :

Chronic Hepatitis B (CHB) infection is defined as the persistence of HBsAg for more than 6 months. Previous HBV infection is characterized by the presence of antibodies (anti-HBs and anti-HBc). Immunity to HBV infection after vaccination is characterized by the presence of only anti-HBs.

HBeAg: It also needs to be established whether the person is in the HBeAg-positive or HBeAg-negative phase of infection (please see the table above), though both require lifelong monitoring, as the condition may change over time. In persons with CHB, a positive HBeAg result usually indicates the presence of active HBV replication and high infectivity. Some HBeAg- negative persons have active HBV replication but are positive for anti-HBe and do not produce HBeAg due to the presence of HBV variants or pre-core mutants.

Measurement of HBV DNA levels :

Plasma HBV DNA concentrations quantified by real-time polymerase chain reaction (PCR) correlate with disease progression and are used to differentiate active HBeAg-negative disease from inactive chronic infection, and for decisions to treat and subsequent monitoring. HBV DNA concentrations are also used for optimal monitoring of response to antiviral therapy, and a rise may indicate the emergence of resistant variants.

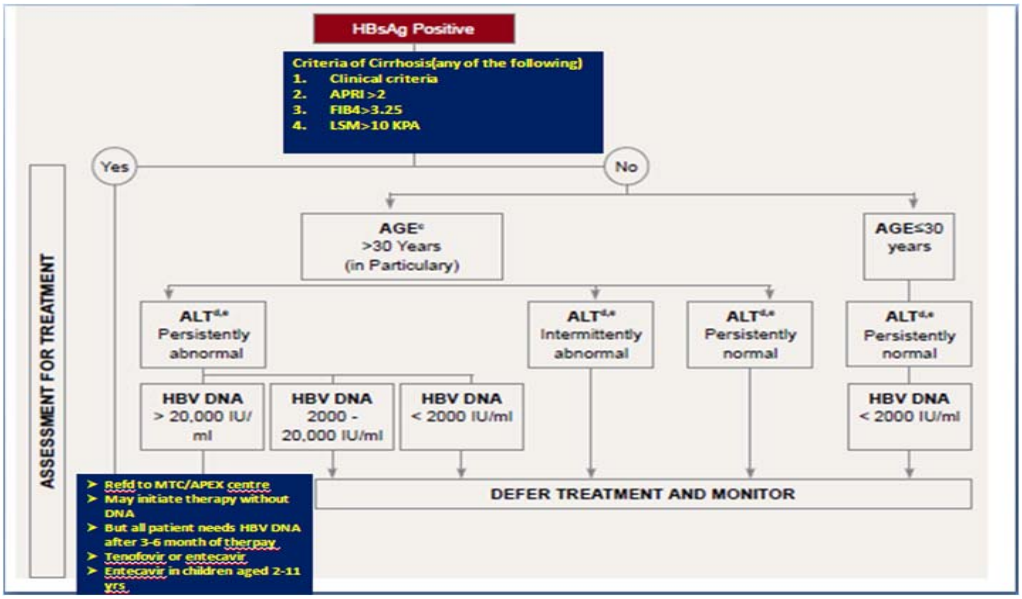
Assessing severity of liver disease :

A full assessment should include

- Clinical evaluation for features of cirrhosis and evidence of decompensation, and
- Measurement of serum bilirubin, albumin, ALT, AST, alkaline phosphatase (ALP), and prothrombin time; as well as full blood count, including platelet count.
- Other routine investigations include ultrasonography and alpha-fetoprotein (AFP) measurement for periodic surveillance for HCC, and endoscopy for varices in persons with cirrhosis.

Management aspects of Hepatitis – B :

Approach to a HBsAg positive patient



Whom to treat :

Chronic Hepatitis-B is defined as the persistence of HBsAg beyond 6 months. A repeat HBsAg testing after 6 months is done to confirm chronicity in those with acute hepatitis or a recent risk factor (180 days) for HBV infection and there is no necessity to confirm with a second HBsAg test in completely asymptomatic patients or those with features of fibrosis/cirrhosis/HBV flare.

The decision to treat a patient depends upon the presence of cirrhosis, fibrosis, levels of liver enzymes and platelet count. The HBeAg is not required for assessing the eligibility to initiate treatment and hence will not be used in the program.The persistently elevated ALT under the program is defined as at least 2 values

four weeks apart in the last 6 months, which are above the upper limit of normal.

The extent of fibrosis / cirrhosis can be established using several methods. APRI (AST-to-platelet ratio index) and FIB 4 are recommended as the preferred non-invasive tests (NIT) to assess for the presence of cirrhosis (APRI score >2: FIB 4 >3.25 in adults). The APRI score more than 1.5 or FIB-4 score more than 1.45 correlates with significant fibrosis (Stage F2). Transient elastography (eg, FibroScan) may be the preferred NITs in settings where they are available and cost is not a major constraint. A mean cut-off of e"12.5 kPa may be used to diagnose cirrhosis and e"8.0 to diagnose significant fibrosis.

PATIENT CATEGORY	PREFFERED DRUG
(1) All adults, adolescents and children aged 12 years or older	Tenofovir or Entecavir (as they have a high barrier to drug resistance)
(2) Woman of childbearing age	Tenofovir may be preferred in the eventuality of a pregnancy. Entecavir is not recommended in pregnancy
(3) Children aged 2–11 years.	Entecavir is recommended
(4) Age > 60 years; bone disease due to chronic steroid use or use of other medications that worsen bone density, history of fragility fracture, osteoporosis; altered renal function with eGFR<60 mL/min/1.73 m2 or albuminuria >30 mg/ 24 hr or moderate dipstick proteinuria or Low phosphate (<2.5 mg/dL) or in patient on hemodialysis	Entecavir may be preferred over Tenofovir
(5) Patients with reduced renal function or bone disease bone toxicities	TAF is the drug of choice. Entecavir is contraindicated
(6) Patients who have been exposed to lamivudine	Tenofovir is preferred as there is chance of entecavir resistance

Treatment :

What to treat with?

