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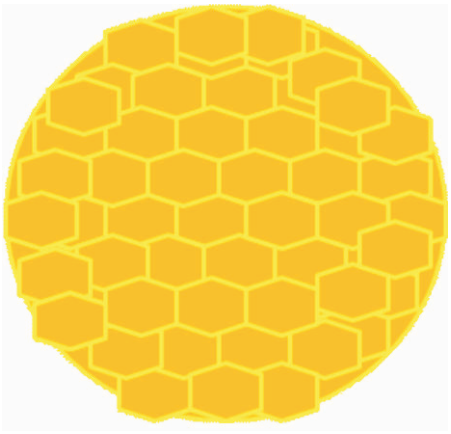

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SRI RAMACHANDRA JOUNRAL OF MEDICINE
JAN - JUNE 2015

CONTENT

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From the Editor's Desk

Dear Readers,

I have great pleasure in bringing out the Jan-June 2015 issue of Sri Ramachandra Journal of Medicine.

Dr. M. Ravi has brought out a general important article on "Predatory Journals".

The highlight of this University journal is its multidisciplinary nature and representing all faculties from various specialities.

As usual we have 2 original articles, 1 review article and 5 case reports.

Wishing you an enjoyable reading.

P.V. VIJAYARAGHAVAN
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Original Article

DEVELOPMENT OF FORMULATION AND IN-VITRO EVALUATION OF CAPECITABINE LOADED FE_3O_4 NANOPARTICLES MODIFIED WITH PLGA-PEG POLYMER FOR COLON CANCER TREATMENT

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ABSTRACT

Background and Objectives*: This present study was to investigate the synthesis of Fe_3O_4 nanoparticles by chemical precipitation method. The Fe_3O_4 nanoparticles were coated with the polymers PLGA-PEG and loaded with the drug capecitabine for the targeting of colon cancer which will be distributed in the large intestine by applying, the external magnetic field. It gets localized in the area of colon cancer cells. After the applied external magnetic field, the iron oxides (Fe_3O_4 nanoparticles) get heated to 37ºC - 40ºC and the tumour cell gets destroyed. The Fe_3O_4 nanoparticles were also called as super paramagnetic Iron oxide nanoparticles. They were very smart materials and mostly used for the applications in medicine like targeted drug delivery system, diagnostic cancer imaging and their therapeutic applications.*

Results: *Formulation was characterized by XRD and FTIR spectroscopy. The Fe_3O_4 nanoparticles were confirmed by Vibrating Sample Magnetometer (VSM) and also the Entrapment Efficiency (%EE) of the drug was calculated. Further, the particle size can be found by particle size analyzer (PSA). The surface morphology of the formulations was analyzed by SEM analysis. Moreover, the in-vitro analysis was done by using Franz Diffusion Cell (FDC) method. The stability studies of the formulations were done at three different temperatures and it shows good stability in refrigerator conditions (4ºC).*

Conclusions: *Moreover, by using this novel drug delivery system we can minimize the dose frequency, side effects, adverse effects, minimal dose, sustained/ prolong in action and to obtain proper patient compliance.*

Keywords: *Capecitabine, colon cancer, Fe_3O_4 nanoparticles, PLGA-PEG, super paramagnetic and iron oxide nanoparticles.*

SRJM 2015;8:1-8

INTRODUCTION

Cancer was a generic term used for a large group of diseases that can affect all over the body. Further terms used were malignant tumours and neoplasm. Cancer was a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008. The main types of cancer were: Lung (1.37 million deaths), stomach (736 000 deaths), liver (695 000 deaths), breast (458 000 deaths) and cervical cancer (275 000 deaths).[1] Among all of these, colon cancer estimated around the world was 1.23 million; new cases which were clinically diagnosed; whereas, 608,000 people were dead due to colon cancer in year 2008.[2] As per the genetic analysis, both colon cancer and rectum cancer were same.[3] In case of 50 year old people, the symptoms and signs were as follows: loss of appetite, loss of weight, nausea or vomiting, worse constipation, blood in the stool and anaemia or bleeding in the rectum were also a risk factors for the old age peoples.[4-6]

Generally, colon cancer occurs in more than 75-95% people with no genetic risk or little age. It mainly occurs in old age people, especially in male gender due to their intake of fat, alcohol, obesity, smoking and also due to lack of proper physical exercise.[7-10] It was a disease that originates from the epithelial cell lining the colon or rectum of the gastro intestinal tract (GIT) most often result as a mutation in the Wnt signaling pathway that artificially increases signaling activity. The mutation occurs in the adenomatous polyposis coli (APC) gene of the intestinal crypt stem cells, as that produce the adenomatous polyposis coli (APC) protein which was responsible for the accumulation of the ß-catenin protein; without adenomatous polyposis coli (APC), ß-catenin accumulates into high levels and translocates into the

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nucleus, binds to Deoxyribonucleic acid (DNA), and activates the transcription of genes that were normally important for stem cell renewal and differentiation but when inappropriately expressed at high levels can cause cancer. Some mutation happens in ß-catenin (CTNNB1), by increasing the ß-catenin because of mutations that blocks its degradation. Analogous to APC such as AXIN1, AXIN2, TCF7L2, or NKD1 was also causes this degradation block.[11-13]

To prevent colorectal cancer, we have to increase the consumption of whole grains, fruits; vegetables and physical exercise can reduce the risk of colon cancer, also avoiding the intake of red meat will be helpful.[14-16] Currently a number of targeted drug delivery strategies with a less amount of toxicity being evaluated. In the future, it was likely that targeted approach will have a significant impact on the diagnostic evaluation of tumours. Capecitabine drug was approved by Food and Drug Administration (FDA) for the treatment of colorectal cancer in the year 2005. Capecitabine was a pro-drug which can be enzymatically gets converted into 5-fluorouracil in the tumour cells. This 5-fluorouracil inhibits the Deoxyribonucleic acid (DNA) synthesis and gradually slows down the tumour cells growth. Colorectal cancer was one of the leading cancer in United States and whereas it was in 4th position worldwide. The drug Capecitabine has 38 - 45 minutes of half life with frequent administration of dose and cause more side effects and adverse affects.[17] Polymers such a s poly-(lactide-co- glycolide) (PLGA) or poly-(lactic acid) (PLA) were commonly used for fabricating nanoparticles due to their excellent biocompatibility and biodegradability. However, despite their versatility, a major draw- back relating to their applicability was rapid clearance from the bloodstream by the mononuclear phagocytic system (MPS), following direct administration to the blood circulation, resulting in reduced drug therapeutic effect. As a consequence, the concept of long-circulating nanoparticles using amphiphilic copolymers has emerged. Vesicles made of amphiphilic copolymers were the most promising polymeric devices for oral insulin delivery, since they were able to maintain a prolonged hypoglycaemic effect after oral administration.

In the plan of an amphiphilic copolymer, the introduction of PEG was conceived with the intention of making nanoparticles coated with PEG chains more stable when in contact with physiological fluids. The literature widely reports on the ability of PEG to repel opsonin proteins leading to the formation of long circulating nanoparticles.

In order to overcome the above said problems, the core shell nanoparticles with modified PLGA-PEG polymers were synthesized and to fulfil the patient compliance by maintaining the drug release for prolonged or sustained in action. Mechanism of action behind the core shell nanoparticles was Fe_3O_4 nanoparticles coated with poly (lactic-co-glycolic acid) - poly ethylene glycol (PLGA -PEG) polymers and further attached with Capecitabine drug. The iron oxide present in these formulations was nothing but super paramagnetic materials which was converged to tumour cells by applying external magnetic field. After the applied magnetic field the iron oxides (Fe_3O_4 nanoparticles) get heated to 37ºC - 40ºC and the tumour cells get destroyed.

MATERIALS AND METHODS

Capecitabine was purchased from Burgeon Pharmaceutical Company- Chennai as a gift sample. Poly-(lactide-co-glycolide) (PLGA) and Poly ethylene Glycol (PEG 7000 - 14000 Daltons) were purchased from the sigma Aldrich-Bangalore. Ferric Chloride, Ferrous chloride and Ammonium hydroxide (25%) were purchased from SD fine chemicals- Mumbai. All other chemicals used were of analytical grades.

Preparation of Fe_3O_4 nanoparticles:

In a traditional hydrothermal production method, 6.1 g of $FeCl_3.6H_2O$ and 4.2 g of $FeSO_4.7H_2O$ were dissolved in 100 ml deionized water in a four-neck flask followed by adding 10 ml of 25% ammonium hydroxide solution with continuous stirring at 55ºC under a nitrogen (N_2) atmosphere and adjust the pH to 11.0-12.0 with ammonium hydroxide. The temperature was raised to 65ºC and the system was then mixed for 2 hours maintaining at same temperature. Then, the pH was adjusted to 6-7 by adding dilute hydrochloric acid solution and the temperature was gradually raised to 80ºC with constant stirring for an hour. Later the pH slowly reduces to 3.4-4.0.[18] The dark black precipitate were collected by filtration and carefully washed with deionized water and air dried. The Fe_3O_4 nanoparticles were collected and stored for further use.

Preparation of capecitabine loaded Fe_3O_4 nanoparticles modified with PLGA-PEG polymer was done by modified nanoprecipitation Method:

The freshly prepared Fe_3O_4 nanoparticles of 5mg/ 5ml in water were taken and sonicated. At the same time the Poly-(lactide-co-glycolide) (PLGA) polymer (120mg) was dissolved in acetonitrile (5ml) and the poly ethylene glycol (PEG) polymer (10mg) in distilled water (5ml). The organic phase solution of polymer was added

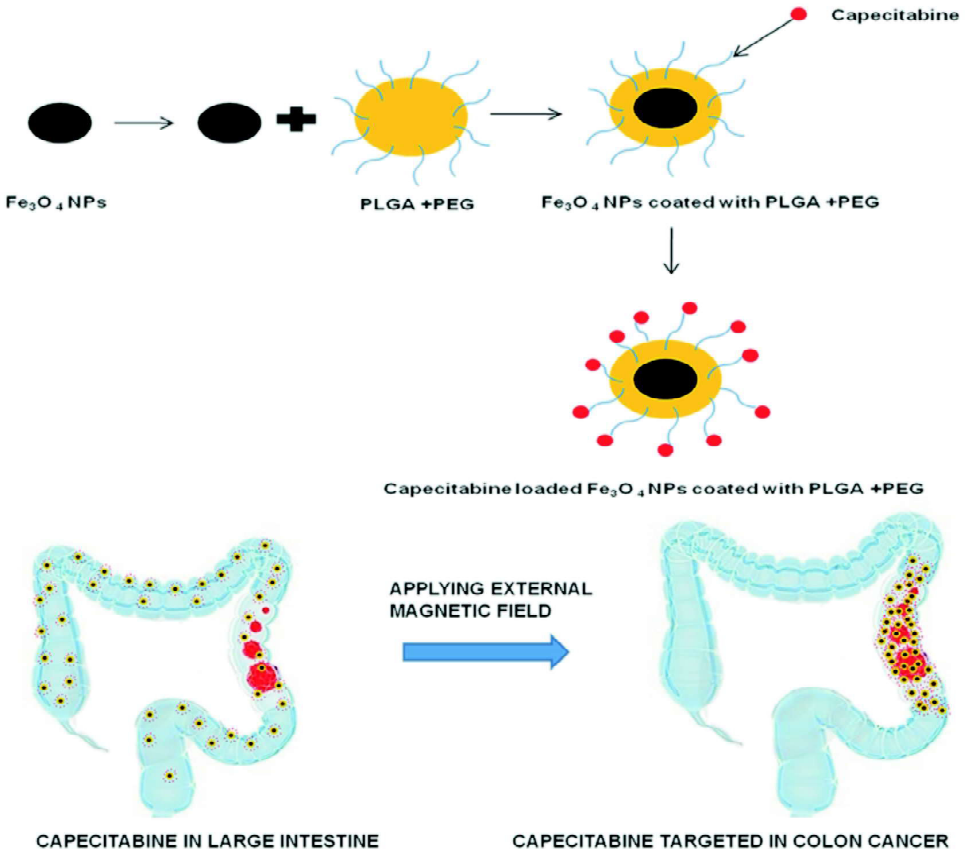

Fig.1 : Illustrates that the synthesis of Fe_3O_4 nanoparticle coated with Polymer (PLGA) Poly-(lactide-co-glycolide) and Poly Ethylene Glycol (PEG) loaded with Capecitabine and the Capecitabine nanoparticles were distributed in the large intestine by applying, the external magnetic field. It gets localized in the area of colon cancer cells.

drop wise into aqueous phase solution with a continuous stirring in a homogenizer at 18000rpm condition until the full organic solvent evaporates i.e. (W/O). The double emulsion method [19] was adapted with slight modification, and the water phase containing the surfactant was added. Later, the drug Capecitabine was loaded into the above formulation and the stirring was continued for about an hour. The plain formulation was also prepared without adding drug to the formulation.[20] The different ratios of polymer were taken; the procedure was repeated and optimized (Fig.1).

RESULTS

Entrapment Efficiency

The procedure of entrapment efficiency was to determine the drug entrapped into the copolymer. The prepared formulations were centrifuged in 2 ml eppendroff tube at 15000 rpm for about 30 minutes. Then the samples were separated into supernatant and precipitated one. The precipitated samples were redispersed by using buffers and recentrifuged for 3-4 times to get entrapped drug alone. The samples were then subjected for the analysis of UV visible spectrophotometer at 240 nm. The capecitabine concentration was analyzed and calculated as shown in Fig.2.[21-22]

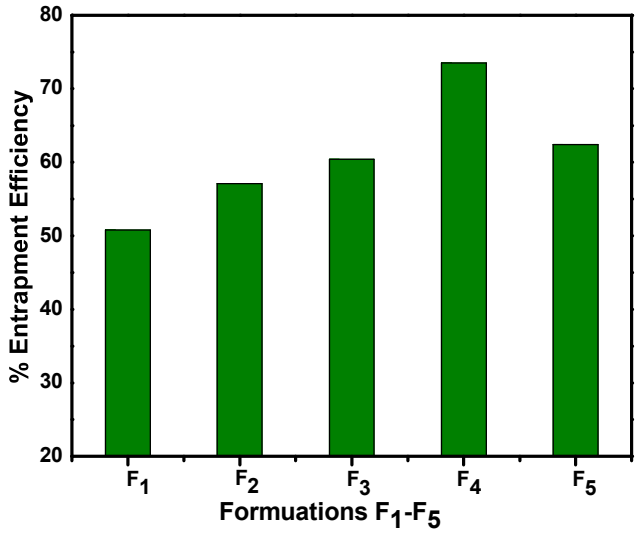

Fig. 2: Indicates the % Entrapment Efficiency (EE) of the formulations F1-F5.

%EE = Total amount of Drug - Entrapped drug / Total amount of drug X100

X-Ray Diffraction analysis (XRD)

The crystallinity of the drug, polymer, iron oxide and formulations were analyzed by powder X-ray diffraction technique in the range of 10º-90º by using the PAN alytical Xpert pro X-ray diffractometer

instrument. Fig shows that 3(a) capecitabine 3(b) Poly-(lactide-co-glycolide) (PLGA) polymer 3(c) Fe_3O_4 nanoparticles 3(d) capecitabine loaded Fe_3O_4 nanoparticles modified with PLGA-PEG polymer (F4). Fig 3(a) shows the sharp peaks of the pure drug capecitabine was at 20.56º, 25.26º, 36.12º and 40.14º. Fig 3(b) shows the broad peak of polymer PLGA around 23.41º. The Fe_3O_4 nanoparticles Fig 3(c) shows the sharp peak at 26.83º, 28.06º, 31.83º, 45.55º, and 75.39º. Further, the prepared formulations Fig 3 (d) show the broad peak at 24.40º. The XRD analysis was done for amorphous and crystalline state of samples. As PEG 2000 was available as flakes, we were unable to perform the XRD for PEG polymer. On the whole, the XRD results shows that the X-ray diffraction signals cannot penetrate into the prepared formulation as that of in the pure drug and Fe_3O_4 nanoparticles where it shows were in crystalline form.

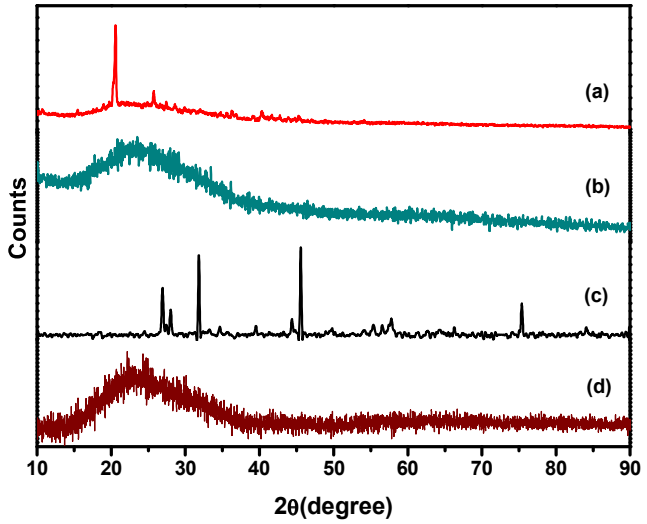

Fig. 3: X-Ray Diffraction patterns (XRD) of (a) shows the Capecitabine pure drug (b) polymer Poly-(lactide-co-glycolide) (PLGA) (c) Fe_3O_4 nanoparticles (d) Capecitabine loaded Fe_3O_4 nanoparticles modified with PLGA-PEG polymer (F4).

Fourier Transform Infra Red Spectroscopy (FTIR)

The FTIR spectrum shows the interaction between the pure drug, polymer and Fe_3O_4 nanoparticles. The spectrum scanning range from 400-4000cm-1 and the resolution was 1cm-1. From the Fig.4(a) shows the characteristics peaks of pure drug capecitabine at the wave number of 3521 cm-1 shows O-H stretching where the free hydroxyl group was present, the characteristic peak at 3253 cm-1 indicates the N-H stretching vibrations, at 2968cm-1 shows the C-H stretching, at wave number 2859 cm-1shows the presence of aldehyde group (C-H=O), at 1773 and 1627 cm-1 indicates the presence of C=O carbonyl group of stretching vibrations, at 1500cm-1shows the

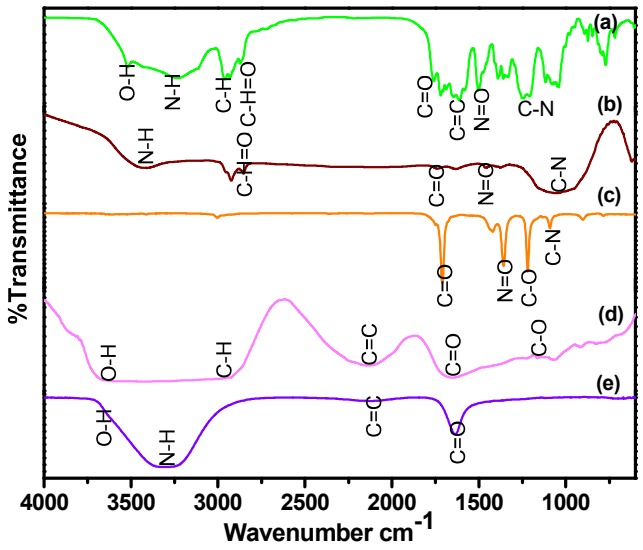

Fig. 4: Fourier Transform Infra Red Spectroscopy (FT-IR) of (a) the Capecitabine pure drug, (b) Fe_3O_4 nanoparticles, (c) Poly Ethylene Glycol (PEG) 2000, (d) polymer Poly-(lactide-co-glycolide) (PLGA) and (e) Capecitabine loaded Fe_3O_4 Nanoparticles modified with PLGA-PEG polymer (F4).