There are various antiviral agents recommended for treatment of CHB. The details are described in the National Treatment guidelines. However, the following table summarizes the recommendations:

Recommended drugs for the treatment of CHB and their doses in adults

Drug	Dose
Tenofovir disoproxil fumarate (TDF)	300 mg once daily
Entecavir (adult with compensated liver disease and lamivudine naive)	0.5 mg once daily
Entecavir (adult with decompensated liver disease)	1 mg once daily
Tenofovir alafenamide fumarate (TAF)	25 mg once daily

Selection of antiviral drug for CHB :

Drugs with a low barrier to resistance (lamivudine, adefovir or telbivudine) are available but not recommended as they lead to drug resistance.

The formulations for children are not currently approved, as and when they become available and approved, the above recommendation will be useful.

Monitoring the treatment :

The disease is complex and has sequelae, resolution as well as drugs side effects. Hence, three types of monitoring is necessitated:-

1) Monitoring for disease progression and treatment response in persons with CHB prior to, during and post-treatment

2) Monitoring for tenofovir or entecavir side-effects

3) Monitoring for hepatocellular carcinoma

Hepatitis B infection and pregnancy :

- Perinatal transmission is the most common route of HBV transmission.
- In the absence of prophylaxis, a large proportion of viraemic mothers, especially those who are seropositive for HBeAg, transmit the infection to their infants at the time of, or shortly after birth.
- The risk of perinatal infection is also increased if the mother has acute hepatitis B in the second or third trimester of pregnancy or within two months of delivery.
- Although HBV can infect the fetus in utero, this appears to be uncommon and is generally associated with antepartum hemorrhage and placental tears.
- The risk of developing chronic infection is 90%

following perinatal infection (up to 6 months of age) but decreases to 20–60% between the ages of 6 months and 5 years.

- All pregnant women with HBV should be evaluated for the need of treatment for hepatitis B and any associated liver disease, and given advice about prevention of transmission.
- Only a proportion of those with hepatitis B virus infection (pregnant or otherwise) need treatment.
- Hepatitis B in a pregnant woman is not a reason for considering termination of pregnancy.
- Similarly, the need for caesarean delivery should be decided based on obstetric indications, and not on the presence of HBV infection.
- Administration of hepatitis B vaccine to pregnant women with HBV provides no benefit either to the mother or the baby.

The goal of treatment in highly viremic mothers is to lower the serum HBV DNA level by several log10 IU/mL by the time of delivery to minimize the chance of newborn infection. The choice of antiviral agent is limited to those that are safe in pregnancy and include TDF, telbivudine, and lamivudine. These agents can be continued postpartum if necessary, but breastfeeding is not recommended in this setting.

Care of the baby :

Immunoprophylaxis of hepatitis B virus infection

- The newborn baby should be administered a timely first dose (the 'birth dose') of hepatitis B vaccine (monovalent) as soon as possible after birth, ideally within 24 hours and it will be better the earlier it can be administered.
- As HBIG is costly and has limited availability so under the program, HBIG will be made available and should be administered for preventing mother to child transmission of HBV (0.5 ml or 100 international units, intramuscular), this should be done as soon after birth as possible (and within 12-24 hours) and in anterolateral aspect of mid-thigh other than the one in which hepatitis B vaccine has been administered.

Breast-feeding :

A mother who has hepatitis B may breast-feed her baby, unless there is an exuding injury or disease of the nipple or surrounding skin. The advantages of breast-feeding far outweigh the risk, if any, of transmission of hepatitis B to a baby who has received hepatitis B vaccine.

Timing of testing :

If it is felt that the baby needs to be tested for hepatitis B, this should be done only after 1 year of age. Any positivity before this age is difficult to interpret and may resolve spontaneously over time.

Last but not the least -Prevention of HBV infection

- The risk of HBV infection may be higher in HIV-infected adults, and therefore all persons newly diagnosed with HIV should be screened for HBsAg and immunized if HBsAg is negative.
- Those already infected with HBV (HBsAg positive) do not benefit from HBV vaccine.
- PLHIV who have already suffered from HBV in the past and have developed protective titre of Anti-HBs antibody (>10 mIU/mL) also do not require HBV vaccine.
- Response to HBV vaccine is lower in persons with HIV or with a low CD4 count, and a meta-analysis has shown that a schedule of four double (40 ìg) doses of the vaccine provides a higher protective anti-HBs titre than the regular three 20 ìg dose schedule
- Besides this, all infants born to HBV positive women need to be immunized within 24 hours of birth (Dose - 0) followed by 6, 10 & 14 weeks (dose – 10 ìg IM) and HBIG – (0.5 ml or 100 international units, intramuscular), this should be done as soon after birth as possible (and within 12-24 hours) and in a limb other than the one in which hepatitis B vaccine has been administered.