N=O bending vibrations and further at 1245cm-1shows the C-N bending vibrations. From the Fig. 4 (b) the Fe_3O_4 nanoparticles shows the characteristic peak at 3417cm-1 indicates the N-H stretching vibrations, at 2853cm-1 the presence of aldehyde group (C-H=O) with stretching vibrations, at 1754 cm-1 shows the presence of C=O carbonyl group with stretching vibrations, at 1457cm-1 indicates the (N=O) nitro groups with bending vibrations and at 1044 cm-1 shows the presence of C-N group with bending vibrations. The Fig. 4 (c) Poly Ethylene Glycol (PEG) 2000 shows the characteristic peaks at 1706cm-1 indicates the presence of C=O carbonyl group with stretching vibrations, at 1354cm-1shows the N=O group of bending vibrations, at 1241cm-1 shows the presence of N-O bending vibrations and at 1093cm-1shows the peak of C-N group bending vibrations. The Fig. 4 (d) Poly-(lactide-co-glycolide) (PLGA) polymer shows the characteristic peak at 3648cm-1 indicates the free O-H hydroxyl group with the stretching vibrations, at 2962cm-1 shows the C-H stretching vibrations, at 2131cm-1 shows the C=C stretching vibrations, at 1664cm-1 indicates the C=O carbonyl group with bending vibrations and at 1165cm-1 shows the presence of the C-O bending vibrations. Moreover, the Fig.4(e) shows the characteristic peaks of formulation (F4) at 3660cm-1 indicates the free O-H group of stretching vibrations, at 3308cm-1 shows the N-H stretching vibrations, at 2113cm-1 indicates the presence of C=C stretching vibrations and at 1627cm-1 shows the presence of C=O bending vibrations.

Overall, the FTIR spectrum of the pure drug, Fe_3O_4 nanoparticles and formulation (F4) shows no interaction between the molecules. The functional groups present in the drug and polymer also exists in the formulation but without any interactions between them.

Vibrating Sample Magnetometer Analysis (VSM)

The Vibrating Sample Magnetometer (VSM) was used to measure the magnetic property of the iron oxide nanoparticle. It initiates that there was hysteresis in all the M versus H curves. The magnetization of the synthesized Fe_3O_4 nanoparticle increases with increase

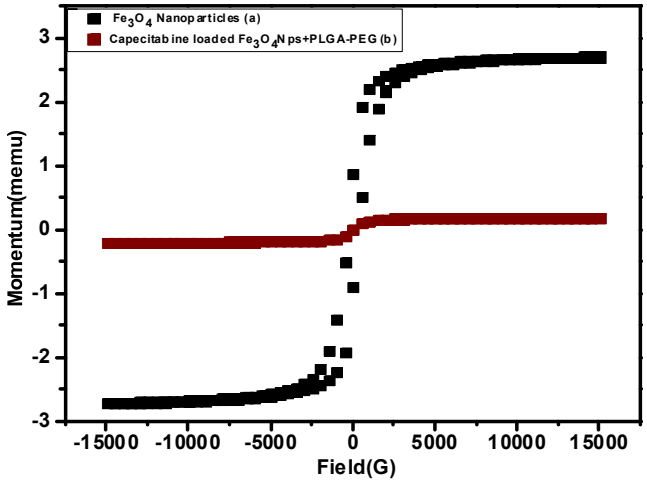

Fig. 5: Vibrating Sample Magnetometer (VSM) Analysis spectrums of Fe_3O_4 nanoparticles and the Formulation F4.

in magnetic field strength.[23] Magnetization using Vibrational Sample Magnetometer (VSM) shows a Saturation Magnetism (Ms) around positive 2.7119 emu as shown in the Fig. 5.

Particle Size Analyzer (PSA)

The particle size of the formulation was taken by using the Malvern particle size analyzer. The drop of the sample was taken and diluted to 1ml with D.I water and placed in the sample cuvette. The prepared formulation F4 was taken for particle size analysis and found the average particle size of the formulation as around 252 nm. The total particle size range of the formulation F4 was 150 to 400 nm as shown in the Fig. 6.

Scanning Electron Microscope (SEM)

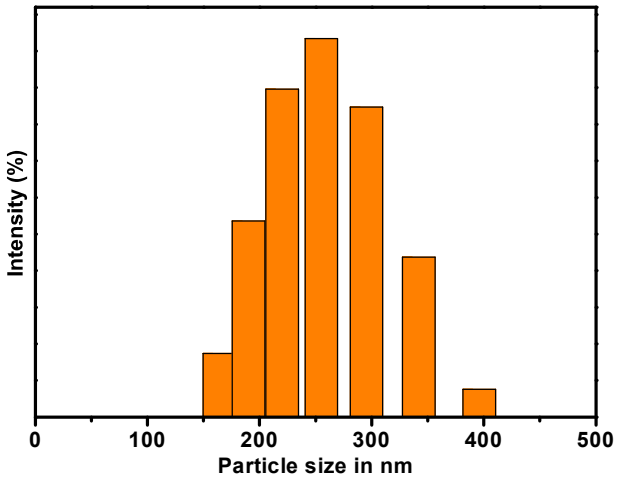

Fig. 6: Particle Size Analysis (PSA) of the formulation (F4) shows the particle ranges 150nm to 400nm.

The particle size of the formulation of capecitabine loaded Fe_3O_4 nanoparticles modified with PLGA-PEG polymer (F4) were viewed and photographed using Scanning Electron Microscopy (SEM) (QUANTA 3D

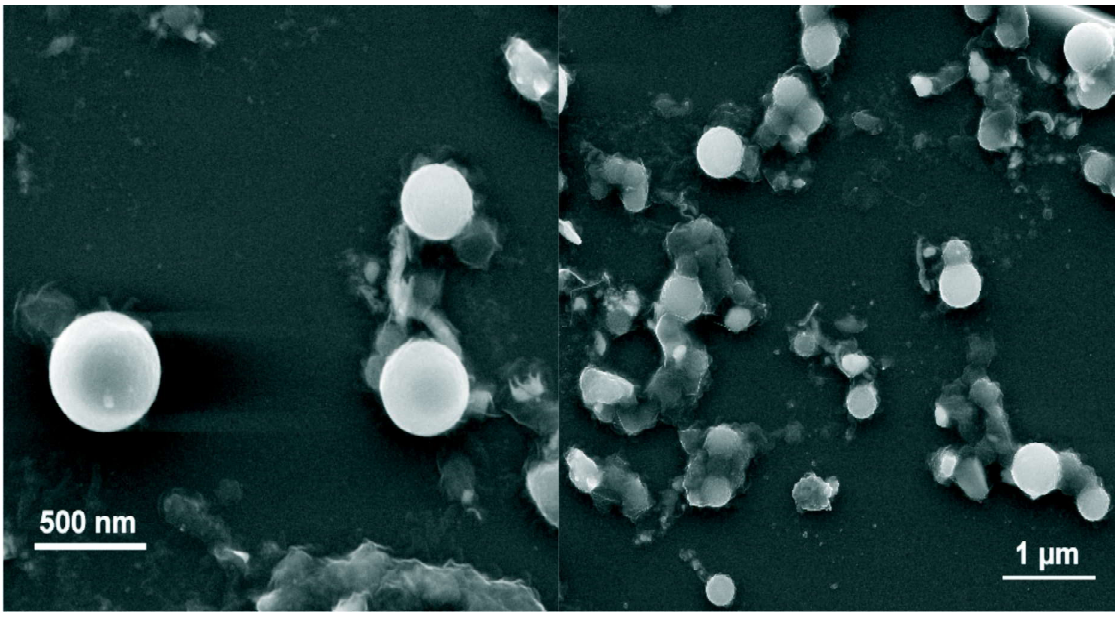

Fig. 7 (a) and (b): Scanning Electron Microscopic image (SEM) of the formulation (F4). The Fig.7 (a) shows the particles were round, spherical and discrete in shape.

FE- SEM). The prepared nanoparticles were dropped on a double sided carbon tape. The solution evaporated slowly at room temperature. The image was captured on SEM mode at desired magnification. The SEM images shows the particles of capecitabine loaded Fe_3O_4 Nanoparticles modified with PLGA-PEG polymer (F4) was in spherical shape and size approximately around 100nm to 500nm as mentioned in the Fig. 7.

In-vitro release study analysis

The In-vitro drug release was studied majorly by using Franz diffusion cell (FDC). The FDC enable to analyze the drug movement across a membrane using a two-compartment model. The donor compartment contains the dose; while the recipient compartment contains phosphate buffer saline (PBS) pH 7.4 and a non-rate limiting membrane (semi permeable) separates the compartments and supports the dose. Thus, drug release profiles can be produced for controlled-drug release formulations.[24-25] The samples were withdrawn from the sampling port at a regular time intervals and the drug release was quantified using UV spectrophotometer as shown in the Fig. 8.

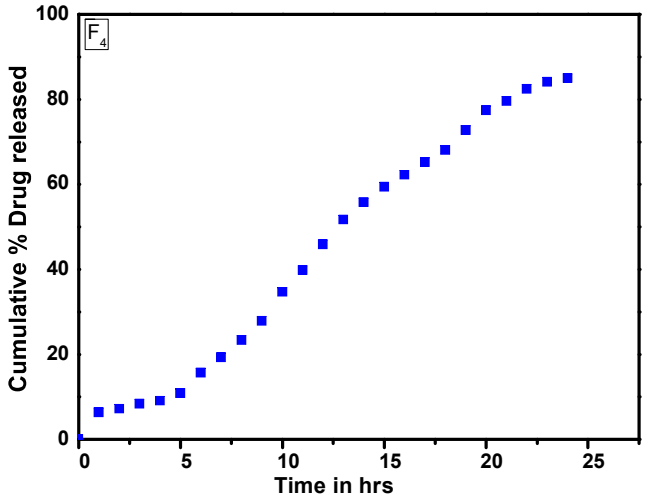

Fig. 8: In-vitro release study analysis of formulation (F4) were plotted Cumulative (%) of drug release Vs Time

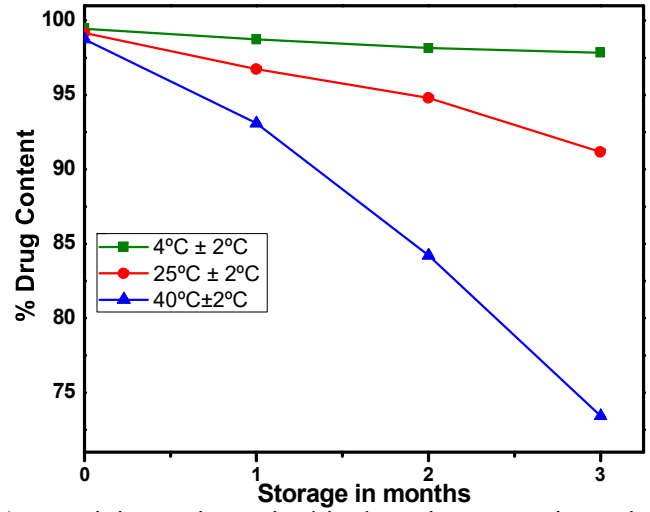

Fig.9: Stability study graph of the formulation (F4) shows the degradation of drug content during the storage for the period of 3 months at different temperature conditions.

Stability Studies

The prepared formulations (F4) were carried out for stability studies by storing at 4°C ± 2°C refrigeration temperature conditions, 25°C ± 2°C room temperature conditions and 40°C ± 2°C high temperature conditions for a period of 3 months as per ICH (International Conference on Harmonization) guidelines. The obtained data was shown in the Fig. 9.

DISCUSSIONS

From the Fig.2 the prepared formulation F4 shows the maximum entrapment efficiency of 73.2%, whereas the F5 shows the entrapment efficiency around 62.17%, F3 shows the entrapment about 60.26%, and F2 and F1 shows around 56.92% and 50.80% respectively. When compared to all other formulations F1, F2, F3 and F5, the F4 shows maximum entrapment efficiency. The best formulation F4 was chosen for the drug release study. The yields of the F1-F5 formulations were found to be 40-45%. The XRD spectrum (Fig. 3) of the pure drug and Fe_3O_4 nanoparticles shows sharp peaks and it confirms the crystal nature. Whereas the formulation F4 shows the amorphous state due to XRD signal which was unable to pass into the composites as that of individual drug materials. The FTIR spectrum (Fig. 4) clearly explains that there was no interaction between the drugs, polymer with that of the formulation F4. The VSM data (Fig. 5) reveals that magnetic property of the Fe_3O_4 nanoparticles, which was positive 2.7119 emu. While the formulation F4 has positive 0.19299 emu, due to the Fe_3O_4 nanoparticles get coated into the polymer its magnetic strength gets reduced. The particle size analyzer (Fig. 6) shows the average particle size, which was around 252 nm. The total formulation F4 shows the particle size was around 150nm - 400nm. The SEM images (Fig. 7(a) and (b)) shows the particles were uniform and spherical in shape. The particles in images show around 100 to 500nm in range. The In-vitro release studies (Fig. 8) shows the graph of sustained release with 84% of drug released in 24 hours.

In stability studies (Fig. 9) the refrigeration conditions (4°C ± 2°C) storage data shows good stability, at room temperature (25°C ± 2°C) it shows better stability whereas, the temperature at (40°C ± 2°C) shows the degradation gradually.

The Fe_3O_4 nanoparticles were prepared successfully by chemical precipitation method. Then,

the Fe_3O_4 nanoparticles formulation was loaded with capecitabine drug by modifying the nanoprecipitation method. The prepared formulation was optimized, selected as best formulation from the entrapment efficiency. The XRD shows no crystalline in the formulation and FTIR shows no interaction between the drug and polymers. The particle size analyzer indicates the uniform size of the particles present in the formulation. The SEM images shows discrete and spherical in shape. The above prepared formulation was more stable in refrigeration conditions. Moreover, the in-vitro release study shows the maximum drug release with prolonged release to attain its bioavailability. By this novel drug delivery system we can able to minimize the dose frequency, side effects, adverse affects, minimal dose and to obtain proper patient compliance.

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REFERENCES

1. Globocan, 2008; IARC, 2010.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–917.
3. Cancer Genome Atlas Network, Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487(7407):330-37.
4. Alpers DH, Kalloo AN, Kaplowitz N, Owyang C, Powell DW. Principles of clinical gastroenterology. Yamada T, editor. John Wiley & Sons; 2011 Sep 7.
5. Astin M, Griffin T, Neal RD, Rose P, Hamilton W. The diagnostic value of symptoms for colorectal cancer in primary cwere: a systematic review. Br J Gen Pract.2011; 61:e231-43.
6. Adelstein BA, Macaskill P, Chan SF, Katelaris PH, Irwig L. Most bowel cancer symptoms do not indicate colorectal cancer and polyps: a systematic review. BMC Gastroenterol. 2011; 11:65.
7. Watson AJ, Collins PD. Colon cancer: a civilization disorder. Digest Dis 2011; 29: 222-8.
8. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, et al. Colorectal cancer. Lancet. 2010; 375 (9719): 1030-47.
9. Lee IM, Shiroma Eric J, Lobelo Felipe, Puska P, Blair Steven N, Katzmarzyk Peter T. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. The Lancet. 2012;380(9838):219-29.
10. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol 2011; 22(9): 1958-72.
11. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993;363(6429):558-61.
12. Wen-feng G, Hong-zhi S, Shuang Z, Zhe-feng N, Xiao-Yun M , Yasuo T, et al. Down regulated inhibitor of growth 3 (ING3) expression during colorectal carcinogenesis. Indian J Med Res. 2014; 139:561-67.
13. Markowitz SD, Bertagnolli MM. Molecular Origins of Cancer: Molecular Basis of Colorectal Cancer. N Engl J Med 2009; 361:2449-60.
14. Campos FG, Logullo Waitzberg AG, Kiss DR, Waitzberg DL, Habr Gama A, Gama Rodrigues J. Diet and colorectal cancer: current evidence for etiology and prevention. Nutr Hosp 2005; 20: 18-25.
15. Doyle VC, Logullo Waitzberg AG, Kiss DR, Waitzberg DL, Habr Gama A, Gama Rodrigues J. Nutrition and colorectal cancer risk: a literature review. Gastroenterol Nur. 2007; 30:178-82.
16. Harriss DJ, Atkinson G, Batterham A, George K, Cable NT, Reilly T Haboubi N, Renehan AG. Lifestyle factors and colorectal cancer risk (2): a systematic review and meta-analysis of associations with leisure-time physical activity, Colorectal Dis 2009;11:689-701.
17. Talbot SM, Neugut A. Epidemiological trends in colorectal cancer. Curr Clin Oncol 2002;1:23-46.
18. Lu SS, Liu S, Zu QQ, Xu XQ, Yu J, Wang JW, et al. In Vivo MR Imaging of Intraarterially Delivered Magnetically Labeled Mesenchymal Stem Cells in a Canine Stroke Model. PLoS ONE. 2013 ;8:54963.
19. Stolnik S, Illum L, Davis SS. Long circulating micro particulate drug carriers. Adv Drug Deliv Rev 1995;16:195-214.

20. Yang J, Park SB, Yoon HG, Hun YM, Haam S. Preparation of polye-caprolactone nanoparticles containing magnetite for magnetic drug carrier. Int J Pharm 2006;324:185-90.
21. Savva M, Duda E, Huang L. A genetically modified recombinant tumor necrosis factor-alpha conjugated to the distal terminals of liposomal surface grafted poly-ethylene glycol chains. Int J Pharm 1999; 184: 45-51.
22. Govender T, Garnett M, Stolnik S, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J Control Release 1999; 57: 171-85.
23. Masala O, Seshadri R. Magnetic properties of capped, soluble MnFe2O4 nanoparticles, Chem Phys Lett 2005; 402:160 - 64.
24. Kaur SP, Rekha R, Hussain A, Khatkar S. Preparation and Characterization of Rivastigmine Loaded Chitosan Nanoparticles. J Pharm Sci Res 2011; 3:1227-32.
25. Craparo EF, Cavallaro G, Bondì ML, Mandracchia D, Giammona G. PEGylated nanoparticles based on polyaspartamide. Preparation, physico-chemical characterization and intracellular uptake. Bio macromolecules 2006; 7: 3083-92.

DEGRADATION OF TWO HYDROCARBONS BY A MARINE *STREPTOCOCCUS* SP. ISOLATED FROM GULF OF MANNAR, SOUTHEAST COAST OF INDIA

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ABSTRACT:

Aim of the study: *Gulf of Mannar and Palk Bay regions of the Southeastern coast of India have been reported to experience hydrocarbon related pollution. The present study was thus carried out to screen hydrocarbon degrading bacteria from these locations with a notion that these polluted areas may harbor some oil-utilizing bacteria.*

Methodology: *A total of 123 bacteria were isolated and those which were screened as lipase producers were selected for biosurfactant production. The lipase [+] isolates were tested for oil spread, tilted glass slide and modified drop collapse assays in determining the efficacy of biosurfactants. The isolate/s which has least MEC for these assays was chosen for tributyrin degradation and analyzed on GC-MS. The potent isolate was identified up to the genus level using standard tests.*

Results: *Out of all the bacteria, isolate K from Gulf of Mannar that produced biosurfactant had least MEC of 150, 500 and 250 µg/ mL for oil spread, tilted glass slide and modified drop collapse assays respectively. It was observed that tributyrin was degraded to more than 96% by the bacterium in a GC-MS spectrum.*

Conclusions: *The potent isolate was identified as a Streptococcus sp and to our knowledge this is the first report of marine Streptococcus which possessed biosurfactant properties. We propose that this study may pave a way to identify biosurfactant producing bacteria from marine realm too.*

Keywords: *Biosurfactants, Gas Chromatography-Mass Spectrometry (GC-MS), marine, Streptococcus sp, tributyrin.*

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INTRODUCTION

Oil pollution in the marine environs is considered as most devastating than any other problems related to pollution because of the deleterious effects on marine life. This may be because of two factors: i) from the oil itself and ii) effects associated with clean-up operations. Gulf of Mannar and Palk Bay areas located on the South eastern parts of India are considered one of the highly biodiversified areas which support a huge group of organisms at various levels. Unfortunately these two areas are categorized as sensitive because there have been reports on oil-pollution due to shipping, by and large. Though the shipping lines are far away from the gulf area, oil spills occurring from ships/tankers moving toward Colombo which cross Gulf of Mannar, would result in oil pollution.[1] Apart from this, oil pollution also occurs from terrestrial sources along the shorelines in areas as cited in literature pertinent.[2, 3] Immediate to an oil spill, when all the organisms are categorized to be stressed, a few groups of bacterial population emerge, survive and grow well in oil polluted areas. It is widely documented that bacteria thriving in oil-polluted areas secrete extracellular enzymes like lipases to utilize hydrocarbons especially with a triglyceride base. Some bacteria, in addition, may also produce biosurfactants for accelerated lipid recovery.[4-7] Biosurfactants are categorized as surface-active substances synthesized by living cells, mainly bacteria and are believed to reduce surface tension and stabilize emulsions. There have been many studies checking biosurfactant-producing marine bacteria.[8-11] However, only a very few studies have been done on identification of isolates of Gulf of Mannar and Palk Bay. Hence, with a notion that Gulf of Mannar and Palk Bay may harbor oil-degrading bacteria, the present investigation is centered to screen bacteria from these areas for lipase and/ or biosurfactant production and to check their bioactivities to degrade hydrocarbon sources, with reference to olive oil and tributyrin. Also, this study attempts to determine the extent of tributyrin degradation using Gas Chromatography-Mass Spectrometry [GC-MS] analysis and to preliminary identify the potent isolate/s using biochemical tests.