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Drug Corner

Baricitinib : Delineating a New Treatment Option in COVID-19

Rajesh Venkitakrishnan[1], Praveen Valsalan[2], Paramez AR[3], Subin Ahmed[4], Akhilesh Kunoor[5], Sophia Philip[6], Monika Chinda[7] On behalf of Cochin Thoracic Society

The later phase of COVID-19 in humans is characterized by declining viral duplication and may be associated with an boisterous hyperinflammatory response in a minority of subjects. This occurs due to dysregulated systemic immune over-activation, which is described as Cytokine Release Storm (CRS). CRS is one of the causative factors leading to development of Acute Respiratory Distress Syndrome (ARDS) and it also leads to organ failure in severe COVID-19 patients. Thus, it is of utmost importance to halt the progression of CRS rapidly and effectively. One of the channels involved in the inflammatory cascade is the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signalling pathway. This makes JAK inhibitors (JAK-i) a potential and powerful therapeutic approach in COVID-19 patients[1]. Baricitinib (C16H17N7O2S) is a reversible Janus-Associated Kinase (JAK) inhibitor (JAK1/JAK2) and is a tiny molecule. More than 65 nations have licenced it for the treatment of individuals with moderate to severe Rheumatoid Arthritis (RA) and the USFDA and DCGI have now approved it for emergency use in COVID-19 patients. It exerts its benefits as an immunomodulatory drug by interrupting the signalling of multiple cytokines. It might exhibit an antiviral effect by targeting the factors of the human cell that help in the endocytosis of the virus. However, Baricitinib can lead to reoccurrence of acute infections and might increase the chance of thromboembolic events in patients of COVID-19. Furthermore, one needs to be vigilant with the renal functions (measured with the eGFR), Absolute Lymphocyte Count (ALC), Absolute Neutrophil Count (ANC) and platelet count while administering Baricitinib[2]. This article reviews the available data on Baricitinib with a keen focus on anti-cytokine and anti-viral action, pharmacological profile and current clinical evidence in COVID-19.

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Key words **: Baricitinib, JAKi, COVID-19, severe acute respiratory syndrome, cytokine release syndrome.**

Introduction and Current Global COVID-19 Scenario :

In December of 2019, a seafood wholesale market in Wuhan, China, discovered a group of patients who developed idiopathic pneumonia. Later, by sample sequencing of these patients, a hitherto undescribed beta-corona virus was discovered. Human airway epithelial cells were used to isolate a Novel Coronavirus, which was later designated as SARS-CoV-2. On 11 March, 2020, the World Health Organization declared the disease as pandemic and it continues to create havoc across many countries. It has affected more than 170 million people in the world, with nearly 30 lakhs dying of the disease till date[5]. In India, the total number of cases have spiked up to 2.9 million. Mortality is also rapidly rising with the second wave and nearly 3.5 lakhs people in the country have lost their life to COVID-19[6]. The rapid increase of the cases in India and worldwide has been linked to rapid mutation and emergence of variants of the virus. The recently named variant of SARS-CoV-2 "Delta variant" which had initially impacted India, is now present in 60 countries and rapidly spreading everywhere[7].

Current treatment strategies and therapeutic gaps

Despite the fact that the COVID-19 vaccine programme continues in India and around the world, the disease burden is steadily increasing. It is critical to find effective medicines, especially in nations where vaccination rates are low. Because of the pandemic's sudden outbreak, timebound discovery of new medications is challenging. As a result, repurposing of current medications to manage COVID-19 is an appealing concept, especially the drugs which are already approved for other indications, since they have well-established safety profiles. The central data repository, called the CORONA project, which was launched in the year 2020, has the data of 443

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medications tested on 34,000 patients. There are only four drugs in the database (Baricitinib, Remdesivir, Tocilizumab and Dexamethasone) ranked as grade A (shown to be effective)[8]. Baricitinib is one such repurposed drug that is effective in combination with Remdesivir in hospitalized COVID-19 patients requiring additional oxygen, invasive mechanical ventilation or extracorporeal membrane oxygenation[4] and deserves special mention as one of the newest addition to the COVID-19 tool kit.

Baricitinib – basic pharmacology

In COVID-19 patients, there are three phases of the disease: early infection phase, inflammatory phase and the hyperinflammatory phase[9]. In the later phase, when the virus titres start declining, vigorous immune response predominates, which may lead to release of proinflammatory cytokines and chemokines. Despite the lack of clear evidence that they are involved in the pathogenesis of COVID-19, elevated serum cytokines and chemokines have been linked to ARDS and multiorgan failure in COVID-19 patients.Serum levels of proinflammatory cytokines were found to be increased in severe COVID-19 patients, including IL-2, IL-4, IL-6, IL-7,IL-10, TNF-α, and IFNγ. Amongst these, many cytokines employ one distinctive intercellular signalling pathway mediated by JAKs (Fig 1). Thus, inhibiting the cytokine storm by blocking the JAK-STAT pathway, constitutes an attractive therapeutic target in the treatment of COVID-19[12].

Baricitinib is a small reversible JAK1/JAK2 inhibitor that was first licenced for the treatment of adult patients with moderate to severe rheumatoid arthritis in over 65 countries[2]. In November, 2020, the US FDA granted an emergency use approval for Baricitinib in combination with Remdesivir to treat adults and pediatric patients aged 2 years and older requiring supplemental oxygen or invasive mechanical ventilation or Extracorporeal Membrane Oxygenation (ECM)[3]. On 1st May 2021, CDSCO also gave similar approval to use Baricitinib in suspected or laboratory confirmed COVID-19 with similar clinical profiles[4]. As illustrated in Fig 2, Baricitinib's anti-inflammatory actions are mediated via reversible JAK inhibition. It may possibly have antiviral properties since it prevents virus entry into the host cell by inhibiting AP2-associated protein kinase 1 (AAK1) and to a lesser extent, cyclin G187 associated kinase (GAK), which are responsible for virus endocytosis.

Oral formulation of Baricitinib is available. Baricitinib has an oral bioavailability of approximately 80%. Food has no significant clinical impact on the drug's bioavailability. About 50% of the drug is plasma protein bound. Around 75% of the administered amount was excreted out through urine, whereas about 20% was eliminated in the faeces[3]. Healthy volunteers have a half-life of 6 - 9 hours but rheumatoid arthritis patients have a half-life of 12 hours and those with severe renal impairment or end-stage renal disease have a half-life of 19 hours[2].

Clinical studies on Baricitinib :

The ACTT-2 (Adaptive COVID-19 Treatment Trial - 2) trial was a double-blind, randomized, placebo-controlled clinical trial conducted in hospitalized individuals with confirmed SARS-CoV-2 infection. It compared treatment with Baricitinib plus Remdesivir (n=515) to placebo plus Remdesivir (placebo group; n=518).All of the patients were given Remdesivir for 10 days and either Baricitinib or placebo for 14 days. The primary outcome was the length of time to disease recovery. Clinical state at day 15 was rated on an 8-point ordinal scale as the secondary outcome. Remdesivir was given intravenously on day 1 at a loading dose of 200 mg, then 100 mg every day until the patient was discharged from the hospital or died. Baricitinib was administered orally or via a nasogastric tube at a dose of 4 mg/day, or 2 mg/day if renal function was impaired (eGFR, 60 ml/min/1.73m^2).

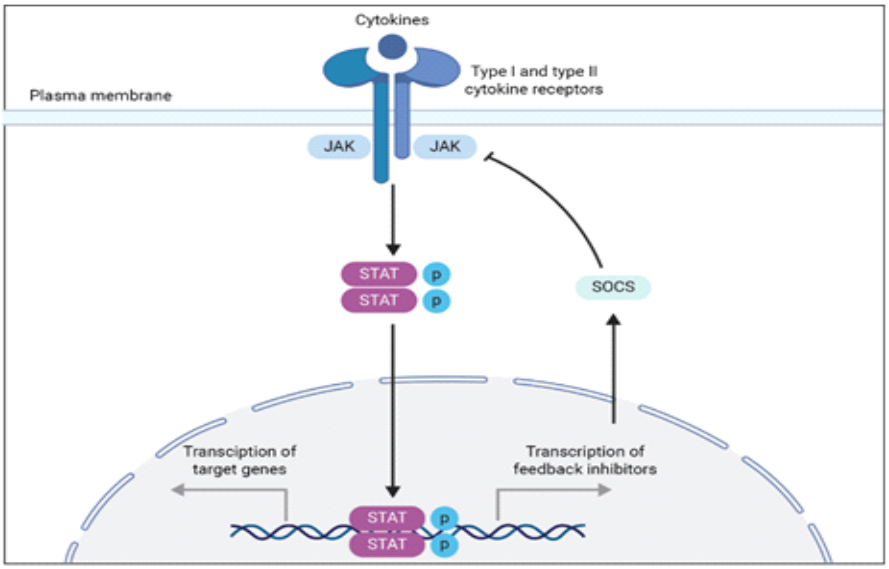


Fig 1 — JAK-STAT pathway

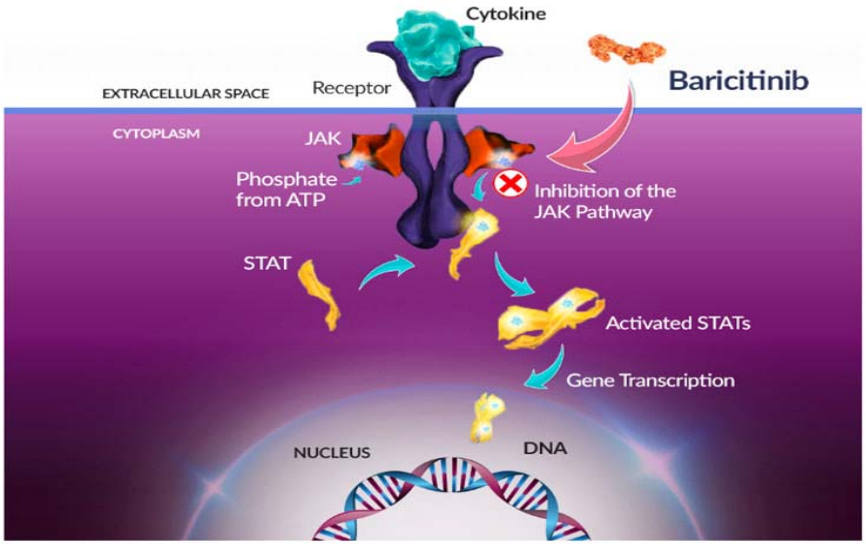


Fig 2 — Inhibition of JAK -STAT Pathway

When compared to Remdesivir alone, patients who received the combination therapy experienced substantial reduction in median time to recovery from 8 to 7 days (12.5% improvement)[p=0.047] (Fig 3). In the treatment arm, individuals on high-flow oxygen and non-invasive ventilation recovered 44 percent faster than those in the control arm (Fig 3). Patients who received Baricitinib in combination with Remdesivir had a better clinical state at Day 15 than patients who received Remdesivir alone [p=0.044]. When compared to Remdesivir alone, the proportion of patients who progressed to ventilation (non-invasive or invasive) or died by day 29 was lower in Baricitinib plus Remdesivir (23% *versus* 28%) [p=0.039]. By Day 29, the proportion of patients who died was 4.7 percent for Baricitinib in conjunction with Remdesivir *versus* 7.1 percent for Remdesivir, a 35 percent drop in the proportion of patients who died. As indicated in Fig 5, adverse events and serious adverse events were reported in 41% and 15% of patients treated with Baricitinib in combination with Remdesivir, respectively, compared to 48 percent and 20% of patients treated with Remdesivir. Infections and venous thromboembolism occurred in 6% and 4% of patients in the treatment arm, respectively, compared to 10% and 3% of individuals receiving Remdesivir. For Baricitinib-treated patients, no new safety signals have been detected[10].