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MATERIALS AND METHODS

Collection and processing of samples:

Sub-surface marine water samples (2-4 m deep) were collected from Gulf of Mannar (lat. 9° 16' long. 79° 7') and Palk Bay (lat. 9° 17' long. 79° 7') of Rameswaram coast of Tamil Nadu, India, in sterile

screw-capped bottles and were brought immediately to the laboratory for microbiological processing. Bacteria of both the stations were briefly extracted by shaking 10 mL of the seawater samples vigorously in 90 mL of filtered and autoclaved seawater. Thereafter dilutions up to of 10^4 were spread on ZoBell Marine Agar (ZMA) (5 g peptone, 1 g yeast extract and 15 g bacteriological agar in 1L filtered and autoclaved seawater) supplemented with 10% tributyrin (HiMedia) in duplicates. Colonies with varying morphotypes were quadrant streaked to obtain pure cultures and each of them were checked for lipase activity. Only the colonies showing considerable zones of clearance [a minimum of 10 mm diameter] from each station were furthered for testing biosurfactant activities.

Extraction of biosurfactants:

Extraction of crude biosurfactant precipitates was done for all lipase [+] isolates as per method of Jenneman et al., (1983).[12] Loopful of bacterial cultures were inoculated in Erlenmeyer flasks containing 100 mL of ZoBell Marine Broth (ZMB) and were shaken at 250 rpm at 37°C for 3 days. Thereafter cultures [1 OD_{660}] were briefly spun at 10,000 x g at 4° C for 10 min and pelleted down. The supernatants were acidified using 6 N hydrochloric acid to pH 2.0 and incubated overnight at 4°C. A precipitate containing biosurfactant was collected by spinning the solution at 15,000 x g for 20 min. Following this, the precipitate was dissolved in distilled water and the extract yield for each of the isolates was noted and expressed as mg/100 mL. To check activities, a known concentration of biosurfactant (5 mg/ mL) was prepared as a stock solution and concentrations were standardized for each of the assays.

Assays to determine biosurfactant properties:

Oil spread, Tilted glass slide, Modified drop collapse:

Petridishes were filled with 50 mL of distilled water and to this, 20 μL of olive oil was spread uniformly. Further, 100, 120, 150, 160, 170, 180, 190 and 200 μg/ 10 μL concentration ranges of biosurfactant precipitates of each of the isolates were added on top of the oil to observe its disintegration. Disruption of oil as a clear zone indicated biosurfactant action.[13] In the case of tilted glass slide method, biosurfactant preparation of different concentrations such as, 100, 200, 300, 400, 500 and 600 μg/ 10μL were placed at the one end of the slide coated uniformly with olive oil. Dripping of the biosurfactant precipitate along the oil coating indicates positive for surfactant action.[14] Whereas for the case of modified drop collapse method, 10μL of olive oil was placed on the slide and biosurfactant preparation in different concentrations of 100, 200, 300, 400, 500, 600 and 700 μg/ 10 μL were added at the centre of oil drop. Biosurfactant producers were detected as collapsing oil droplet within a min as described by Bodour and Miller-Maier (1998).[15] All these experiments were performed in triplicates and the mean values $\pm$SD is noted. A positive control such as Sodium Dodecyl Sulfate (SDS) and controls without biosurfactant preparation/ SDS [1 μg/ 10 μL] were also run simultaneously. Minimum Effective Concentrations (MECs) were noted for each of the tests which denote the least concentration of surfactant to elicit a visible oil droplet spread/ drip/ collapse.

Evaluation of other properties of the isolates (Hemolytic activity and growth on Rhodamine B Agar plates)

All the isolates showing positive for lipase production were tested for hemolytic properties as per Abouseouda et al., (2008)[16] and Gandhimathi et al., (2009).[17] Briefly the cells were grown at 37° C for 48 hrs on blood agar plates to evaluate possible cell lysis. To check growth in Rhodamine Agar, lipodial solution of tributyrin and ZMA were prepared and mixed together with Rhodamine B solution and set on sterilized petriplates. The isolates were patch-inoculated and incubated at 37° C for 30 hrs. Cultures were examined on a transilluminator for fluorescence which indicated positive for degradation of hydrocarbons and release of free fatty acids.[18]

Gas chromatogram-Mass Spectrometry analysis of tributyrin degradation and identification of the potent isolate.

Isolate, K which exhibited potent tributyrin as well as olive oil degrading activities was cultured in ZMB amended with 10% (v/v) tributyrin and after 5 days of incubation at 37° C the culture broth was submitted to GC-MS (GC-FID, VARIAN-CP-3800) to find out whether or not there has been a degradation of tributyrin. Standard tributyrin and a control [uninoculated with K cultures] were also run simultaneously in GC-MS for comparison. Isolate K was identified up to the genus levels using the protocols outlined in Bergey's Manual of Systematic Bacteriology. These included observation of morphology [pigmentation, Gram staining, shape, endospore staining], growth on Mac Conkey Agar, IMViC, gas and acid production from various sugars, casein and gelatin hydrolysis, catalase test and growth with Tween20 and Tween 80.

RESULTS AND DISCUSSION

The Total Heterotrophic bacterial count from Gulf of Mannar was 15.7 x 10^2 and that of Palk Bay was 14.5x10^2 CFU/ 100μL of seawater. A total of 123 isolates were screened out of which only 7 from Gulf of Mannar and 9 from Palk Bay recorded positive for the production of extracellular enzyme, lipase (Fig. 1). Occurrence of lesser percentage of oil degrading bacteria of the total Bacterial Load is not uncommon because not many bacteria utilize hydrocarbons as their sole source of energy.[19, 20] Not only that, when microorganisms grow in such oil-rich environs, they must acquire many adaptations to utilize chained fatty acids which ultimately becomes a prerequisite for their growth.[21-24] In the present work, a maximum zone of clearance of 33 and 30 mm diameter was conferred by the isolates O and K (Gulf of Mannar), whereas, the isolates of Palk Bay did not exhibit more zones of clearance on tributyrin agar plates. In general, a maximum zone of only 20 mm was elicited by isolate 9 at the Palk Bay regions.

production is as follows: N > O > I > K > Q in Gulf of Mannar and 19 > 1 > 7 in Palk Bay with maximal yields from N (32mg/ 100mL) and 19 (20mg/ 100mL) (Fig. 1). As indicated before here again, only a very small population of the total heterotrophic load is categorized as biosurfactant producers in general. In fact, a study showcases seawater-derived biosurfactant producers contributed only 0.5% of the total heterotrophic bacterial load[29] and the current study recorded 6.5%. There was an overall strong positive correlation between the potent lipase producers with biosurfactant yield ($r = +0.54$). However, it was also found that isolates, M and R from Gulf of Mannar and 2, 9, 10, 14, 15 and 16 isolated from Palk Bay did not produce any biosurfactants though recorded positive for lipase production, indicating quite a few of lipase [+] isolates may not produce biosurfactants too. This indicates that biosurfactant may enhance the degradation of hydrocarbons together with extracellular lipases.[5-7]

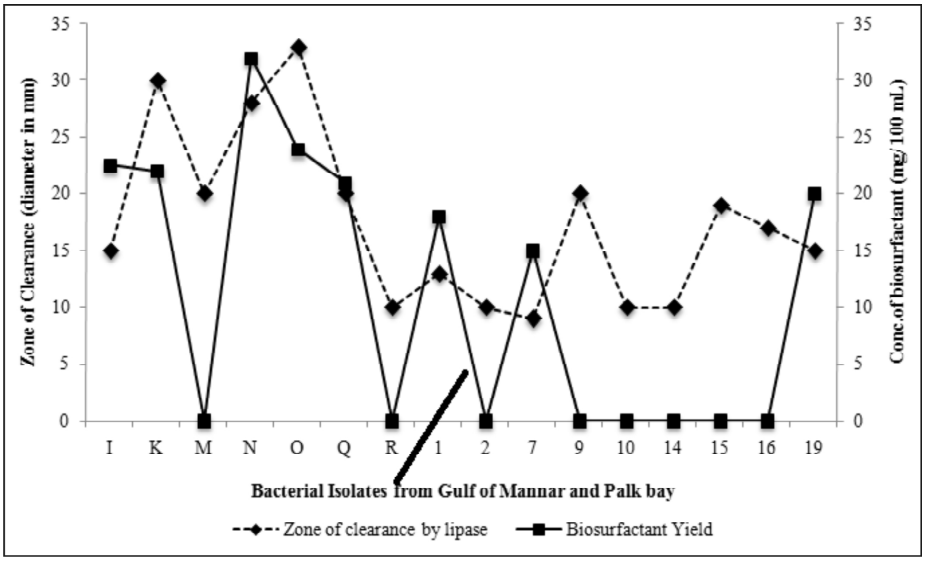

Fig. 1. Crude biosurfactant yield of bacterial isolates from waters of Gulf of Mannar and Palk bay

Preliminary lipase screening experiments were used as base for selection of isolates for mass production of biosurfactants based on literature pertinent because lipase secreting bacteria are by and large considered to secrete biosurfactants too.[25-28, 5] Based on zones of clearance on tributyrin supplied medium, only isolates, I, K, M, N, O, Q and R from Gulf of Mannar station and 1, 2, 7, 9, 10, 14, 15, 16 and 19 from Palk Bay were selected for production of biosurfactants.. In Gulf of Mannar region, most of the isolates categorized as lipase [+] yielded good amount of biosurfactants. The order of biosurfactant

Isolates K, O and Q had olive oil spreading capabilities by displacing them vigorously on the surface of water, at MECs of 120, 110 and 102 μg/ 10 μL respectively (Fig. 2). For tilted glass slide assay, strains K and O showed activities with MECs of 500 and 230 μg/ 10 μL respectively. For the modified drop collapse method, which is a tough test to analyze the efficacy of biosurfactants [owing to the piercing biosurfactant into a drop and breaking], isolate K had MEC of 250 μg/ 10 μL (Fig. 3). A few studies have only qualified isolates for biosurfactant activities by using a known volume of bacterial extracellular fluid than the

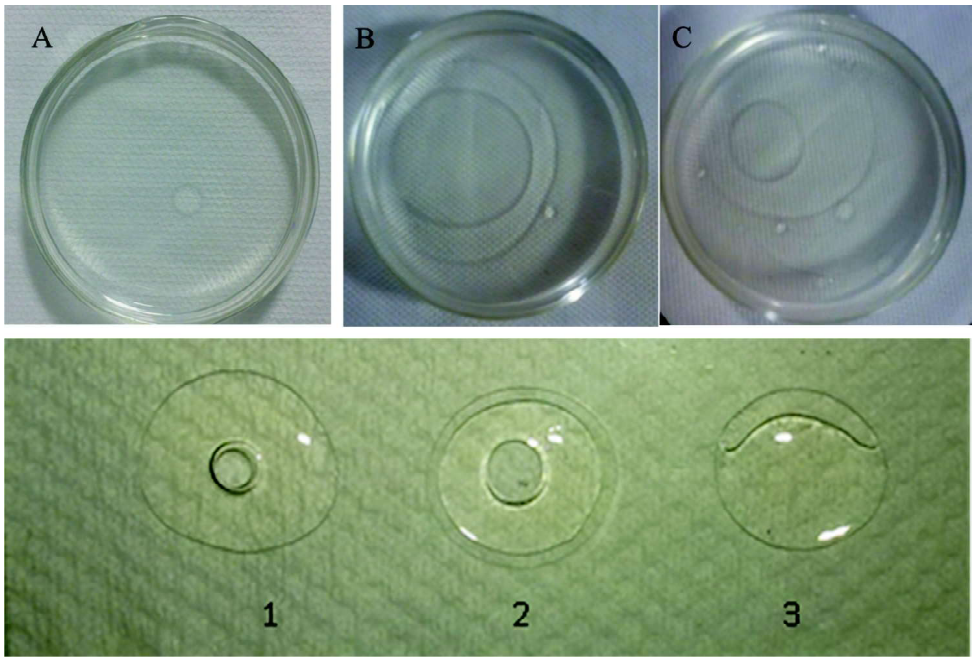

Fig.2 : Displacement of olive oil by biosurfactants of strain K in oil spread method. (a) Control-oil droplet in water (b) Positive control - SDS (1 μg) and (c) Biosurfactants of isolate K (120 μg). Disrupting the oil droplet on water by the biosurfactant of strain K (1)Water (2) SDS (2 μg) (3) Strain K (250 μg). (*correlation between oil spread and drop collapse $r = +0.67$ and between tilted glass slide and drop collapse- $r = +0.20$)

extracted biosurfactant rather.[15,20] In general, is always justified to use drop collapse as a primary method to detect biosurfactant producers and thereafter countercheck by oil spread to determine biosurfactant concentration.[30]

It is in general said all the three tests correlate positively, ie., when a biosurfactant is able to spread oil, it might also drip and collapse it as well.[31, 20] We indicate that all the three tests showed good correlation among each other, ie., between oil spread and drop collapse methods ($r = +0.67$), tilted glass slide and drop collapse ($r = +0.20$). Similar conclusions with positive correlation between all the three tests were reported in a few previous investigations[31,20] as well. Hence

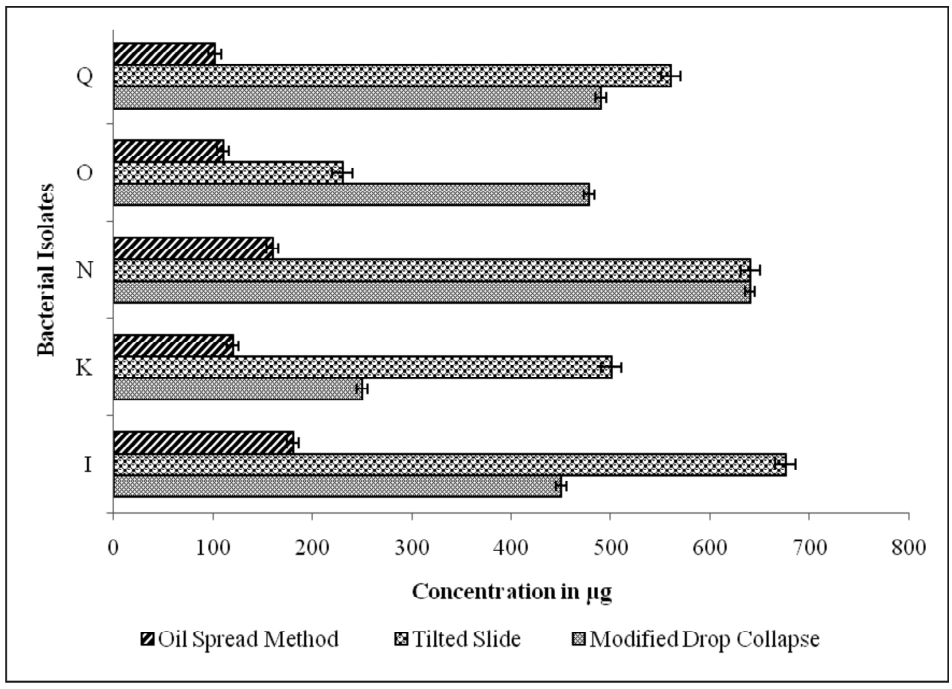

Fig. 3 : Minimum Effective Concentration (MEC) of the crude precipitate showing bioactivities

we suggest that all the three tests which show good positive correlation be used as a tool to evaluate biosurfactant properties. The supernatants when assayed for biosurfactant activity (data not shown) did not prove any positive results indicating that the biosurfactants were pelleted down. All the bacterial strains which were potent lipase producers were found to be Gram positive [60% being cocci and 40% rods] (data not shown).

Evaluation of other properties of the isolates

Hemolysis has been always been looked upon as a screening method for the isolation of biosurfactant producing strains.[32, 33] In the present study, all the isolates which possessed biosurfactant properties have shown hemolysis as blood agar plates as well showing a fair positive correlation ($r = +0.54$) as given in Fig. 4. However, there are also reports to mention that hemolytic activity is not always associated with biosurfactant property.[30] This could be true due to the presence of virulence factors of the hemocytes and weak infusibility of certain biosurfactants. Thus, it is not clear whether or not blood agar lysis could be used as a screening methodology to identify biosurfactant (+) isolates. However, such screening could only be used as rapid methods, in which isolates with a positive result should be tested for other tests like oil spread, titled glass and drop collapse methods to confirm surfactant properties.

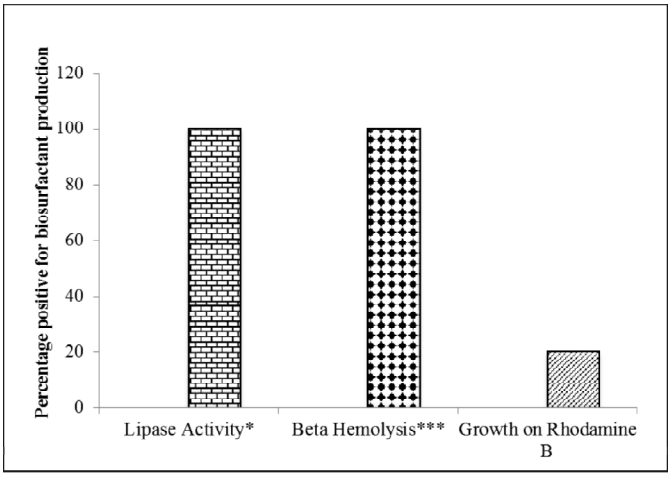

Fig. 4 : Percentage of lipase, hemolytic and Rhodamine B positive strains possessing biosurfactant properties. (*r* values: * +0.54; *** +0.89)

It is essential to find out whether or not the potent biosurfactant producers grow on Rhodamine B agar. Rhodamine B forms colored complexes with many acidic materials, especially when uranyl ion is present. According to Feigl (1956)[34], uranyl ions form complexes with fatty acids (breakdown product of fats and hydrocarbons) and completely ionize them. Thus protons released from fatty acids changes Rhodamine B into cationic form, which complexes with uranyl fatty acid ions which is an orange colored fluorescent compound. Thus many of the bacterial species which do not utilize tributyrin accumulates rhodamine B and only forms pink colored colonies, but do not fluoresce upon UV irradiation[18] and those which utilize hydrocarbons appear as fluorescent orange colored colonies. The current study indicates that isolate I (Gulf of Mannar) and 7, 15, 16 (Palk Bay) showed positive result for the Rhodamine test (Fig. 5) and put together, only 20% of the biosurfactant [+] isolates fluoresced when grown on Rhodamine B. These results contradict with a few other studies which suggest the usage of Rhodamine B agar for selective isolation of biosurfactant producing bacteria.[35-37] However this contradiction should be substantiated with further tests.

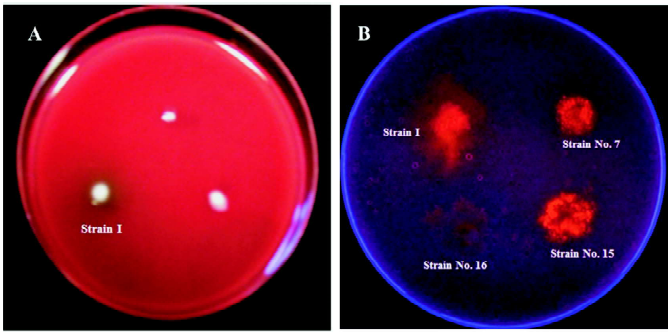

Fig. 5 : Blood Agar plates showing ß Hemolysis (A) and fluorescence in Rhodamine B agar (B).

Gas chromatogram- Mass Spectrometry analysis of tributyrin degradation and identification of the potent isolate.

Based on the zones on tributyrin agar, biosurfactant yield, results of oil spread, tilted slide and modified drop collapse methods, isolate K was chosen for tributyrin degradation studies. Degradation of tributyrin was witnessed by culture K at 5 days of incubation. GC-MS analysis suggested that the isolate K degraded almost all the hydrocarbon present in tributyrin (Fig. 6). Our preliminary results [on olive oil] for biosurfactant assays are confirmed as indicated by the degradation of this hydrocarbon too by the isolate K, as proved by decrease in peak heights. In comparison to the control, the produced surfactant reduced tributyrin to the tune of 96% only after 5 days of incubation. Based on the morphological and biochemical tests as listed in materials and methods [data not shown], isolate K is identified as a species belonging to the genus, *Streptococcus*.

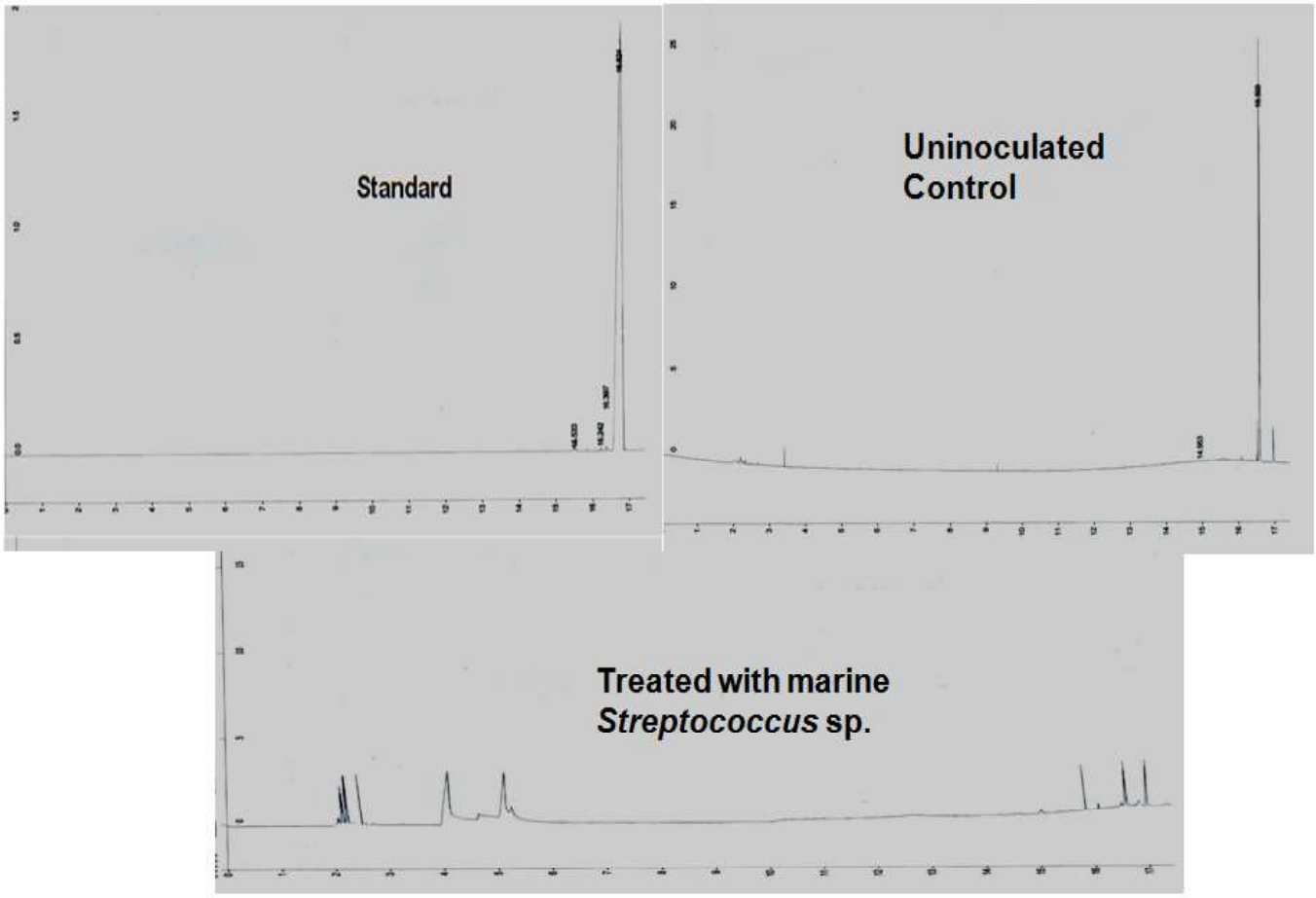

Fig. 6 : Degraded tributyrin on Gas Chromatography- Mass Spectrum (GC-MS)

To our knowledge this is the first work on biosurfactants from marine *Streptococcus* sp. Biosurfactants sourced from *Streptococcus* species are previously reported only from terrestrial sources.[38,39] Surfactants of *Streptococcus thermophilus* were mainly reported to be rich in glycolipid fractions. However marine Streptococcal species may secrete a variety of surfactants and could be looked upon as a new platform for exploitation of potent surfactants. Hence we conclude that more studies on the species level identification, pathogenicity of the isolate, analysis and purification of surfactant from this strain may offer a potent and possibly a novel surfactant. This study makes evident that hydrocarbon degrading bacteria are extant in the Gulf of Mannar regions which can be used to ameliorate oil pollution.

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REFERENCES:

1. Rai N, Pandey IP, Joshi K. Impacts and management of oil pollution along the Indian coastal areas. J Ind Res Tech 2011;1:119-26.
2. Ramadass V, Rajeswari KR. The effect of oil pollution and disposal of domestic sewage on the vitality and diversity of coral reefs of Palk Bay, Mandapam region, South-east coast of India. 2nd International Conference on Environmental Science and Technology IPCBEE IACSIT Press Singapore 2011;6:165-9.
3. Karuppanapandian T, Karuppudurai T, Kumaraguru A.K. A preliminary study on the environmental condition of the coral reef habitat. Int J Environ Sci Tech 2007;4:371-8.
4. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 2000;53:495-508.
5. Rahman KS, Rahman TJ, McClean S, Marchant R, Banat IM. Rhamnolipid biosurfactant production by strains of *Pseudomonas aeruginosa* using low-cost raw materials Biotechnol Progr 2002;18:1277-81.

6. Muthusamy K, Gopalakrishnan S, Ravi TK, Sivachidambaram P. Biosurfactants: Properties, commercial production and application. Curr Sci 2008; 94:736-47
7. Das P, Mukherjee S, Sen R. Antimicrobial potential of a lipopeptides biosurfactant derived from a marine *Bacillus circulans*. J Appl Microbiol 2008;104:1675-84.
8. Abraham WR, Meyer H, Yakimov M. Novel glycine containing glucolipids from the alkane using bacterium *Alcanivorax borkumensis*. Biochim Biophys Acta 1998;1393:57-62.
9. Poremba K, Gunkel W, Lang S, Wagner F. Marine biosurfactants, III. Toxicity testing with marine microorganisms and comparison with synthetic surfactants. Z Naturforsch 1991;46:210-16.
10. Zinjarde SS, Pant A. Emulsifier from tropical marine yeast, *Yarrowia lipolytica* NCIM 3589. J Basic Microbiol 2002;42:67-73.
11. Husain DR, Goutx M, Acquaviva M, Gilewicz M, Bertrand JC. The effect of temperature on eicosane substrate uptake modes by a marine bacterium *Pseudomonas nautica* strain 617: relationship with the biochemical content of cells and supernatants. World J Microbiol Biotechnol 1997;13: 587-90.
12. Jenneman GE, McInerney MJ, Knapp RM, Clark JB, Ferro JM, et al. A halotolerant, biosurfactant producing *Bacillus* species potentially useful for enhanced oil recovery. Dev Ind Microbiol 1983;24:485-92.
13. Morikawa M, Hirata Y, Imanaka TA. Study on the structure-function relationship of lipopeptide biosurfactants. Biochem Biophy Acta 2000;1488: 211-18.
14. Peerson A, Molin G. Capacity for biosurfactant production of environmental Pseudomonas and Vibrionaceae growing on carbohydrates. Appl Microbiol Biotechnol 1987;26:439-42.
15. Bodour AA, Miller-Maier R. Applied of a modified drop collapse technique for surfactant quantification and screening of biosurfactant producing microorganisms. J Microbial Meth 1998;32:273-80.
16. Abouseouda M, Maachib R, Amranec A, Bouderguaa S, Nabia A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination 2008;223: 143-51.
17. Gandhimathi R, Seghal K, Hema TA. Production and characterization of lipopeptide biosurfactant by a sponge- associated marine actinomycetes, *Nocardiopsis alba* MSA10. Bioproc Biosyst Engg 2009;32:825-35.
18. Kouker G, Jaeger K. Specific and Sensitive Plate Assay for Bacterial Lipases. Appl Environ Microbiol 1987;53:211-3.
19. Rashid N, Imanaka T. Efficient degradation of grease using microorganisms. J Chem Soc Pakistan 2008;30:612-7.
20. Karthik L, Kumar G, Rao KVB. Comparison of methods and screening of biosurfactant producing marine actinobacteria isolated from nicobar marine sediment. The IIOAB Journal 2010;1:34-8.
21. Whyte LG, Slagman SJ, Pietratonio F, Bourbonniere L, Koval SF, Lawrence JR. Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus* sp. Strain Q15. Appl Environ Microbiol 1999;65: 2961-8.
22. Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM. Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: Effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 2000;66: 3262-8.
23. Barkay T, Navon-Venezia S, Ron EZ, Rosenberg E. Enhancement of solubilisation and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. Appl Microbiol Biotechnol 1999;65: 2697-702.
24. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 1997;61: 47-64.
25. Thanomsub B, Watcharachaipong T, Chotelersak K, Arunrattiyakorn P, Nitoda T, Kanzaki H. Monoacylglycerols: glycolipid biosurfactants produced by a thermotolerant yeast, *Candida ishiwadae*. J Appl Microbiol 2004;96:588-92.
26. Khopade A, Ren B, Liu XY, Mahadik K, Zhang L, Kokare C. Production and characterization of biosurfactant from marine *Streptomyces* species B3. J Colloid Interface Sci 2012;367:311-18.
27. Colla LM, Rizzardi J, Pinto MH, Reinehr CO, Bertolin TE, Costa JAV. Simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. Bioresour Technol 2010;101:8308-14.
28. Rosenberg E, Ron EZ. High- and low-molecular-mass microbial surfactants. Appl Microbiol Biotechnol 1999;52:154-62.

29. Thavasi R, Sharma S, Jayalakshmi S. Evaluation of Screening Methods for the Isolation of Biosurfactant Producing Marine Bacteria. J Pet Environ Biotechnol 2011;1:1-6.
30. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ. Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Meth 2004;56:339-47.
31. Satpute SK, Bhawsar BD, Dhakephalkar PK, Chopade BA. Assessment of different screening methods for selecting biosurfactant producing marine bacteria. Ind J Mar Sci 2008;37:243-50.
32. Johnson MK, Boese-Marrazzo D. Production and properties of heat stable extracellular hemolysin from *Pseudomonas aeruginosa*. Infect Immun 1980;29: 1028-33.
33. Banat IM. The isolation of a thermophilic biosurfactant producing *Bacillus* sp. Biotechnol Lett 1993;15:591-94.
34. Feigl F. 1956 Spot tests in organic analysis, VII Edition, Elsevier publishers, NY [ISBN No: 9780444597977]
35. Das P, Mukherjee S, Sen R. Biosurfactant of marine origin exhibiting heavy metal remediation properties. Bioresour Technol 2009;100:4887-90.
36. McInerney MJ, Javaheri M, Nagle DP Jr. Properties of the biosurfactant produced by *Bacillus licheniformis* strain JF-2. J Ind Microbiol 1990;5: 95-101.
37. Queiroga CL, Nascimento LR, Serra GE. Evaluation of paraffins biodegradation and biosurfactant production by *Bacillus subtilis* in the presence of crude oil. Brazilian J Microbiol 2003;34:321-24.
38. Busscher HJ, Kuij-Booij DM, Mei HC. Biosurfactants from thermophilic dairy Streptococci and their potential role in the fouling control of heat exchanger plates. Ind J Microbiol Biotechnol 1996;16:15-21.
39. Rodrigues LR, Teixeira JA, Mei HC, Oliveira R. Isolation and partial characterization of a biosurfactant produced by *Streptococcus thermophilus* A. Colloids Surf B 2006;53:105-12.

AN OVERVIEW OF CUBOSOMES - SMART DRUG DELIVERY SYSTEM

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ABSTRACT

Cubosomes are square and rounded particles with internal cubic lattices visible. The invention of cubosomes is a distinctive story and spans the fields of food science, differential geometry, biological membranes and digestive processes. Self-assembled cubosomes act as active drug delivery systems; highly accepted, has got importance after innovation and nomination. Cubosomes are thermodynamically stable; they enclose a structure similar to "Honeycomb" through bicontinuous domains of water and lipid. Inside the surfactant, it is assembled into bilayers and wrapped into a three dimension, periodic and minimal surface, forming a strongly packed structure. Bicontinuous cubic liquid crystalline phase with optically lucid, extremely viscous material and have exclusive structure at the nanometer range. On the whole, cubosome render high importance in nano drug preparations for melanoma treatment outstanding to their potential advantages, including heavy payloads of drug because of increased surface area and cuboidal structures. They have very simple method of preparation; whereas biodegradability of lipids have the capability of encapsulating hydrophobic, hydrophilic and amphiphilic substances meanwhile targeted and controlled release of bioactive agents. Cubosome dispersions are bioadhesive and biocompatible. Because of their properties, cubosome are versatile systems, administrable by different ways such as oral, percutaneous and parenteral. Cubosomes have broad vast applications in many areas and are characterized by various parameters. Consequently, Cubosomes are in move forward of awareness by pharmaceutical development division.

***Key words**: Cubosomes, Hydrophilic, Honeycomb, Drug payloads, Pharmaceutical division.*

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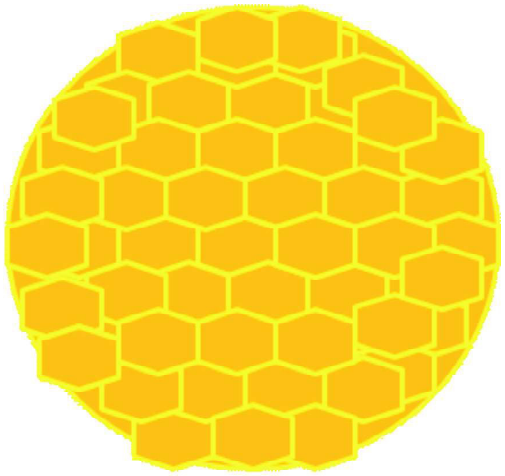
INTRODUCTION

Cubosomes are distinct, sub-micron, nano-structured particles of bicontinuous cubic liquid crystalline phase. They contain identical microstructure as that of its parent with high surface area and their dispersions are less viscous than the parent cubic phase.[1]

Most probably cubosomes are composed of polymers, lipids and surfactants with polar and non polar components hence said as amphiphilic. The amphiphilic molecules are driven by the hydrophobic effect into polar solvent to impulsively identify and assemble into a liquid crystal of nanometre scale (Fig.1). Thus cubosomes are bicontinuous cubic liquid phase enclosing two separate regions of water divided by surfactant controlled bilayers.[3] Further these are similar to liquid crystalline substance with cubic crystallographic symmetry and are optically isotropic, viscous and solid too.[4] The cubic phase can fracture and form colloidally and or thermodynamically stable particulate dispersions.[5] Cubosomes have great importance in nanodrug formulations.



CUBOSOME D

Fig. 1: Shows the bulk cubic phase/stage of Diamond (D) cubic lattices cubosomes.

Advantages of Cubosomes[6, 7]

- Because of their high internal surface area and crystalline cubic structures they have high drug payloads.
- They can be prepared by simple method.
- Posses lipid biodegradability.
- They can encapsulate all 3 types like hydrophilic, hydrophobic and amphiphilic substances.

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- They render bioactive agents with targeted release and controlled release.

Cubosomes retain their stability even at high dilution which is not possible with other liquid crystalline systems because they transform into micelles. Thus, being incorporated into formulations easily.[8] Cubosomes are generally produced by the following method: The bulk cubic phase is dispersed by high energy then colloidally stabilized using surfactant polymers.[9] Thus formed cubosomes are formulated into a product and applied topically/ mucosal surface which are absorbed/released via diffusion. They can be modified with protein molecules also. Moreover, companies like L'Oreal, Nivea, Procter and Gamble are interestingly investigating cubosomes for cosmetics applications which will be efficient and also cost effective for the scale up of this technology.

The disadvantages of the cubosomes are that they do not offer controlled drug delivery on their own compared with polymer based drug delivery. It is very difficult to load water soluble active ingredients during the formation of cubosomes, due to large amounts of water already present in it. Furthermore, researchers find it as a challenging task on production of large scale cubosome due to its high viscosity.

Recently, scientists are interested in preparing cubosomes for the treatment of cancer therapy, topical applications, cosmetics preparations and other drug delivery systems. Even though liposomes, niosomes, nanocochleates, micro sponges, micro particles and other carrier systems have been used as targeted / novel drug delivery systems, the cubosomes are more thermally stable. So, the authors suggest that preparation of cubosomes may be helpful in future by targeting the drug to a particular site and achieve therapeutic efficacy and also improve patient compliance.

Narration / The Past:

Early in 1980's, cubosomes manufacture in large scale was difficult because of their complexity and high viscosity. They are unique as they have viscosity similar to solids. These cubic phases can be broken and spread to thermodynamically/ colloidal particulates which are stable for a longer time. At certain concentrations, some surfactants form cubic phases suddenly when mixed with water. Luzzati and Husson,[10] Lusatia et al., [11] Larsson[12] and Hyde et al.,[13] determined the honey comb structure of cubosomes between 1960 and 1985 as represented in the figure 2 (a) and (b).

The word "Cubosome" was coined by Larsson, since the structure resembles cubic molecular crystals and liposomes. Attempts were made in scaling up the production of cubosomes. In practice very few anticancer drugs are being successfully formulated and characterized as cubosomes.[14]

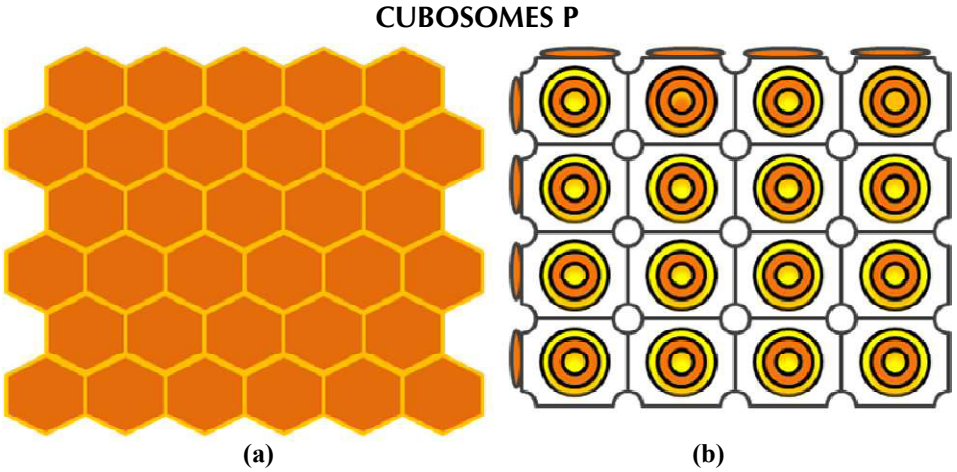

Fig. 2: (a) Shows the honey comb structures, (b) shows the structures of primitive (P) cubic cubosomes which is similar to honeycomb.