In the phase 3 COV-BARRIER tiral, add on Baricitinib to Standard of Care (SoC) was compared to placebo (plus SoC) in hospitalized COVID-19 patients. The standard of treatment (SoC) at the time of study included corticosteroids, antimalarials, antivirals, and/or Azithromycin COV-BARRIER was a Global, randomized, double-blind, placebo-controlled trial which included 1525 people who did not require supplementary oxygen or high-flow oxygen. The primary endpoint was the proportion of patients who progressed to the first occurrence of noninvasive ventilation (including high flow oxygen) or invasive mechanical ventilation or death by day 28.

The primary endpoint of the study was not statistically significant but relevant only by numbers,

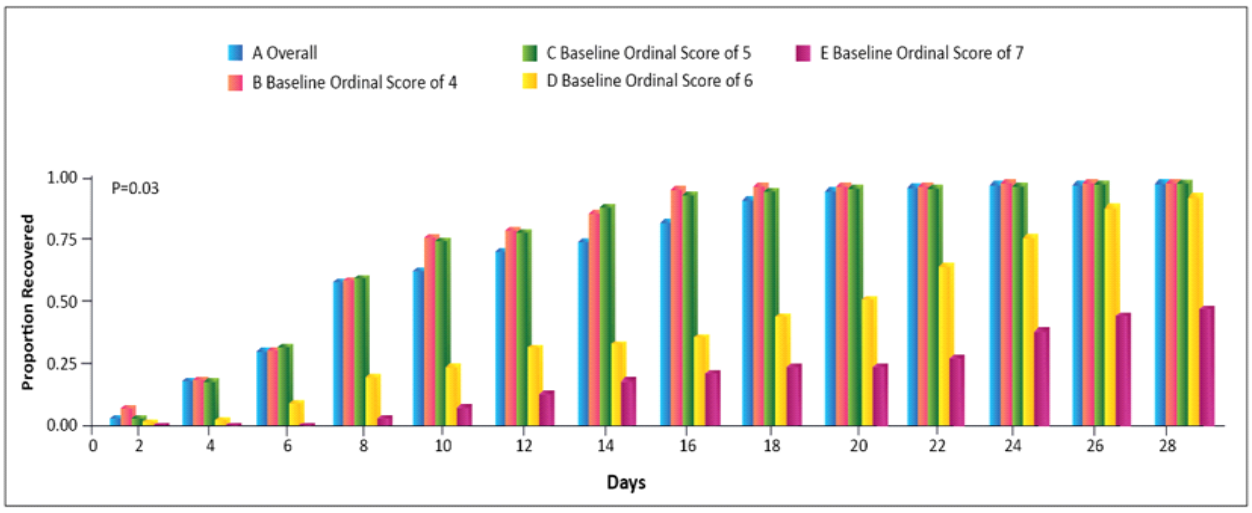
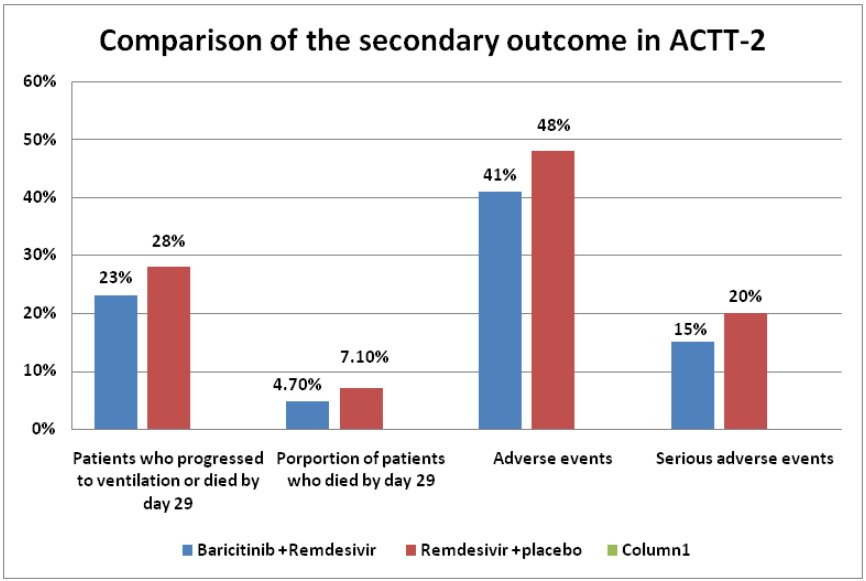


Fig 3 — Primary outcome in ACTT-2 trial (as per the ordinal score)



as seen in the results. Baricitinib-treated patients were 2.7 percent less likely than placebo to advance to ventilation or death (p=0.18). When compared to placebo, patients treated with Baricitinib had a 38 percent lower risk of death from any cause by day 28(p=0.0018). Furthermore, all baseline severity subgroups demonstrated a numerical reduction in mortality in the Baricitinib therapy arm with the highest reduction in individuals receiving noninvasive mechanical breathing at baseline (17.5% for baricitinib *versus* 29.4% for placebo; p =0.0065).There was also a decrease in mortality in the predetermined subgroups who were treated with or without corticosteroids at the start. Both of these trials were a significant step forward for Baricitinib in the treatment of COVID-19. Various further studies would be conducted to determine the molecule's safety and efficacy[11].

In an observational study, patients with moderate to severe SARS-CoV-2 pneumonia were given Lopinavir/ritonavir, HCQ, and Corticosteroids (CS group, n=50) or Corticosteroids and Baricitinib (BCT-CS group, n=62).The change in levels of oxygen saturation determined by pulse oximetry (SpO2)/FiO2 from hospitalisation to discharge was the primary end point. The percent of patients requiring supplementary oxygen at discharge and one month later were secondary end goals[14].

The results of the observational study showed that the BCT-CS group had a larger improvement in the oxygen saturation levels from hospitalisation to discharge than the CS group (p<0.001). When compared to the CS group, a much smaller proportion of patients in the BCT-CS group required supplemental oxygen at discharge (P<0.001) and at one month follow up (P=0.024). The study concluded that in moderate to severe COVID-19 pneumonia patients the combination of baricitinib plus corticosteroids was associated with significant improvement in the pulmonary function[14].

Uncertainties in knowledge :

Baricitinib is being used in rheumatoid arthritis patients since 2014; however, the emergency approval to use in COVID-19 was granted based on the results of ACTT-2 trial[3,10]. As previously discussed this trial used Baricitinib as an add on therapy to Remdesivir and compared outcomes with Remdesivir therapy. The utility of Baricitinib as a monotherapy or its benefits with agents like steroids (Dexamethasone) is less well described. The combination with agents like tocilizumab also needs exploration. There is a substantial need to determine whether patients with profiles that differ from the ACTT 2 trial's inclusion criteria would benefit from JAK inhibitor medications. Venous thromboembolic phenomena is a concern with use of Baricitinib and a specific study to address the magnitude and consequences of this in patients at high risk of thrombotic events (coronary heart disease patients, subjects with a known history of CVA, documented previous DVT etc) may be worthwhile. Need to collect data on the real-world effectiveness of Baricitinib in hospitalised patients with COVID-19 outside a rigorous trial setting may also be considered. The safety parameters would also need to be analysed in post marketing studies, once physicians start using the drug in a larger number of patients.

Positioning in practice :

Till the time more high-quality evidence emerges, the optimum strategy would be to utilise Baricitinib as a combination therapy with Remdesivir in patients having a clinical profile similar to the ACTT 2 study subjects. Thus, the physicians can use Baricitinib in combination with Remdesivir in hospitalised patient with COVID-19 pneumonia who require supplemental oxygen, non-invasive ventilation or high flow oxygen and invasive or ECM.

It can be administered orally or through nasogastric tube depending on the patient's condition. It has an advantage of 4 mg or 2 mg once daily dosing depending on the renal function of the patient. Baricitinib would

help to tackle the cytokine storm in the hospitalised patient and would prevent further worsening of the condition[3].

Future directions :

The cytokines produced by the JAK-STAT pathway that contribute to CRS suggest that blocking the route could be important in the treatment of COVID-19 patients. Various additional JAK inhibitors, such as ruxolitinib, Tofacitinib, and Fedratinib, are now being studied in COVID-19 patients in clinical trials[1] and a comparison of their relative benefits and demerits may be demystified with the publication of these studies. The molecular pathways underlying the inflammatory cascade in COVID-19 may be better brought to light with further studies providing opportunities to target hitherto undescribed pathways and mediators.

Conclusions

This review critically appraises the mechanisms of benefit and published evidences of Baricitinib in COVID-19. It further gives recommendations for positioning of baricitinib in the treatment of COVID-19 in real world setting, pending emergence of further high quality evidence. From the available evidence, Baricitinib in combination with Remdesivir offers an attractive treatment option for patients with COVID-19 and affords multiple benefits in clinical practice. The adverse event profile is favourable and contraindications are few.

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(Answers : Mediquiz 07/2021)

1. ---------- A
2. ---------- C
3. ---------- C
4. ---------- C
5. ---------- A
6. ---------- C
7. ---------- B
8. ---------- D
9. ---------- A
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11. ---------- A
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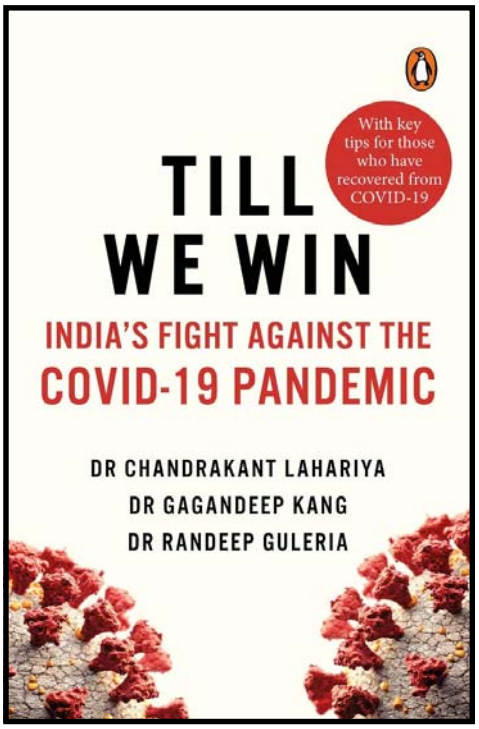
Book Review



"Till We Win" by Dr Chandrakant Lahariya, Dr Gangadeep Kang and Dr Randeep Guleria, Published by Penguin Random House India, 7th Floor, Infinity Tower C. DLF Cyber City, Gurgaon 122002, Haryana, India, 20 Cm x 13 Cm, pp 1-308, Rs 299.00.