Cubosomes and their Uses:

A universal application is as drug delivery vehicles and although bulk cubic phases achieve controlled release frequently, the first patent on cubosomes was to specify its medical and controlled release applications.[15,16] As a result, self-assembled surfactant phases are extensively examined for compatibility through various medical dynamic ingredients and their applications.[17] The quick development of the life-sciences industry is likely to drive beforehand "exotic" delivery vehicles and ingredients interested in broader market places, such as personal care and consumer products.[18] An area under modern development by L'Oreal is the use of cubosomal particle

as oil-in-water emulsion stabilizers and pollutant absorbents in cosmetics.[19-22] They revealed that a second amphiphile, glycerol monooleate and phytantriol have an aqueous phase behaviour sufficiently close to that of monoolein to form cubosomes.

Interests on cubosomes being formulated as cosmetics products like skin care, hair care, antiperspirants has been increased and Nivea had filed patent too.[23-25] In spite of new activity, there resides a need on the practical problems like scale up and material customization with the purpose of necessary to lead formulators to believe using cubosomes in commercial products.

Current Status of Knowledge:

Cubosomes are biocompatible drug delivery system and is a novel approach. The controlled release application of these nanoparticles is of a great significance in cosmeceutical and pharmaceutical fields. Cubosome have become an attractive vehicle

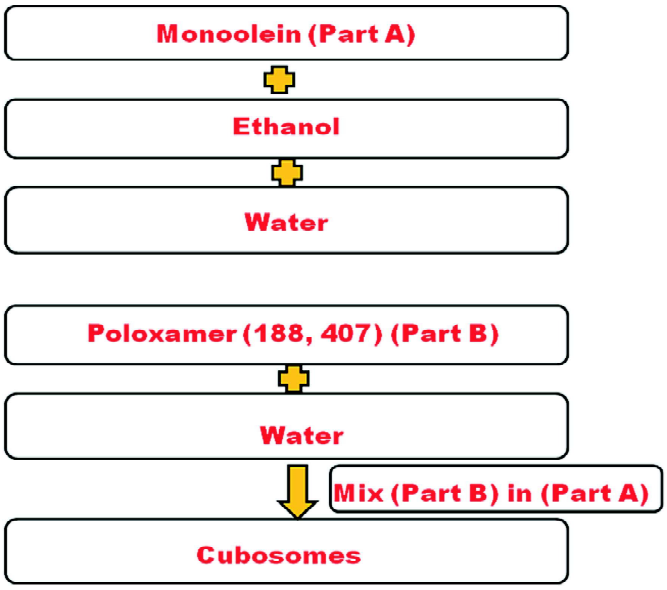

Fig.3: Shows the flow chart preparation of cubosomes formed by dilution of an isotropic solution.

for *in-vivo* drug delivery due to their low cost, versatility and potential for controlled release and functionalization. The personal care industry show particular interest on cubosomes due to their unique features. Deepak Prashar et al. and Patrick T. Spicer et al. studies substantiate the use of cubosome during manufacture / formulation enhanced the flexibility for product development.[26, 27] Cubosome formulations have been revealed to be safe in brain targeted drug delivery reported by Yosra SR Elnaggar et al and also Sergio Murgia et al have successfully exploited single living cell imaging by preparing drug loaded fluorescent cubosomes.[28, 29] Besides, Ruchi Sharma et al have reported the preparation of cubosomal gels of fluconazole which resulted in enhanced pay load, entrapment efficiency, drug permeability compared with conventional gels.[30]

DISCUSSION

Preparation / Manufacture of Cubosomes[31]

There are two methods in preparing cubosomes

i) Bottom up technique (Fig.3)

ii) Top down technique (Fig.4)

Cubosome dispersion formed by dilution of an isotropic solution (Bottom up technique)

Powder cubosomes precursor (Top down technique)

The merits of cubosomes preparations are:

* Easy method of preparation.
* Cost effective procedure.
* Less time consumption.

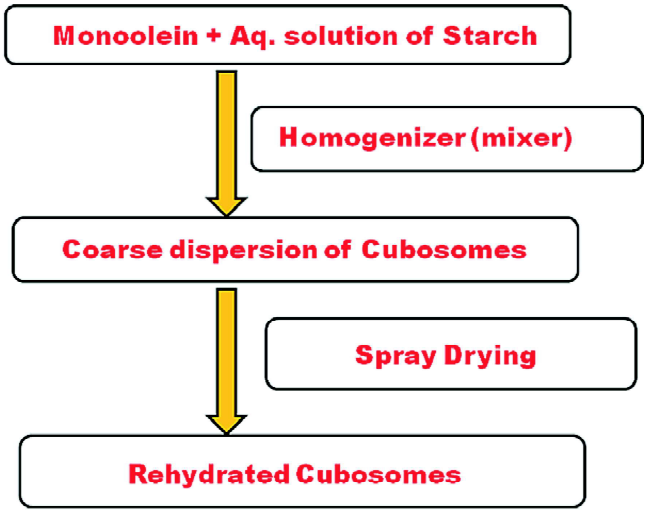

Fig.4: Shows the flow chart preparation of powdered cubosomes precursor.

CONCLUSION

Cubic phase materials formed with simple mixture of biologically compatible lipids and water and are consequently well suited for pharmaceutical and body tissue. The main applications of cubosomes are controlled release of various drugs, in melanoma (cancer) therapy, oral drug delivery systems, intravenous drug delivery systems and topical drug delivery systems. The capability to shape cubosomes both in use, throughout formulation or throughout manufacture offer great extent of flexibility for product development. Furthermore, the narrative or the past reviews states the effectiveness of cubosome as a controlled /sustained release drug carrier.

ACKNOWLEDGEMENT

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REFERENCES

1. Bhosale RR, Osmani RA, Harkare BR, Ghodake PP. Cubosomes. The Inimitable Nanoparticulate Drug Carriers. Sch Acad J Pharm 2013; 2:481-86.
2. Wu H, Li J, Zhang Q, Yan X, Guo L, Gao X et al. A novel small Odorranalectin - bearing cubosomes: preparation, brain delivery and pharmacodynamic study on amyloid-b25-35- treated rats following intranasal administration. Eur J Pharm Bio Pharm 2012;80:368-78.
3. Scriven LE. Equilibrium bicontinuous structure. Nature 1976;263:123-25.
4. Madhurilatha T, Paruchuri SK, Suria Prabha K. Overview of Cubosomes: A Nano Particle. IJRPC 2011; 1:535-41.
5. Spicer P. Cubosome Processing Industrial Nanoparticle Technology Development. Chem. Eng. Res. Des 2005;83:1283-86.
6. Rizwan SB, Dong YD, Boyd BJ, Rades T, Hook S. Characterisation of bicontinuous cubic liquid crystalline systems of phytantriol and water using cryo field emission scanning electron microscopy (cryo FESEM). Micron 2007;38:478-85.
7. Bei D, Meng J, Youan BC. Engineering nanomedicines for improved melanoma therapy: progress and promises. Nanomedicine (Lond) 2010; 5:1385-99.
8. Gustafsson J, Ljusberg Wahren H, Almgren M, Larsson K. Cubic lipid - water phase dispersed into submicron particles. Langmuir 1996;12:4611-13.
9. Landh T. Phase behavior in the system pine needle oil monoglycerides-Poloxamer 407- Water at 20. J Phys Chem 1994;98:8453-67.
10. Luzzati V, Husson F. The structure of the liquid-crystalline phases of lipid water systems. J Cell Biol 1962; 12: 207-19.
11. Luzzati V, Tardieu A, Gulik-Krzywicki T, Rivas E, Reiss-Husson F. Structure of the cubic phases of lipid-water systems. Nature 1968;220:485-8.
12. Larsson K. Two cubic phases in monoolein- water system. Nature 1983; 304: 664 - 65.
13. Hyde ST, Andersson S, Ericsson B, Larsson K. A cubic structure consisting of a lipid bilayer forming an infinite periodic minimal surface of the gyroid type in the glycerol monooleate water system. Z Kristallogr. 1984;168:213-19.
14. Vinod KR, Sravya K, Sandhya S, Banji D, Anbazhagan S, Prameela RA. Tailoring active compounds across biological membranes by cubosomal technology: an updated review. J Chine Pharm Sci 2013; 22:303-11.
15. Longley W, Mcintosh TJ, A bicontinuous tetrahedral structure in a liquid-crystalline lipid. Nature 1983; 303: 612-14.
16. Boyd BJ. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int J Pharm 2003; 260: 239-47,
17. Drummond CJ, Fong C. Surfactant Self-Assembly Objects as Novel Drug Delivery Vehicles. Curr Opin Colloid Interface Sci 2000;4:449-56.
18. Enriquez J, Goldberg R. Transforming Life, Transforming Business: The Life-Science Revolution. Harvard Bus Rev 2000; 96-104.
19. Ribier A, Biatry B. Cosmetic Compositions Comprising a Stable Aqueous Dispersion of Phytantriol-Based Gel Particles Containing a Long-Chain Surfactant as Dispersant and Stabilizer. Eur. Pat. Appl. 1995; 13.
20. Ribier A, Biatry B. Cosmetic or Dermatologic Oil/ Water Dispersion Stabilized with Cubic Gel Particles and Method of Preparation. Eur. Pat. Appl. 1996;16.
21. Ribier A, Biatry B. Oily Phase in Aqueous Phase Dispersion Stabilized by Cubic Gel Particles and Method of Making. Loreal (Paris, FR), USA. 1998.
22. Biatry B. Cosmetic and Dermatological Emulsion Comprising Oily and Aqueous Phase, Eur. Pat. Appl. (Loreal, Fr.), Ep. 2000.
23. Schreiber J, Albrecht H. Cosmetic Cleaning Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases. Ger. Offen. (Beiersdorf AG, Germany) De 2002.
24. Schreiber J, Eitrich A. Deodorant and Antiperspirant Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases. Ger. Offen. (Beiersdorf AG, Germany) De. 2002.
25. Schreiber J, Schwarzwaelder C, Cassier T. Skin Care Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases. Ger. Offen. (Beiersdorf AG, Germany) De. 2002.
26. Deepak P, Dharmesh S. Cubosomes: A Sustained Drug Delivery Carrier. Asian J Res Pharm Sci 2011; 1: 59-62

27. Patrick TS Bicontinuous Cubic Liquid Crystalline Phase and Cubosome Personal Care Delivery Systems. 1-27.

28. Yosra SRE, Samar ME, Doaa AA, Ossama YA. Novel piperine - loaded tween - integrated monoolein cubosomes as brain-targeted oral nanomedicine in Alzheimer's disease: pharmaceutical, biological and toxicological studies. Int J Nanomedicine 2015, 10: 5459-73.

29. Sergio M, Sara B, Angela MF, Sandrina L, Vito L, Valeria M et al. Drug-Loaded Fluorescent Cubosomes: Versatile Nanoparticles for Potential Theranostic Applications, Langmuir 2013;29:6673-79.

30. Ruchi S, Gurvinder K, Deepak NK, Fluconazole loaded cubosomal vesicles for topical delivery. Int J Drug Dev & Res 2015, 7: 032-041.

31. Spicer PT, Small WB, Lynch ML, Burns JL. Dry Powder Precursors of Cubic Liquid Crystalline Nanoparticles (cubosomes).J Nanopart Res.2002; 4:297–311.

POLYMERASE CHAIN REACTION TARGETING MULTIPLE SITES IN EARLY DETECTION OF ABDOMINAL TUBERCULOSIS

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ABSTRACT

Diagnosis of abdominal tuberculosis is challenging as the specimens are paucibacillary in nature. Polymerase chain reaction (PCR) is a diagnostic tool increasingly being used for the detection of Mycobacterium tuberculosis in such specimens. The efficiency of PCR as a molecular method in the detection of Mycobacterium tuberculosis depends on the targeted gene. Use of multiple targets in detection of Mycobacterium tuberculosis by PCR increases the sensitivity of the assay. We report a 57 year old male patient who presented to the tertiary care centre with increased frequency of defecation associated with mucous and pain. The clinical findings were found to be suggestive of tuberculosis. Tissue biopsy following colonoscopy was sent for histopathological investigations and for PCR targeting two multi-copy genes, IS6110 and TRC4. The histopathological findings were consistent with tuberculosis. PCR was performed with the biopsy sample by targeting IS6110, TRC4 and MPB64. PCR targeting IS6110 and MPB64 were negative whereas PCR targeting TRC4 was found to be positive. This case report suggests that PCR for tuberculosis must be performed by targeting multiple genes to increase the case finding among patients with tuberculosis.

Keywords: *Abdominal tuberculosis, Colonoscopy, IS6110, MBP64, MTB, PCR, TRC4.*

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INTRODUCTION

Tuberculosis (TB) affects one third of the global population[1] and is of major global health concern because of its diverse clinical presentations and the diagnostic dilemma it presents. Extra-pulmonary tuberculosis is difficult to diagnose as it deals with paucibacillary specimens. The delay in diagnosis affects disease management and increases the risk for mortality.[2] Therefore, there is an urgent need to reduce the time to laboratory diagnosis of TB and initiate early treatment. In this report, we present the laboratory diagnosis of an immunocompetent patient with abdominal tuberculosis using polymerase chain reaction (PCR) targeting multiple genes.

CASE REPORT

A 57 years old male patient presented to the tertiary care centre with increased frequency of defecation associated with mucous and pain. Frequency of defection was reported to be 5 to 7 episodes per day, was not associated with blood or foul smell. He also reported history of fever with evening rise of temperature for 1 week, cough for 1 month, weight loss for 3 months and loss of appetite for 3 months. There was no history of nausea, vomiting or diarrhoea. Though the patient reported having undergone colonoscopy elsewhere, he did not know the reason and did not give any history of prolonged medication. He had surgery for haemorrhoids, 3 years back. The patient was an alcoholic and smoker. He is not a known case of diabetes mellitus, hypertension or pulmonary tuberculosis. He is not allergic to any food or drugs. There was no family history of tuberculosis.

Tissue biopsy specimen was received in the laboratory following colonoscopy for histopathological investigations and for polymerase chain reaction (PCR) targeting two multi-copy genes, *IS6110* and *TRC4* which are specific for *Mycobacterium tuberculosis*. The impression from colonoscopy revealed a high suspicion of gastro-intestinal tuberculosis (Fig. 1).

The histopathology examination showed extensive ulceration with ileal mucosa and necrotising granulomatous inflammation which was consistent with tuberculosis (Fig. 2).

Prior to the PCR assay, DNA from the specimen was extracted using a standard kit based method (MTB amplification kit, Bangalore Genei) and a nested PCR was performed targeting *IS6110* gene. The same DNA was used for performing conventional PCR targeting *TRC4* gene. An internal control was included in the

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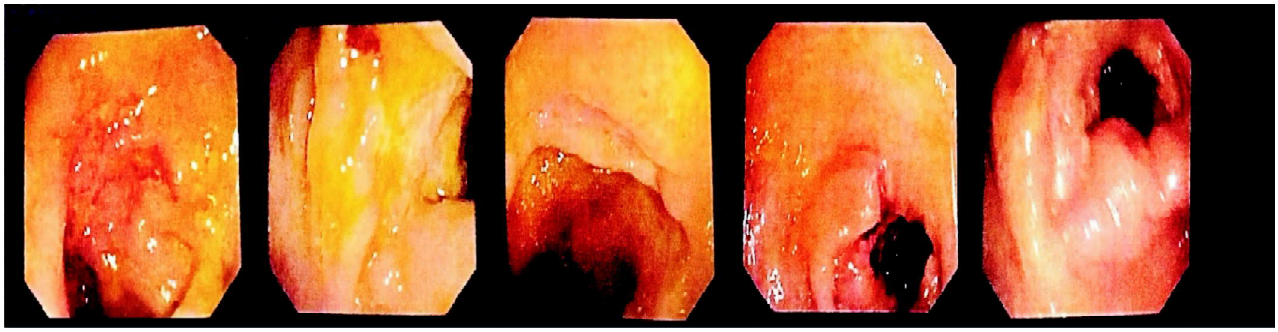

Fig. 1: The colonoscopy findings:The colonoscopy picture shows presence of multiple ulcers in the terminal ileum and colon region.

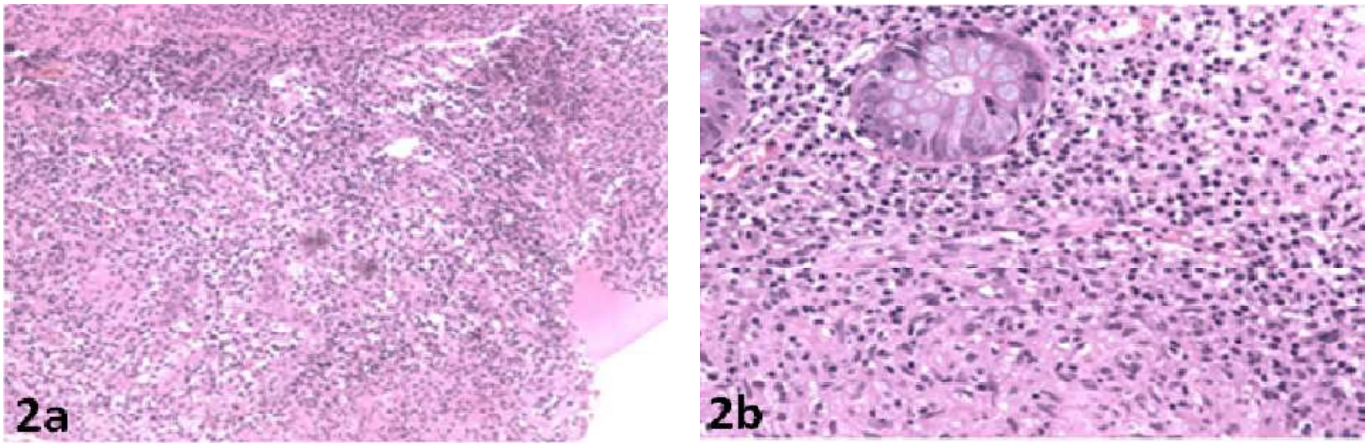

Figure 2a & 2b: The histopathological findings of the biopsy specimen under low and high power objectives: (H & E staining, 2a.100x 2b.400x). The tissue segment shows presence of granulomatous inflammation with epitheloid cells and lymphocytes, granulomas seen along with gastric gland under 2a. Low power objective and 2b. High power objective.

assay to confirm that the result was not false negative due to amplification inhibition. The PCR targeting *IS6110* gene was found to be negative (Fig. 3a), while the PCR targeting *TRC4* gene was found to be positive (Fig. 3b). Another PCR targeting *MPB64* gene was performed with same DNA, but was found to be negative (Fig. 3c).

DISCUSSION

Abdominal tuberculosis (TB) may involve any part of the gastrointestinal tract and with various clinical manifestations such as chronic abdominal pain, fever, weight loss, changes in bowel habits, abdominal mass, ascites, nausea, and vomiting.[3] It can also mimic other diseases and conditions, including inflammatory bowel disease, malignancy, and infectious diarrhoea and it is often difficult to diagnose.[3]

Therefore, clinicians often request for a combination of tests such as radiologic, endoscopic, histological findings, microbiological and molecular techniques. The microbiological culture method is considered the gold standard for the diagnosis of tuberculosis. Solid culture method takes 4-6 weeks, while the liquid culture method takes few days.[1,4] It has been reported that combination of histopathological and microbiological culture techniques can increase the diagnostic rate in over 60 % of patients.[5]

In abdominal tuberculosis, tissue from the affected area is the specimen of choice but these extra-pulmonary specimens are paucibacillary in nature.[1,4] Therefore, most laboratories have adapted to the rapid molecular techniques such as polymerase chain reaction. These assays are rapid and accurate even in paucibacillary conditions. It is a nucleic acid amplification based assay, which target specific genes from the genome of *Mycobacterium tuberculosis*. Several target sites such as *IS6110, TRC4, MPB64, 65kDa, 38kDa, 85B sequence, Pab, devR, GCRS, MTB40*, etc have been evaluated for detection of *Mycobacterium tuberculosis*.[1,2,6,7,8] Among these, *IS6110* the repetitive / multi-copy gene (present in multiple copies in the genome) is the most common gene targeted.[1] Similarly, *TRC4* is a multi-copy gene discovered and patented (235025) by scientists from National Institute for Research in Tuberculosis, Chennai, India.[6] A study reported that targeting the multi-copy gene *IS6110* performed better than targeting the other single copy genes.[7] But, some strains from

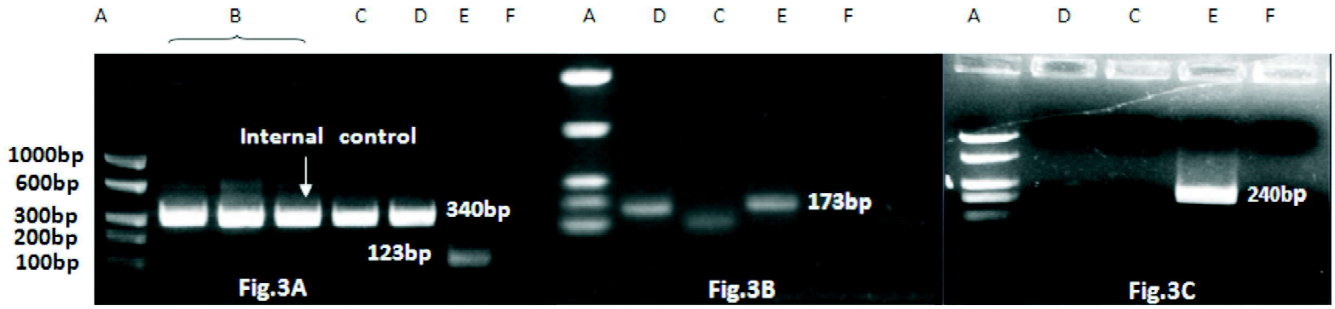

Fig. 3A : IS6110 gel image **Fig. 3B :** TRC4 gel image **Fig.3C :** MPB64 gel image

A. M.wt marker B.Samples C.Negative Control D. Colonoscopy Biopsy E.Positive Control F. NTC (No template control) Gel image shows Fig.3a.PCR targeting IS6110 gene was found to be negative. Fig.3b. PCR targeting TRC4 gene was found to be positive and Fig.3c. PCR targeting MPB64 gene was performed with same DNA, was found to be negative.