In the present pandemic situation when the entire world is suffering from anxiety and fear, when normal life has been incapacitated, 'Till We Win' the book jointly ventured by Dr Chandrakant Lahariya, a leading public policy and health system expert, Dr Gangadeep Kang, a world famous vaccine researcher cum virologist and Dr Randeep Guleria, the Director of AIIMS, Delhi is an appropriate answer to the queries, most people are placing to the health sector.

The book efficiently deals with the history of Pandemic, its inevitability, its impact on the humanity, its presentation, the importance of lockdown, specific health initiatives taken to tackle the situation, balancing the unlock in regard to safety and economy, the challenges faced and the lessons learnt, drug therapy, community participation, the effect of pandemic on the societal and mental health, the problems faced by the health fraternity, the vaccines and the ways to fight to victory.

The book is picturesque, enigmatic, descriptive with positivity and surely will have immense impact not only on the community but the entire global health leadership as well.

Prof Lopamudra (Dhar) Chowdhury[1]
RG Kar Medical College & Hospital, Kolkata 700004 **Prof Jyotirmoy Pal[2]**
[1]Professor, Department of Pharmacology
[2]Professor, Department of Medicine

Letter to the Editor

[The Editor is not responsible for the views expressed by the correspondents]

JIMA : Vol 119, No 6, June, 2021

Sir, — In the editorial in the June issue of JIMA, you have done very well to highlight the role played by the doctors and HCWs. Their dedicated work and sacrifices to save lives cannot be praised enough. Paramedics and also Police have done great work. I would salute them all. Hundreds of doctors have lost lives due to COVID and they deserve appreciation and their families some empathy and condolences.

However, I am sad that IMA lacks the national pride on the handling of the COVID 19 pandemic by India. Western countries praised India for it. Just consider the COVID deaths figure; USA with >12 ICUbeds /100,000, and with a population of 32.82 crores only has had 6.05 lakh death as of today (2-7-2021), while India with less than 1 ICU bed/100,000 population, with 4 times the population of USA, has had only 4.01 lakh deaths –two third of US deaths ! We need to be proud too that India developed its own vaccine against corona virus and was not only the first to immunize its population but was generous enough to share it with other countries. Many countries appreciated it but not the IMA.

Talking of the lack of ICU beds in India, it is true we need more of them. But then, looking at the current scenario, we do not have enough intensivists, ICU nurses, technicians, needed for the existingnumber of ICU beds; they are being managed by junior doctors and untrained nurses. Remember we do not even have enough MBBS doctors! Who will manage the additional thousands of ICU beds?

If the government efforts are not effective, it is largely because ourpeople disobey the protocols. Leaders do not care about their followers' safety. Look at political rallies in West Bengal last month.However, the Kumbhamela, which was well controlled by the government, incidence was only 0.7% +vecases among those tested from the 48.5lakh devotees (The Hindu). We the Indians are averse to rules; look at our road traffic infringements. Majority of road deaths are due to driver's or pedestrian's breaking traffic rules; but they all find fault with the Government, police, roads and so on.

We do not need any aliens from outside; unfortunately, we have enough and more of them amongst us in India itself for centuries. You see them in history and even present days. Insatiable greed for money (and power in some cases) is their DNA. ICU beds being sold for Rs40,000-60,000 in Bangalore, inflating the hospital bill with unnecessary, repeated CT scans and investigations etc, spreading false rumours about vaccinations, huge scams of recent past are only some examples of the actions of such aliens.

I always try to look at the half full glass rather than the half empty glass. My belief is that the only way to counter these aliens is for us all to introspect seriously, and develop a pride for our culture and our nation.IMA must lead the way at least for medical fraternity.

Shimoga, Karnataka **Dr R D Prabhu**

INDIAN MEDICAL ASSOCIATION

Purity of Profession - Parity in Healthcare

DOCTORS' DAY : 1st July, 2021

National President & Hony. Secy. General Paying Homage to Covid Martyrs

Hon'ble Prime Minister, Sh. Narendra Modi Ji

Dr Ketan Desai, Past President, WMA

Dr. V. K. Paul, Member, Niti Aayog

Sh. Rajesh Bhushan, Secretary, MoHFW, GoI

Dr Randeep Guleria, Director, AIIMS, New Delhi

New Delhi

Behala Medical Association, West Bengal

JIMA, Your Health & IMA Hq, Kolkata, West Bengal

Indore, Madhya Pradesh

Glimpse of IMA Protest Day held on 18th June, 2021 and other activities done by IMA States and Local Branches

IMA Behala Branch, West Bengal

Protest in front of IMA Hqs Kolkata Building

IMA Titagarh Branch, West Bengal

IMA Tollygunge Branch, West Bengal

Editor and Secretary of JIMA addressing


INDIAN MEDICAL ASSOCIATION
Alappuzha Branch
IMA National Protest Day
18 june 2021


INDIAN MEDICAL ASSOCIATION
KANNUR BRANCH
IMA NATIONAL PROTEST DAY
18th June 2021
ASHOKA
HOSPITAL


INDIAN MEDICAL ASSOCIATION
TELANGANA STATE
IMA National Protest Day
STOP VIOLENCE ON
PROFESSION AND
PROFESSIONALS
NO MORE VIOLENCE


BLK
Super Speciality
Hospital


INDIAN MEDICAL ASSOCIATION
NORTH PARVUR BRANCH


VASAVYA NURSING HOME
EDUCATION PROGRAMME


INDIAN MEDICAL ASSOCIATION
MUMBAI BRANCH
Blood Donation Camp
Donate Blood Save Life