South India lack insertion sequence *IS6110* in their genome.[1] Thus, targeting only *IS6110* may lead to false negatives and mis-diagnosis. In this report, the negative PCR for *IS6110* region in the absence of amplification inhibition suggests that it could be due to strains that lack even a single copy of *IS6110*.

Few studies have reported using an additional target site such as *TRC4* to increase the diagnostic performance of PCR.[6,9] The negative PCR targeting *MPB64* explains the limitations of targeting a single copy gene; similarly, detection of *Mycobacterium tuberculosis* DNA by PCR for *TRC4* reveals the importance of targeting multiple sites, especially the multi-copy genes.

We conclude that the genome of *Mycobacterium tuberculosis* is wide and complex and requires detection of multiple PCR target sites. The accuracy of PCR assay mainly depends on these target sites. Moreover, PCR positivity can help to rule-in TB in an endemic region like India even when other laboratory tests are not conclusive. We suggest PCR for tuberculosis must be performed by targeting multiple genes to increase the case finding among patients with tuberculosis.

REFERENCE

1. M.P. Sharma & Vikram Bhatia. Abdominal tuberculosis. Indian J Med Res 2004;120: 305-15.
2. Ramadass Balamurugan, Subramanian Venkataraman, K. R. John, Balakrishnan S. Ramakrishna. PCR amplification of the IS6110 insertion element of Mycobacterium tuberculosis in fecal samples from patients with intestinal Tuberculosis. Journal of clinical microbiology 2006;44:1884-6.
3. Gregorio Zubieta-O'Farrill, Juan de Dios del Castillo-Calcáneoa, Carlos Gonzalez-Sanchezb, Eduardo Villanueva-Saenza, Jacob A. Donoghued. Colonic tuberculosis in an immunocompetent patient. International Journal of Surgery Case Reports 2013; 4:359- 61.
4. Phillipo L Chalya, Mabula D Mchembe, Stephen E Mshana, Peter F Rambau, Hyasinta Jaka, Joseph B Mabula. Clinicopathological profile and surgical treatment of abdominal tuberculosis: a single centre experience in northwestern Tanzania. BMC Infectious Diseases 2013; 13:270.
5. Rossato Silva D, Mânica Müller Roth Dalcin.P.T. Factors associated with delayed diagnosis of tuberculosis in hospitalized patients in a high TB and HIV burden setting: a cross-sectional study. BMC Infectious Diseases 2012; 12:57.
6. S Narayanan, V Parandaman, P.R.Narayanan, P.Venkatesan, C.Girish, S. Mahadevan, A Rajajee. Evaluation of PCR Using TRC4 and IS6110 Primers in Detection of Tuberculous Meningitis. J. Clin. Microbiol 2001; 39:2006-8.
7. Neghi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S. Diagnostic potential of IS6110, 38KDA, 65KDA, and 65KDA and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. Indian Journal of Medical Microbiology, 2007; 25(1):43-9.
8. Sahni.A.K.B, Singh. S.P. , Kumar.A, Khan I.D., Comparison of IS6110 and 'short fragment' devR, (Rv3133c) gene targets with phenotypic methods for diagnosis of Mycobacterium tuberculosis, Medical Journal Armed Forces India 2013;69:341-4.
9. R Barani, G Sarangan, T Antony, S Periyasamy, A J Kindo, P Srikanth. Improved detection of Mycobacterium tuberculosis using two independent PCR targets in a tertiary care centre in South India. J Infect Dev Ctries 2012; 6(1):46-52.

SUBGALEAL BLEED AND BILATERAL PROPTOSIS AS AN INITIAL MANIFESTATION OF MILD HEMOPHILIA A IN A CHILD.

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ABSTRACT

A nine year old boy presented with bilateral protrusion of eyes and gross decrease in visual acuity, and was evaluated for a systemic etiology. Radioimaging revealed the presence of subgaleal hematoma and bilateral subperiosteal haemorrhage. In the course of his stay in hospital, he had worsening proptosis, and deteriorating vision due to corneal decompensation and optic nerve ischaemia. After a battery of investigations, a diagnosis of mild Hemophilia A was made. Subgaleal bleed with subperiosteal haemorrhage in a male child should raise the suspicion of mild Hemophilia A. A high index of suspicion, early diagnosis and appropriate therapy would prevent dismal visual prognosis in these children.

***Key words**: Mild Hemophilia A, proptosis, subgaleal hematoma, subperiosteal haemorrhage.*

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INTRODUCTION

Subperiosteal haemorrhage can result from trauma, blood dyscrasias and haematological malignancies. Patients usually present with proptosis of acute onset, visual dysfunction and ophthalmoplegia.[1] Hemarthrosis is usually the presenting feature in Hemophilia A and there is almost always a history of preceding trauma. While subperiosteal haemorrhage as a presenting sign of Hemophilia A has been rarely reported, a combination of subgaleal bleed and subperiosteal haemorrhage as presenting features of mild Hemophilia A has not been reported, to the best of our knowledge. Subperiosteal haemorrhage follows subgaleal bleed and is a poor prognostic indicator, and when accompanied by ophthalmoplegia, compressive neuropathy and corneal exposure, poses a surgical dilemma in the absence of a definitive diagnosis of a bleeding disorder.

CASE REPORT

A 9 year old boy presented with bilateral proptosis of recent onset, associated with diminution of vision and frontal headache. Child had an episode of trivial injury of slip and fall while playing at school, one month ago, following which he had no complaints related to the trauma. There was no history of pain, fever, similar episodes in the past, swelling of knees or any other bleeding tendencies. He was the elder of two siblings, born of a third degree consanguinous marriage, and had achieved developmental milestones appropriate for age, and maintained a good scholastic performance.

He had a visual acuity of counting fingers close to face in the right eye, and 2/60 on the left eye. Ocular examination revealed bilateral severe proptosis, periocular ecchymosis, corneal xerosis with peripheral corneal gutterring, and total external ophthalmoplegia [Fig.1]. A relative afferent pupillary defect (RAPD) was demonstrated in the right eye. Fundus of the right eye was not visualised, whereas left fundus was normal.

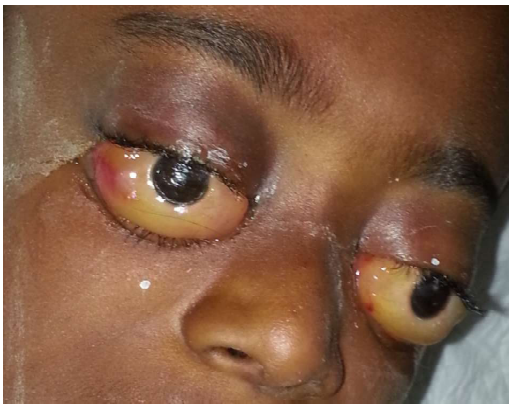

Fig.1: Clinical picture of the male child with bilateral extensive proptosis and severe exposure keratitis

Magnetic Resonance Imaging (MRI) brain showed extensive subgaleal and superior subperiosteal bleed [Fig. 2]. Simultaneous systemic evaluation was done, which revealed a near normal coagulation profile and haematological assay (platelet count -3.40, PT-15.9, aPTT -37.2) initially. Normal bone marrow study and

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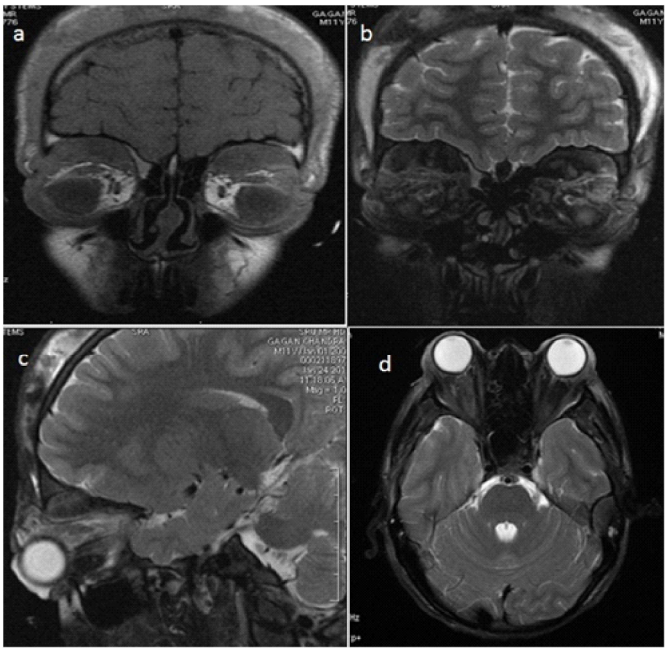


Fig.2 :(a,b) Coronal T1 T2 weighted images reveal subgaleal haemorrhage and bilateral relatively symmetrical areas of subperiosteal haemorrhages involving superior aspect of orbit. (c) Sagittal T2 weighted image showing extensive proptosis with adjacent extraoocular muscles, and optic nerve displaced inferiorly. (d)Axial T2 weighted image reveals subperiosteal haemorrhages with diffuse scalp haemorrhage.

ultrasonogram abdomen, ruled out acute myeloid leukemia and neuroblastoma respectively. Cerebral angiogram showed no evidence of arteriovenous malformations. Frequent instillation of topical lubricants and antibiotics was done to prevent corneal decompensation.

Inferring from the initial normal coagulation profile, the subgaleal bleed and the subperiosteal haemorrhage were thought to result from spontaneous venous bleed. Patient had increased orbital pressure and resultant orbital compartment syndrome as evidenced by bilaterally stretched optic nerves in MRI and RAPD in the right eye, along with constant corneal exposure and progressive corneal decompensation. Hence, after obtaining informed consent from parents, bilateral lateral canthotomy and subperiosteal drainage were performed under general anaesthesia. 40 cc of subperiosteal altered blood from the right side, and 30 cc from the left side were aspirated. Approximately 1cm of the lateral orbital margin was removed on both sides, involving the frontozygomatic suture to decompress the orbit and provide space for the regression of proptosis.

Post orbital decompression, the orbital pressure reduced remarkably and visual acuity improved to 6/60 (right eye) and 2/60 (left eye), pupillary reactions were restored, along with minimal improvement of extraocular movements. Unfortunately, the corneal decompensation progressed and bilateral amniotic membrane graft with cyanoacrylate glue application under general anaesthesia was done to prevent corneal perforation.

Despite aggressive ocular therapy, child developed bilateral exudates in the anterior chamber with peripheral ring infiltrates in the left eye. Microbiological studies of the corneal scrapings, showed no growth of organism. Topical antibiotic therapy with 2 hourly instillation of Besifloxacin 0.6% was initiated . Bilateral Bscan ultrasonography and Visually evoked Potential (VEP) showed normal study. Informed consent was obtained, and bilateral lateral tarsorrhaphy was done under general anaesthesia, to arrest the deterioration of the corneal status.

In view of persisting subgaleal bleed despite repeated blood transfusions and fresh frozen plasma infusions, child was suspected to have a haematological disorder. Repeat blood investigations revealed an abnormal Activated Partial Thromboplastin Time (aPTT - 47.2 seconds) value and low Factor VIII (11.0%) assay, confirming the diagnosis of Mild Hemophilia A. The delay in diagnosis was attributed to the initial near normal coagulation profile. Despite best efforts by a multidisciplinary team, child developed right corneal perforation and lost perception of light in the right eye. [Fig.3a] Left eye had resultant central corneal opacity with healed peripheral gutter, and maintained at 2/60. [Fig. 3b]

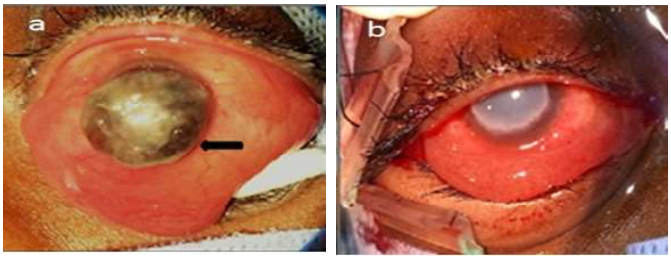


Fig.3: (a) Right eye demonstrating severe exposure keratitis and corneal perforation (arrow). (b) Left eye with central corneal opacity and healed peripheral gutter.

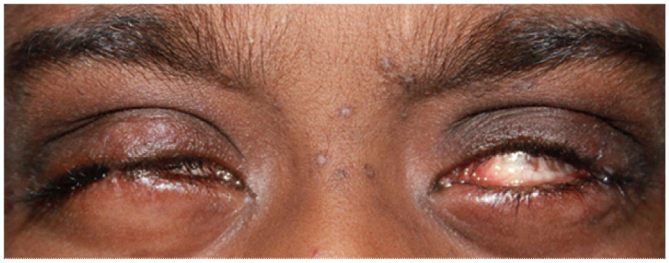
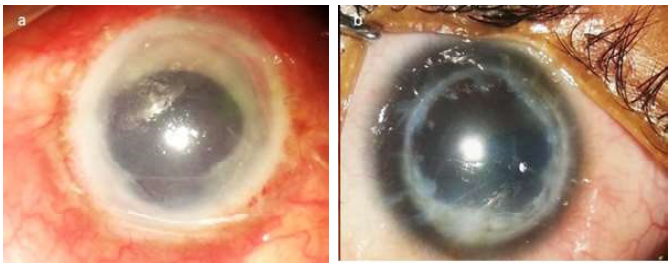


Fig.4: Resolving proptosis post bilateral lateral tarsorrhaphy



a b

Fig.5: Postoperative picture of right (a) and left eye (b) respectively following right anterior vitrectomy and sclerocorneal graft, and left penetrating keratoplasty.

After replacement therapy with Factor VIII, the proptosis regressed after 2 weeks. [Fig.4] With the consent of the parents, patient underwent right corneoscleral graft with Vitrectomy and Ahmed glaucoma valve implantation (due to a high probability of trabeculectomy failure), followed by left Penetrating Keratoplasty, under general anaesthesia.[8] Postoperatively, he had a visual acuity of counting fingers at 1 metre right eye, with residual vitreous haemorrhage, and 6/18 left eye respectively. [Fig. 5]

DISCUSSION

Hemophilia A is an X linked, recessive disorder caused by deficiency of Factor VIII (F VIII), which may be inherited or arise from spontaneous mutation.The coagulation cascade comprises of an intrinsic and extrinsic pathway which aids in the formation of a stable fibrin clot at the site of injury. Factors VIII and IX when activated , cooperate to cleave and activate factor X, which is vital for conversion of fibrinogen to fibrin.[2] Hemophilia is classified based on the plasma procoagulant level as severe (F VIII < 1% of normal) , moderate (1 - 5% of normal) and mild (5 - 40% of normal).[3] Severe hemophilia presents in children younger than 1 year with spontaneous bleeding whereas mild Hemophilia presents in children older than 2 years with bleeding only after significant trauma or surgery. Activated partial thromboplastin time (aPTT) is usually significantly prolonged in severe cases, but may be near normal in mild to moderate hemophilia.

Proptosis is usually a late occurrence in cases of subgaleal haematomas, the reason being the anatomical configuration of the orbital septum, which acts as a barrier between the facial and orbital structures. The orbital septum extends from the periosteum to the upper eyelid, blending anteriorly with the levator aponeurosis. This continuity is lost at the lateral canthal region, creating a communication between the subgaleal space and the periorbital space.[4]

Subperiosteal bleed could be spontaneous or could result from trauma, bleeding diathesis, vascular diseases, lymphangiomas and cavernous hemangiomas.[5] Mild hemophilia A should be thought to be the causal factor in a male child who presents with subgaleal bleed and proptosis arising from subperiosteal haemorrhage. Factor XIII deficiency[4] and Christmas disease (Factor IX deficiency)[6] are known to have produced subgaleal and orbital hematoma. Guirgis et al have reported subperiosteal haematoma as an initial manifestation of Christmas disease.[6] Poor vision at presentation, RAPD, external ophthalmoplegia and exposure keratopathy at presentation are all poor prognostic indicators for visual recovery. In the absence of subgaleal bleed, prompt replacement therapy with Factor VIII and supportive therapy can cause resolution of proptosis. However factor substitution therapy could be ineffective in patients with autoantibodies to Factor VIII.[7] Orbital decompression needs to be resorted to when there is an orbital compartment syndrome.[4]

A high index of suspicion, appropriate haematological assay and prompt replacement of the deficient factors can prevent adverse visual prognosis in children who present with subgaleal and bilateral subperiosteal haemorrhage .

ACKNOWLEDGEMENTS

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REFERENCES

1. Jeevesh Mallik, Anil Kumar, CB Sahay, TJ Minj. Orbital subperiosteal hematoma associated with frontal and subfrontal extradural hematoma- A case report . Indian Journal of neurotrauma 2013;10: 45-7
2. Kliegman, Stanton, St.Geme, Schor, Behrman. Nelson textbook of paediatrics. In.19th ed. Philadelphia. Elsivier Saunders 2011; 1699-701.
3. Preston FE, Kitchen S, Jennings I, Woods TA, Makris M. SSC/ISTH classification of hemophilia A: can hemophilia center laboratories achieve the new criteria? J Thromb Haemost 2004;2:271-4.
4. Natarajan MS, Prabhu K, Braganza A, Chacko AG. Posttraumatic subgaleal and orbital hematoma due to factor XIII deficiency. J Neurosurg Pediatr 2011;7:213-7.

5. CR Stewart, JF Salmon, Z Domingo, A D Murray.Proptosis as a presenting sign of extradural haematoma. Br J Ophthalmol 1993;77:179-80.
6. Guirgis MF, Segal WA, Lueder GT. Subperiosteal orbital hemorrhage as initial manifestation of Christmas disease (factor IX deficiency). Am J Ophthalmol 2002;133: 584-5.
7. A. Pakala, J. Thomas, P. Comp. Hemophilic Pseudotumor: A Case Report and Review of Literature. International Journal of Clinical Medicine. 2012;03:229-33.
8. Thomas R, Braganza A, Chandrasekhar G, Honavar S, Mandal AK, Ramakrishnan R, et al. The role of artificial drainage devices in glaucoma surgery. Indian J Ophthalmol. 1998;46:41-6.

BILATERAL SYNCHRONOUS RENAL MALIGNANCIES OF VARIED HISTOLOGY (BSRMVH): IPSILATERAL RENAL PELVIC UROTHELIAL CARCINOMA AND CONTRA-LATERAL CHROMOPHOBE TYPE RENAL CELL CARCINOMA - A RARE CASE REPORT AND REVIEW OF LITERATURE

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ABSTRACT

Urothelial malignancies of the renal pelvis and renal cell carcinoma are the most common urological malignancies. However, simultaneous bilateral renal malignancies are uncommon. Even more uncommon is synchronous bilateral renal neoplasms of dissimilar histologies.

In our report, we present a very rare case of a 42 year old male, who presented with painless total hematuria with passage of clots and left loin pain. On evaluation, he was diagnosed to have urothelial malignancy of left kidney and renal cell carcinoma of the right kidney. He underwent initial left nephrostomy placement, followed by left radical nephroureterectomy and right radical nephrectomy.

This case is presented for its rarity (first ever such case published in Indian literature), the diagnostic dilemma faced, the challenges that it posed in offering an appropriate treatment and the lessons learnt.

Key words: *Urothelial malignancy, renal cell carcinoma, synchronous malignancy.*

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BACKGROUND

Renal cell carcinoma and transitional cell carcinomas are commonly encountered urological malignancies. It is not uncommon to individually make a diagnosis of renal cell carcinoma and urothelial malignancies. However, the combination, that too synchronously in contra lateral kidneys Bilateral Synchronous Renal malignancies of varied histology (BSRMVH), is a rare entity.

The overall incidence of bilateral renal malignancies is 1 to 4% for renal cell carcinomas and 3.5% for transitional cell carcinomas.[1,2] Cancers of varied histological patterns are extremely rare. So far, only 13 such cases are being reported in literature.

In a large population-based study, one of the most salient and novel finding was that the risk of bilateral renal cell cancer depends profoundly on age at first diagnosis. Patients first diagnosed before age 40years were at a 17-fold higher risk compared with patients first diagnosed at the age of 60 years or older.[3]

Patients with bilateral multifocal renal cell carcinoma are at increased risk of developing locally recurrent or de novo tumors after nephron-sparing procedures. The main reason for recurrence after nephron-sparing surgery is likely to be the presence of multifocal disease, which is identified in 4 to 25% of the radical nephrectomy specimens.[4]

In this report, we present a case of a middle aged obese male who presented with BSRMVH. The rarity of this presentation, the dilemma and challenges faced in diagnosis and management are being discussed here.

CASE REPORT

A 42-year-old male reported with painless total hematuria with passage of amorphous clots of two months duration. He also had vague pain in the left loin pain for 15 days. The loin pain was dull aching, ill localized, continuous and non-radiating.

On examination there was vague abdominal fullness on the left loin. Left renal angle was full. There was mild tenderness on deep palpation of the left loin. Ultrasound of abdomen showed a well-circumscribed right upper polar tumour and left side grossly dilated hydronephrotic kidney with thinned out cortex.

His hemoglobin level was 10 gms%. Total count was 13,500 cells/cub mm, with predominant neutrophilia. His temperature at presentation was 100.4F. The serum creatinine was 1.6 mg% and blood urea levels were 48 mg/dl.

Contrast Enhanced Computed Tomography (CECT) of Kidneys showed left pelvi ureteric junction (PUJ) obstruction with hydronephrosis and a heterodense enhancing lesion in the right renal

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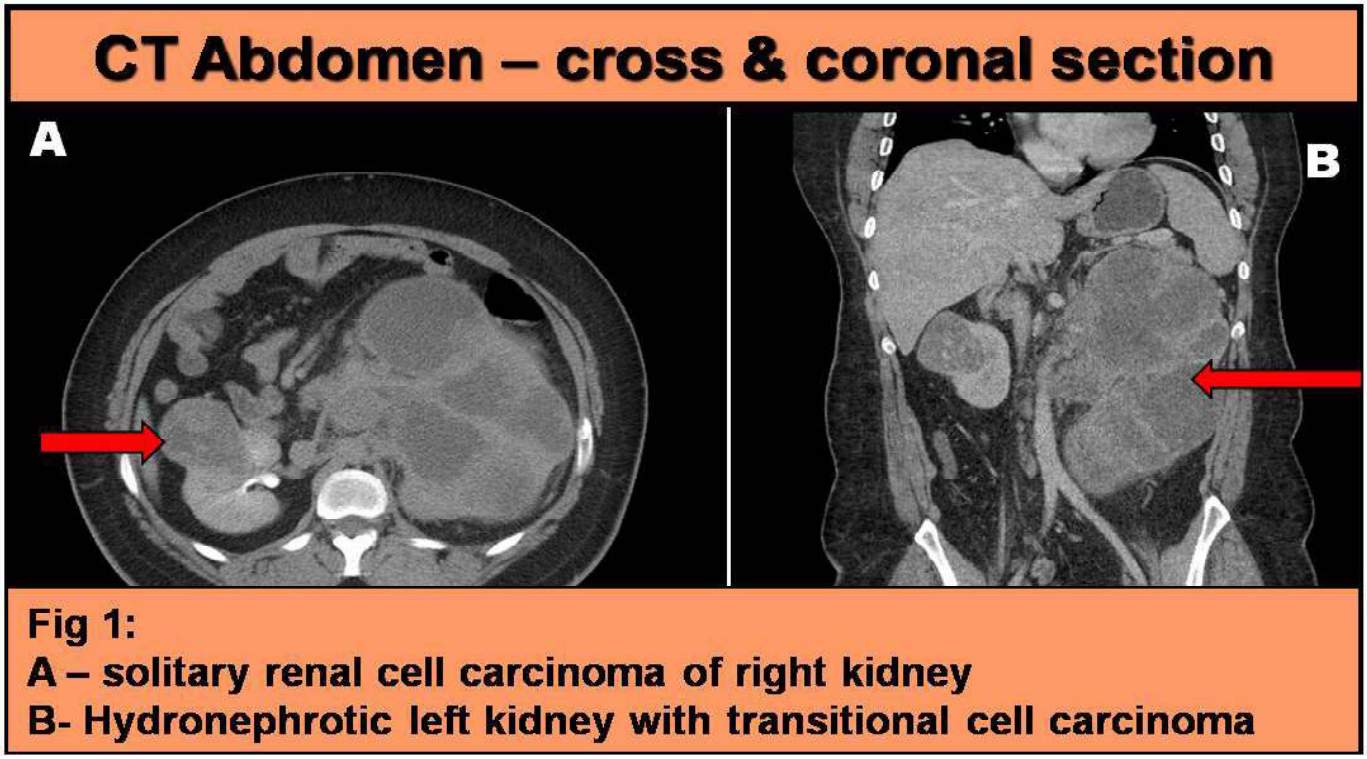

Fig 1:
A – solitary renal cell carcinoma of right kidney
B- Hydronephrotic left kidney with transitional cell carcinoma

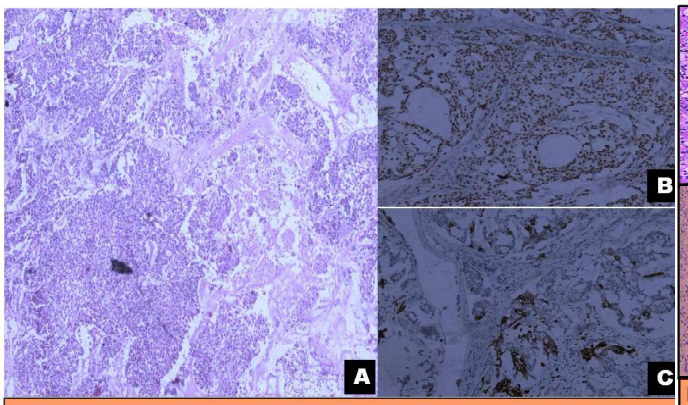

Fig 2: A – Urothelial Carcinoma High power (H & E 200 X)
B – P63 Positive
C – CD 10 negative

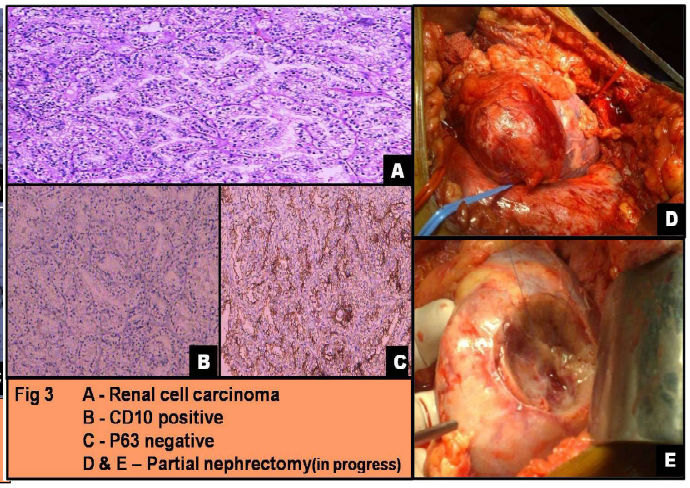

Fig 3 A - Renal cell carcinoma
B - CD10 positive
C - P63 negative
D & E – Partial nephrectomy(in progress)

interpolar region suggestive of Bosniak type IV cystic lesion with left paraaortic lymphadenopathy (Fig 1). Nuclear scintigraphy showed decreased uptake in the left kidney compared to the right. After an initial diagnosis of left PUJ obstruction and right renal cell carcinoma was made, patient was posted for left double J stenting and right partial nephrectomy. During left side stenting, retrograde pyelogram revealed an irregular upper ureteric margin and a tight narrowing at the pelvi ureteric junction that was impossible to be negotiated with a hydrophilic guide wire. Diagnostic ureteroscopy revealed only a red glow at the level of upper ureter and the renal pelvis could not be entered. Hence left percutaneous nephrostomy (PCN) was done as a temporary external diversion procedure. On PCN placement, about 1200 ml of thick brownish, gelatinous fluid was drained. Initially an infected PUJ obstruction was thought of, but the urine culture was sterile. Cytology of the drained fluid was positive for urothelial malignancy.

He subsequently underwent Left nephroureterectomy with excision of the nephrostomy tract. Histopathology revealed a high grade urothelial malignancy of renal pelvis (Fig 2 A,B,C). Patient was explained about the need of residual ureteral stump removal along with the cuff of bladder on left side, but he was not willing for the same. One month later he underwent nephron sparing surgery on the right side. In the immediate post operative period his serum creatinine rose upto 1.6mg/dl, later it reached a nadir of 1.1mg/dl. Histopathology revealed chromophobe renal cell carcinoma T1bN0 Fuhrman Grade 1 (Fig 3

A - E). Patient subsequently was offered chemotherapy using Paclitaxel and Gemcitabine.

DISCUSSION

Bilateral synchronous renal malignancies of varied histology are extremely rare. So far, following a thorough medline search, only a very few cases are reported in literature. If we exclude those diagnosed by autopsy, we believe we are presenting the eighth overall and the first ever such case being reported from our country. A majority of those reported earlier were from Japanese literature.

The first case of BSRMVH was reported by Villegas et al in 1967, where the diagnosis was made at autopsy.[5] Gillis et al reported the first case of BSRMVH in a live patient in 1971.[6] Joczi reported a similar case in 1976 where a radical nephrectomy was done for renal cell carcinoma and a partial nephrectomy was performed for transitional cell carcinoma.[7] However, the patient survived without any recurrence for the next 8 years. Sung Kyu reported a case of a 63 year old male who presented with left renal pelvic carcinoma and right renal cell carcinoma and subsequently underwent left nephrectomy and lymphadenectomy and planned for angio infarction of the right renal mass.[8]

Smoking has been the most important and significant predisposing factor for development of renal cell carcinomas and urothelial malignancies. Various occupational hazards, industrial chemicals, ingestion of large amounts of phenacetin and family history have all been considered to be contributory factors for development of urothelial malignancies.[9,10] However synchronous occurrence of malignancies of varied histology has been a rare entity.

The biological behavior of tumors of varied histology, the various grades and stages at which they present, presence of other co-morbid and predisposing factors and the need for a long term follow-up in such cases pose a significant challenge in prognosticating as well as managing such tumors.[11]

Our patient had a left radical nephroureterectomy and a right partial nephrectomy done. However, he needed long term follow up for the residual left ureteral stump and bladder recurrences.

The purpose of this case report is to highlight the rarity of our presentation, need for a high index of suspicion whenever any form of an abnormal fluid is drained from the kidney and the diagnostic dilemmas and challenges that one could face in making an appropriate decision regarding management.

REFERENCES

1. Bratslavsky G, Linehan WM. Long-term management of bilateral, multifocal, recurrent renal carcinoma. Nat Rev Urol 2010;7:267-75.
2. Kang CH1, Yu TJ, Hsieh HH, Yang JW, Shu K, Shiue YL. Synchronous bilateral primary transitional cell carcinoma of the upper urinary tracts: ten patients with more than five years of follow-up. Urology 2004;63:380-2.
3. Wiklund F, Tretli S, Choueiri TK, Signoretti S, Fall K, Adami HO. Risk of bilateral renal cell cancer. J Clin Oncol 2009;27:3737-41.
4. Grimaldi G, Reuter V, Russo P. Bilateral non-familial renal cell carcinoma. Ann Surg Oncol 1998; 5:548-52.
5. Villegas A C. Bilateral primary malignant renal tumor of dissimilar histogenesis: report of 2 cases and review of literature. Urol 1967;98:450-5.
6. Gillis DJ, Finnerty P, Maxted WC. Simultaneous occurrence of hypernephroma and transitional cell carcinoma with development of transitional cell carcinoma in the opposite kidney: case report. J Urol 1971;106:646-7.
7. Jozsi B P, Wise H A, Quilter T N. Bilateral simultaneous kidney tumours of dissimilar cell type: a case report with emphasis of operative approach. JUrol 1976;116:655-7.
8. Hong S K, Jeong SJ, Lee SE. A case of renal transitional cell carcinoma associated with synchronous contralateral renal cell carcinoma. J Korean Med Sci 2001;16:108-10.
9. Ando M, Arisawa C, Okano T. A case of renal cell carcinoma associated with synchronous contralateral renal pelvic cancer and bladder tumor. Int J Urol 1996;3:310-2.
10. Adachi Y, Kubota Y, Irisawa C, Watanabe H, Ishii N, Suzuki K et al. A case of simultaneous contralateral renal cell carcinoma and ureteral transitional cell carcinoma. Urol Int 1991;46:67-9.
11. N Srinath, US Dinesh, GP Sharma. A case of synchronous renal cell carcinoma (Rt) and transitional cell carcinoma urinary bladder. Medical Journal Armed Forces India 2006;62:190-91.

AN INTERESTING CASE OF ENDOMETRIAL STROMAL SARCOMA CLINICALLY MIMICKING FIBROID UTERUS

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ABSTRACT

The diagnosis of uterine sarcoma in women undergoing hysterectomy with a clinical diagnosis of fibroid uterus is very low. Uterine sarcomas are clinically indistinguishable from leiomyomas since both typically present with abnormal uterine bleeding, pelvic pain and a pelvic mass. A high degree of suspicion is essential to diagnose these tumours. Endometrial Stromal Sarcomas(ESS) are rare, constitute 10% of all uterine sarcomas and 0.2% of all uterine malignancies. The mean age of presentation is 42 to 58 years. Uterine sarcomas are most commonly diagnosed following myomectomy or hysterectomy. In a premenopausal woman with bleeding disproportionate to size of uterus and significant pain, sarcoma is suspected. We report a case of ESS in a 42 year old woman with fibroid uterus following hysterectomy.

Key words: *Endometrial Stromal Sarcomas, Hysterectomy, Leiomyomas.*

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INTRODUCTION

The diagnosis of uterine sarcoma in women undergoing hysterectomy with a clinical diagnosis of fibroid uterus is 0.2 to 0.5 percent.[1] Uterine neoplasms with mesenchymal differentiation includes leiomyoma, leiomyosaromas and endometrial stromal tuomors. Endometrial Stromal tumors are rare and can be benign or malignant. Endometrial Stromal Sarcomas(ESS) constitute 10% of all uterine sarcomas and 0.2% of all uterine malignancies.[2] The International Society of Gynecologic Pathologists and the World Health Organization, classifies uterine sarcomas as purely nonepithelial or mixed epithelial-nonepithelial type. The usual clinical presentations are vaginal bleeding, pelvic pressure symptoms (eg,pressure, urinary frequency, constipation), enlarged uterus, or abdominal distension. Distinguishing leiomyoma and sarcoma in the preoperative period is difficult. Only rare cellular variants of leiomyoma progress to sarcoma. Diagnostic modalities like Ultrasound examination, magnetic resonance imaging, computed tomography, or positron emission tomography cannot reliably distinguish between a sarcoma and leiomyoma, endometrial cancer, lymphoma, intravenous leiomyomatosis, or adenomyosis. Endometrial sampling will yield the correct diagnosis in some, but not all patients.[3] We report this case to highlight the fact that even clinically innocent looking lesions may turn out to be malignant on histopathology.

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CASE REPORT

A 42 year old lady presented to gynaecology department with complaints of dysmenorrhoea of increasing intensity for the past 6 months. She essentially did not have menstrual complaints except dysmenorrhoea. Per abdomen examination showed a uterine mass of 16 weeks size. Speculum examination showed a healthy cervix and pap smear was taken. On pelvic examination she had a 16 weeks size uterus. Baseline investigations were done and were within normal limits. Transvaginal ultrasonogram showed a 9.7 x 6.9 x 8.0 cm intramural fibroid in anterior right lateral wall and endometrial thickness was 8.5 mm (Fig.1). Pap smear was negative for intraepithelial lesion or malignancy. Endometrial aspiration showed disordered proliferative pattern. With a provisional diagnosis of fibroid uterus total abdominal hysterectomy (TAH) with ovarian conservation was planned.

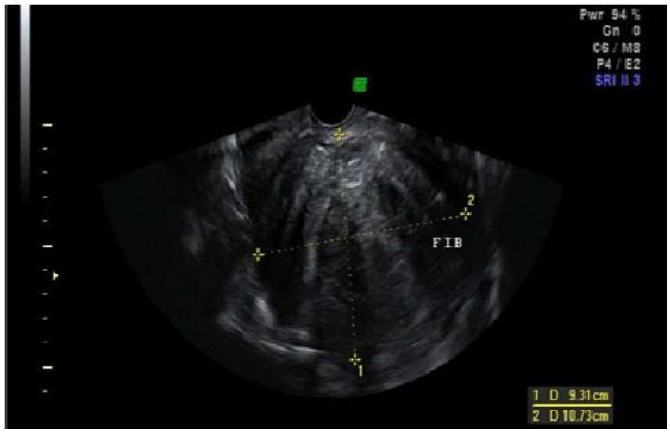

Fig. 1 : TVS showing anterior wall intramural fibroid 9.3 x 10.7cm.

Intraoperatively TAH with right salpingo oopherectomy was done as right adnexa was adherent to uterus and left ovary was conserved. (Fig.2). On cut section fibroid with degenerative changes was observed and didnot arouse a suspicion for frozen section. Histopathological examination of the specimen showed a tumor composed of small round to oval cells with uniform size and shape(Fig. 3). There were only 3 mitosis/10 HPF with no nuclear atypia or necrosis. With these features a diagnosis of Endometrial stromal sarcoma -low grade was given. Full thickness of myometrium was involved. By immunohistochemistry Estrogen Receptor and Progestrone Receptor was positive. The positivity of

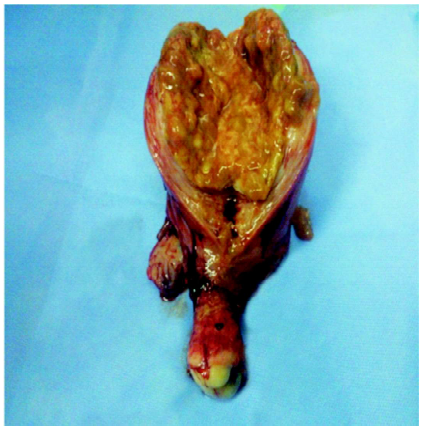

Fig. 2: Picture showing cut section of uterus with the mass and right adnexa.

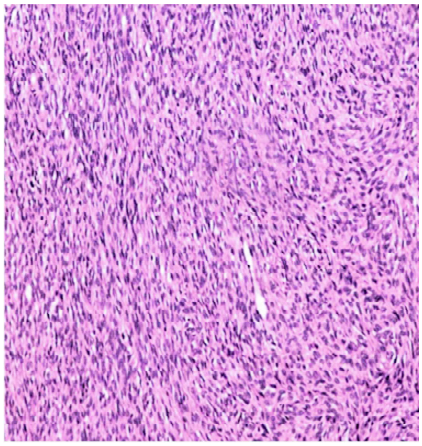

Fig.3: Endometrial stromal sarcoma -Photomicrograph showing the tumour with small round to spindle cells(H &E X 100)

these markers can be used as a targeted therapy. Also C-Kit (CD 117) was done to rule out a GIST (Gastro intestinal Stromal Tumor).