INDIAN MEDICAL ASSOCIATION
MUMBAI BRANCH
WELCOME DELEG


INDIAN MEDICAL ASSOCIATION
THRISSUR BRANCH
IMA NEDUMBASSERY
IMA THRISSUR
OXYGEN
CONCENTRATOR


Save
The
Saviours
I.M.A. KODERMA BRANCH


INDIAN MEDICAL ASSOCIATION
JHARKHAND STATE BRANCH

Report of IMA Doctors' Day observed by IMA (HQs) on 1st July 2021

The Doctors Day is being celebrated in remembrance of Dr. B.C. Roy for the altruistic services rendered by him for the country in all walks of life as a clinician, as a politician, as an administrator, as an ethnic academician and as a great social reformer.

In his Presidential address, Dr. J.A. Jayalal said that our country is observing the Doctors' Day with great zeal and enthusiasm, but Doctors Day of 2021 is of special importance. In this covid-19 pandemic, the entire fraternity, right from the day one had involved in the war against corona from the front line to save millions of people from the clutches of severe covid-19 and in the bargain lost more than 1500 of its proactive veterans and dynamic young Doctors as martyrs of covid-19.Hence, National President informed the audience that Hon'ble Prime Minister of India will be addressing the medical fraternity at 3.10 pm to greet the Doctors on the occasion of the National Doctors' Day.

In the Special Address for the Doctor s community, Hon'ble Prime Minister Shri Narendra Modi that said this day is celebrated in the memory of Doctor BC Roy, is the symbol of the highest ideals of our medical fraternity. He thanked the Doctors on behalf of 130 crore Indians for their services during the difficult times in last one and half years of the pandemic. Some of the salient points of his address were - "Doctors are called a form of God, and for a good reason. When the nation is fighting the COVID-19 pandemic, doctors have worked tirelessly to save lakhs of lives. There have been those doctors who laid down their lives saving the lives of others. He paid homage to all those doctors who made the supreme sacrifice. The budgeted allocation for health is more than doubled to over □ 2 lakh crores. We are always thankful to doctors and healthcare workers for their service, and hence we have brought and enhanced laws for their protection too. We have also seen in recent times that many doctors and medical practitioners have actively promoted yoga and wellness. Yoga and wellness is accepted the world over and it is encouraging to see how many people are now actively keen about their well-being and fitness".

The National President, IMA whole-heartedly thanked the Hon'ble Prime Minister on behalf of the Indian Medical Association for boosting the morale of the doctors who are tirelessly service our fellow men during this Covd pandemic. He also assured that we will stand together and work hand-in-hand with the Government.

The National President, Dr. J.A. Jayalal and Hony. Secretary General, IMA Dr. Jayesh Lele along with other leaders paid Floral Tribute to IMA Covid Martyrs.

Dr. Ketan Desai, Past President, WMA and Past National President, IMA, Dr. V.K. Paul, Member, Niti Aayog, Govt. of India, Shri Rajesh Bhushan, Secretary Health, MoHFW and Dr. Randeep Guleria, Director, AIIMS thanked the medical fraternity for their wonderful and excellent work done by them during the Covid-19 era and shared their views.

Shri Om Birla, Hon'ble Speaker, Lok Sabha, Shri G Kiran Reddy, Hon'ble Minister of State, Ministry of Home Affairs, Shri Vijay Rupani, Hon'ble Chief Minister of Gujarat, Shri Shashi Tharoor, Hon'ble Member of Parliament, Lok Sabha, Shri Anil Vij, Hon'ble Health Minister, Haryana and Shri Udhayanidhi Stalin, MLA, Tamilnadu sent their video messages motivating and encouraging the medical fraternity. They all thanked medical fraternity for their dedicated services rendered to the citizens of this country during this Covid Pandemic.

Messages were also received from Shri Manoj Sinha, Hon'ble Lieutenant Governor, Jammu & Kashmir, Smt. Droupadi Murmu, Hon'ble Governor of Jharkhand, Shri Uddhav Thackeray, Hon'ble Chief Minister, Government of Maharashtra, Shri Naba Kisore Das, Hon'ble Minister, Health & Family Welfare, Government of Odisha, Shri Vivek Thakur, Hon'ble Member of Parliament, Rajya Sabha and Shri M K Stalin, Hon'ble Chief Minister of Tamil Nadu

On this occasion, IMA HQs also announced Safe Motherhood Week progrmame from 1st to 7th July 2021 thereby providing a Safe Motherhood facility to all the women of the county. A week's long Webinar was jointly conducted by IMA Standing Committee for Safe Motherhood, IMA Standing Committee for Women Doctors and IMA Standing Committee for Mission Pink Health. It was appreciated and attended by large number of participants across the country

The following Awards were announced on this occasion: -

(1) Covid Warrior Award were announced on the occasion of Doctors' Day to recognize the members who have contributed during this pandemic

(2) An amount of Rs.10 lakhs each was announced to the Nine family members of Covid Martyrs.

(3) IMA Doctors' Day Appreciation Awards.

On this occasion, IMA also honoured the Past National Presidents and Hony. Secretary Generals present during this occasion for their exemplary services.

The meeting was attended by large number of members across the country on virtual mode.

Dr. J.A. Jayalal
National President, IMA

Dr. Jayesh Lele
Honorary Secretary General, IMA

Our Respectful Homage

Bharat Ratna Dr. Bidhan Chandra Roy

Born : 1 July 1882 ▲ **Died : 1 July 1962**

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