Medical oncologist opinion was obtained. Computerised tomography plain and contrast was done which showed a post hysterectomy status, left ovary 4.2x2.4x4.3 cms and no significant lymph node enlargement and no evidence of extrauterine spread (Fig. 4) Oncologist advised revision surgery to remove left ovary and for pelvic lymphadenectomy. In the event of no surgery, to decide for chemoradiation. The patient was administered 6 cycles of chemotherapy with Paclitaxel, Adriamycin and Cisplatin. External Radiation Therapy was given to deliver a dose of 50.4 Gy in 28 fractions. Patient had a good response and is on a regular follow up.

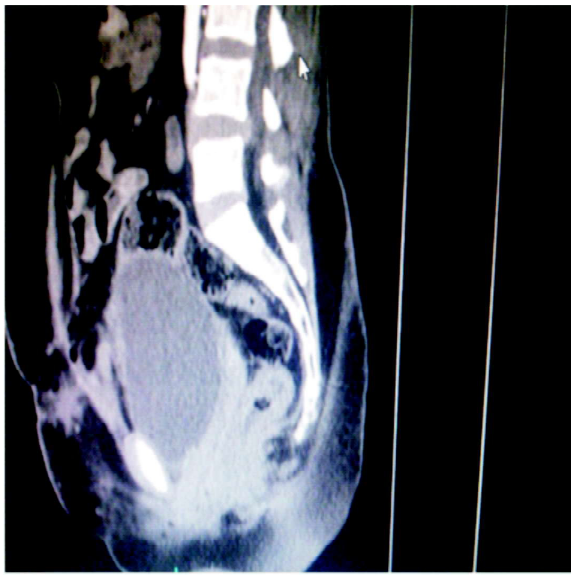

Fig. 4 : Computerised tomography of abdomen and pelvis showing no evidence of extra uterine spread in the post operative period.

DISCUSSION

The American College of Obstetricians and Gynecologists advises that there is a risk of uterine sarcoma in women taking tamoxifen.[4] Post menopausal status , pelvic irradiation and a childhood history of Retinoblastoma are other risk factors.[5] The overall survival rate at 10 years is 65 to 76 percent. Uterine sarcomas are referred to as homologous or heterologous. The majority are homologous and they differentiate in ways similar to normal uterine tissues. It includes those arising from endometrium (ESS-endometrial stromal sarcomas), muscle (leiomyosarcoma), or sarcomas of nonspecific supporting tissue (eg, connective tissue, blood vessels, lymphatics). Heterologous tumors contain elements with non-native differentiation like skeletal muscle, cartilage, bone. The World Health Organization classifies endometrial stromal tumors into three categories: endometrial stromal nodule (ESN), endometrial stromal sarcoma (ESS), and undifferentiated endometrial sarcoma (UES). ESN is a benign entity that can be cured with simple hysterectomy. Endometrial stromal sarcoma - ESS is a low-grade sarcoma with metastatic potential. They exhibit myometrial and/or vascular invasion[6] ESS is characterised by distinctive

finger-like projections that invade the myometrium, veins, and lymphatics. Histologically, they are characterized by densely uniform stromal cells with minimal cellular pleomorphism, mild nuclear atypia, and rare mitotic figures. Low-grade ESSs have less-frequent mitosis (< 3 per 10 high-power fields) and they do not show hemorrhage and necrosis. They are immunoreactive for the estrogen and progesterone receptors (ER and PR).They are typically immuno-histochemically positive for CD10 and negative for desmin and h-caldesmon.[7] Endometrial stromal sarcoma (ESS) has a non-specific appearance on ultrasound, typically characterized as a heterogeneous hypoechoic endometrial mass, which can show extensive myometrial involvement. On magnetic resonance imaging (MRI), these tumors appear as large masses with or without evidence of myometrial invasion. The characteristic pattern of ESS consists of worm-like tumor projections along the vessels or ligaments, which are best visualized on MRI with diffuse weighted imaging.[8]

ESS are staged according to the 2010 International Federation of Gynecologic Oncology (FIGO) staging system. If sarcomas are diagnosed following hysterectomy for a fibroid uterus it is essential to do imaging to look for metastasis. Further surgery is not needed if the imaging is negative. If extrauterine disease is detected, surgical staging and cytoreduction are performed only if there is no extraabdominal disease and the intraabdominal metastases are resectable. Lymphadenectomy is per-formed only in patients with preoperative evidence of enlarged lymph nodes (based on imaging), intraoperative findings of lymphadenopathy, and those with extrauterine disease.[9]

Observation is needed for patients with surgical stage I ESS. For women with surgical stage II to IV ESS, adjuvant endocrine therapy is needed. Radiotherapy may be administered (in addition to endocrine therapy) to reduce the risk of a locoregional recurrence rather than chemotherapy. For patients with recurrent or metastatic ESS who progress despite endocrine therapy chemotherapy is given. Hormone therapy with medroxy progesterone, tamoxifen, gonadotropin releasing hormone (GnRH) analogues and aromatase inhibitors are suggested for low-grade ESS stage 3-4 and for recurrent disease.[10] Available treatment combinations include gemcitabine plus docetaxel and doxorubicin-based regimens. As these tumors have a tendency for late recurrence, long-term follow up is essential. It shall be once in 3 months for the first year and half-yearly for next 4 years. Thereafter annual follow up is recommended. Our case had no evidence of metastasis on computerised tomography and was treated with radiotherapy and chemotherapy to prevent metastasis. She is advised to come for a regular follow up in future.

This case is highlighted to show the significance of histopathological examination in all clinically benign and innocent looking lesions, as a correct diagnosis will help in the proper management of the patient.

REFERENCES

1. Leung F, Terzibachian J.J, Gay C. et al. Hysterectomies performed for presumed leiomyomas: should the fear of leiomyosarcoma make us apprehend non laparotomic surgical routes? Gynecol Obstet Fertil 2009;37:109-14.
2. Chan JK, Kawar NM, Shin JY, Osann K, Chen LM, Powell CB, et al. Endometrial stromal sarcoma: A population based analysis. Br J Cancer 2008;99:1210–5.
3. Bansal N, Herzog TJ, Burke W, Cohen CJ, Wright JD. The utility of preoperative endometrial sampling for the detection of uterine sarcomas. Gynecol Oncol 2008;110:43–8.
4. Yildirim Y, Inal MM, Sanci M, Yildirim YK, Mit T, Polat M, et al. Development of uterine sarcoma after tamoxifen treatment for breast cancer: report of four cases. Int J Gynecol Cancer 2005;15:1239–42.
5. Yu CL, Tucker MA, Abramson DH, Furukawa K, Seddon JM, Stovall M, et al. Cause-specific mortality in long-term survivors of retinoblastoma. J Natl Cancer Inst 2009;101:581–91.
6. Hendrickson, M.R., Tavassoli, F.A., Kempson, R.L. Mesenchymal tumors and related lesions. in: F.A. Tavassoli, P. Devilee (Eds.) World Health Organization Classification of Tumors. Pathology and Genetics: Tumors of the Breast and Female Genital Organs. France: International Agency for Research on Cancer, Lyon; 2003:233.
7. Rush DS, Tan J, Baergen RN, Soslow RA. h-Caldesmon, a novel smooth muscle-specific antibody, distinguishes between cellular leiomyoma and endometrial stromal sarcoma. Am J Surg Pathol 2001;25:253-8.
8. Tamai K, Koyama T, Saga T, Morisawa N, Fujimoto K, Mikami Y, et al. The utility of diffusion-weighted MR imaging for differentiating uterine sarcomas from benign leiomyomas. Eur Radiol 2008;18: 723-30.
9. Tropé CG, Abeler VM, Kristensen GB. Diagnosis and treatment of sarcoma of the uterus. A review. Acta Oncol 2012;51:694-705.
10. Reich O, Regauer S. Hormonal therapy of endometrial stromal sarcoma. Curr Opin Oncol 2007;19:347-52.

AN INTERESTING POP UP IN THE GALLBLADDER

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ABSTRACT

Gallbladder polyps can be a cause of concern as some are premalignant lesions. Risk factors for malignancy include patients older than 60 years with a single, sessile polyp more than 1cm in size and associated with gall stones. Lipoma is one type of benign polyp of the gallbladder and is a rare entity. Limited number of cases has been published in the literature. We present a case of lipoma of the gallbladder in a 48 year old male patient.

Keywords: *Gallbladder, lipoma, polyp.*

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INTRODUCTION

Gallbladder polyps comprise of 4.3-6.9% of all gallbladder lesions. They are broadly classified into non-neoplastic and neoplastic polyps. Lipoma of the gallbladder is a benign neoplastic lesion. The incidence of lipoma of the gallbladder is unknown as very few cases have been documented in literature.

CASE REPORT

A 48 year old male presented to the outpatient department with right hypochondrial pain. Following clinical work up patient was found to have a gallbladder polyp and underwent open cholecystectomy.

Grossly the gallbladder measured 4cm in length. The wall thickness ranged from 0.3-1cm. The mucosa was granular and bile stained with a well defined sessile polyp measuring 2x2x1 cm was seen (Fig. 1). Cut surface of the lesion was greasy yellow.

Microscopically a fairly circumscribed lesion was seen along the muscularis propria and serosa which was composed of mature adipocytes is seen (Fig. 2). No other significant pathological findings were noted. Thus a diagnosis of ' Lipoma of the Gallbladder' was rendered.

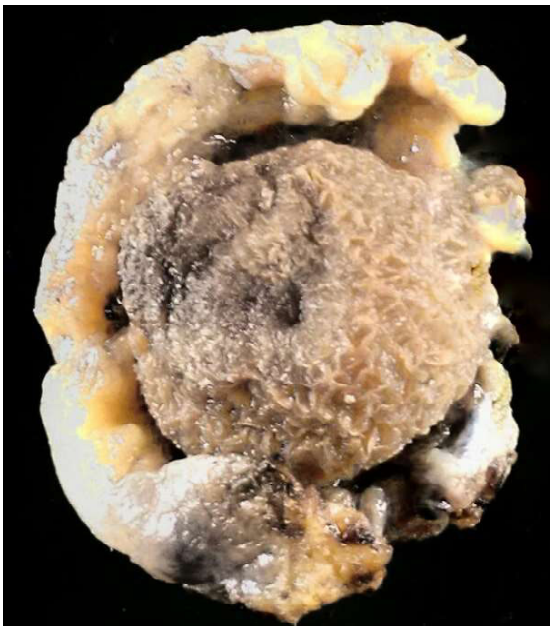

Fig.1: Gallbladder with a single sessile polyp

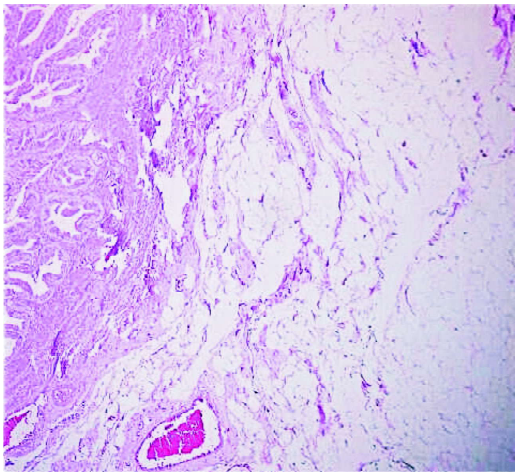

Fig. 2: Microscopy (40x) shows a collection of mature adipocytes along the muscularis propria and serosa

DISCUSSION

Gallbladder polyps are outgrowths protruding from the mucosal surface It represents a wide spectrum of lesions which are broadly classified into benign and malignant. Benign gallbladder polyps are subdivided into- 1) pseudotumors (cholesterol polyps, inflammatory polyps, cholesterolosis and hyperplasia), 2) epithelial (adenomas) and 3) mesenchymal (fibroma, lipoma, hemangioma). Malignant gallbladder polyps include gallbladder carcinoma.[1]

Gallbladder polyps account for 4.3- 6.9% of the gallbladder lesions.[1] Little is known about the risk factors of gallbladder polyps. Review of literature

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shows an association with congenital polyposis syndromes like Peutz Jeghers polyp, Gardners syndrome, altered fat metabolism and chronic hepatitis B. Age, gender, obesity diabetes and other medical conditions have not shown consistent relationship in the development of gall bladder polyps.

Gallbladder polyps are usually asymptomatic and found incidentally on routine ultrasound imaging. In some cases they can present with right hypochondrial pain, dyspepsia, vomiting or complications like obstructive jaundice, cholecystitis or pancreatitis. In a retrospective analysis of gallbladder polyps on abdominal ultrasound, 64% were diagnosed during a work-up of unrelated illness, 23% had abdominal symptoms and 13% had elevated liver function tests. In our case the patient had right hypochondrial pain.

Cholesterol polyps may detach and behave clinically as a gallstone, causing complications like biliary colic or even pancreatitis. Other complications include acalculous cholecystitis and hemobilia.

There has been an increasing trend in the incidence of gallbladder carcinomas. Epidemiological studies showed the highest incidence in northern India, followed by Pakistan, Ecquador, Korea, Japan and Europe.[2] The most plausible cause for carcinoma is the dysplasia-carcinoma sequence. Though less than 3% of the polyps particularly adenomatous polyps can be premalignant or malignant, routine follow up is required.[3] Risk factors of malignancy include age greater than 60 years, single sessile polyps with a size more than10mm and those associated with gallstones.[4]

Percutaneous ultrasound is a useful imaging modality. When the features of the polyp are obscured like in the presence of stones endoscopic ultrasound is more useful .[5,6]

In the current case, ultrasound imaging showed a partially contracted gallbladder with minimal wall thickening, few small polyps largest measuring 3 mm. As the patient was symptomatic, cholecystectomy was done and grossly a single sessile soft yellow poylp measuring 2x2x1 cm was identified. Microscopy revealed a fairly circumscribed lesion composed of mature adipocytes along the muscularis propria and serosa of the gallbladder.

Lipomas of the gastrointestinal tract more commonly involve the small bowel and arise from the submucosa. Lipomas can also be seen arising from intramural and extramural sites. Limited literature is available on lipomas of the gallbladder. Gallbladder is one of the rarer locations and as they lack a submucosa, the lipomas if present more commonly arise from the wall.

In our case the patient was below 60 years of age with multiple polyps largest measuring 3mm on ultrasound with no associated gallstones. As the patient was symptomatic cholecystectomy was done. On gross examination there was a single sessile polyp of 2 cm size, which microscopically showed features of lipoma. Histopathology aided in confirming the benign nature of the lesion.

J Shah studied 31 polypoidal lesions of the gallbladder diagnosed by ultrasound. and concluded that ultrasound is useful in early detection of polypoidal lesions of the gallbladder but requires histopathogical correlation to differentiate malignant from non-malignant polyps, greater than 5 mm in size and in whom long term follow up cannot be completed.[7]

CONCLUSION

Lipoma of the gallbladder presenting as a polyp is a rare entity. Gallbladder polyps presenting in patients as a single sessile mass greater than 5mm or as multiple lesions need surgical intervention and histopathological correlation.

REFERENCES

1. Andren-Sandberg A. Diagnosis and management of gallbladder polyps. N Am J Med Sci 2012; 4: 203-11.
2. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 2006;118:1591-602.
3. Roa I, De Aretxabala X, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer. J Surg Oncol 2006;93:615-23.
4. Terzi C, Sokmen S, Seckin S, Albayrak L, Ugurlu M. Polypoid lesions of the gallbladder:report of 100 cases with special reference to operative indications. Surgery 2000;127:622-7.
5. Azuma T, Yoshikawa T, Araida T, Takasaki K. Differential diagnosis of polypoid lesions of the gallbladder by endoscopic ultrasonography. Am J Surg 2001;181:65-70.
6. Humphris JL, Jones DB. Subepithelial mass lesions in the upper gastrointestinal tract. J Gastroenterol Hepatol 2008;23:556-66.
7. Shah J. Postoperative Histopathology Findings of Ultrasonographically diagnosed gallbladder polyp in 32 patients. The Internet Journal of Third World Medicine. 2010;9(1).

PREDATORY JOURNALS - A NOTE OF CAUTION

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The primary need to publish research, as we all know, is to share new findings as can be useful contributors to science and society. Publications also help in the researchers obtaining recognition as well as a satisfaction that they have contributed significantly, in an accepted manner. The need to publish has become even more relevant today than it was a few years ago due to several other factors; from qualifying for certain academic UG and PG programs, for obtaining PhD qualification, for elevation in career and for obtaining research funds being the predominant driving factors. Well, when several factors are involved, it is inevitable that a dilution of sorts will occur, from the quality of publications and the quality of journals/publishing houses too. The past decade has seen an alarming raise in the number of publishing houses with journals of doubtful quality with dubious means of attracting, soliciting and publishing "scholarly Research". Several thousands of such journals now exist capitalizing on the "Open Access" model of sharing research. Such journals are considered not appropriate for publishing meaningful research as can contribute to knowledge generation and sharing. In fact, such journals add junk to scientific literature and dilute the very philosophy of scientific publications.

Some of the ways in which such predatory journals entice gullible authors are by providing confusing facts about the Impact factors, the speed of peer review, manner in which peer-review is conducted and by using the "Open Access" model of publishing. Some examples of confusing parameters are the "Google-based Impact Factor, The International Scientific Indexing (ISI) Impact Factor, claims about being evaluated by leading indexing portals when not actually included and including links to leading indexing portals such as PubMed in their websites irrelevantly. These journals invariably charge publication/processing fees and some of them even have premium payment options for rapidly accepting and publishing manuscripts!

There are several sources of information regarding predatory journals. One of the best sources is as provided by Jeffrey Beall, the librarian of Auraria library, University of Colarado, Denver, USA. His web site https://scholarlyoa.com/publishers/ provides valuable information about predatory journals and a list of suspicious publishing houses and the journals. The list is constantly being updated and three main criteria for identifying these journals are by evaluating using the guidelines of following Committees/Codes/ Guidelines:

* Committee on Publication Ethics (COPE)
* Code of Conduct for Journal Publishers
* Principles of Transparency and Best Practice in Scholarly Publishing

It is therefore important to identify good journals before one gets to submit a manuscript towards publication. Some of the factors that can be useful in the identification of reputed journals include the following:

1. Indexing
2. Journal ranking (Impact Factors, etc)
3. Peer review process
4. Composition of the editorial board

1. Indexing

Some of the indexing portals that reputed journals are included in include PubMed, MEDLINE(r), EMBASE, Science Citation Index, Scopus, EMBiology, BIOSIS, Current Contents/Life Sciences, Elsevier BIOBASE, CNRS/Pascal and Chemical Abstracts.

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In addition to above, MCI has also included Index Copernicus in the database list for Faculty promotion (reference of the MCI letter dt. 22/07/2015 and can be accessed on www.mciindia.org/ circulars/ Circular-03.09.2015-TEQ-Promotion-Publication.pdf website). Also UGC website Also, UGC INFONET Digital library consortium has also given a list of resources and journals (http://inflibnet.ac.in/econ/ oaeresources.php)

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2. All dental journals subscribed by Indian national medical library

3. All journals published in speciality associations , there are nine associations publishing its journals

4. Indian Dental Association Journals (All India)

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Refer: www.dciindia.org.in/Admin/NewsArchives/A-8752.pdf

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2. Journal ranking by Impact Factors

As of now, the only universally accepted Impact Factor is as provided by the Journal Citation Reports(r) from Thomson Reuters. All other Impact Factors as claimed by several predatory publications are not validated or accepted universally.

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4. Composition of the editorial board

The composition of the editorial board, the qualifications and experience of the members should be appropriate to the scope and policies of the journal.

Hence, it is always useful to identify an appropriate journal before submitting one's manuscript. After all, a lot of effort would have gone into one's research and it is only good to have a journal of quality that matches with that of one's research for manuscript submission. Well, the answer for "Where to publish?" lies in "Why to publish?"

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Collaboration with The University of California - Berkeley

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