Premium Editing Sample

Case Report

Symptoms of Acute Appendicitis ~~Masquerading~~ Overlapping with Distal Intestinal Obstruction Syndrome in Adult Cystic Fibrosis

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Commented [A1]: Thanks for providing this opportunity to assist you with this manuscript. I have edited the text for language, grammar, and improved clarity. I have also checked the manuscript for conformance with the formatting guidelines provided. In the cases where additional information is required from you, I have added comments to bring them to your attention.
Should you have any concerns, please feel free to get back to me.
My best wishes for your success with the manuscript.

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Abstract

~~Overshadowed by Sino-pulmonary infections,~~With the improved life expectancy in c~~C~~ystic f~~F~~ibrosis (CF) patients, there has been an increase in ~~commonly affects~~ gastrointestinal ~~organs~~ manifestations because of secretory and motility dysfunction. Infrequently, these changes can result in d~~D~~istal i~~I~~ntestinal ~~O~~obstruction s~~S~~yndrome (DIOS), an ~~more and more~~increasingly diagnosed gastrointestinal condition~~entity~~ in adult ~~Cystic Fibrosis~~CF patients. We present the~~a~~ case of a 22-year-old man~~le~~ who presented to our hospital with right lower quadrant abdominal pain. ~~with~~ Despite the suspicion of acute appendicitis, the patient ~~and~~ was subsequently diagnosed ~~as~~ with DIOS. Our case highlights the importance of considering DIOS as a differential diagnosis ~~of~~ for right lower quadrant abdominal pain in CF patients, especially ~~for~~ by physicians working at community hospitals that~~which~~ may not have a CF~~ystic Fibrosis~~ care program available.

1. Introduction

Cystic ~~F~~fibrosis (CF) is a genetic disease ~~of~~ that affects multiple organs. ~~With~~ Because of advancements~~ing~~ in the managementing of CF ~~patients~~, patients can now ~~often~~ survive ~~become~~ to adulthood~~s~~ [1]. However, the i~~I~~mproved life expectancy among adult CF patients has ~~given rise~~led to an increase in extrapulmonary, notably gastrointestinal, man~~-~~ifestations, which ~~did not happen~~ was previously uncommon. Distal ~~I~~intestinal o~~O~~bstruction ~~S~~syndrome (DIOS) continues to be a rising complication in adult CF patients, presenting as~~with~~ acute abdominal pain ~~like~~ and mimicking an acute abdominal emergency.

We report the case of a 22-year-old man with a history of CF who presented to our hospital with right lower quadrant abdominal pain. Despite the initial suspicion of acute appendicitis, he was subsequently diagnosed with DIOS.

2. Case ~~Report~~Presentation

A 22-year-old Turkish-origin man~~le~~ with a past medical history of ~~Cystic Fibrosis~~CF presented with a one-day

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I have, instead, added information that has been used in the introduction to serve as background here. Please review this change.

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history of right lower quadrant abdominal pain. He described a sharp periumbilical pain that continued to worsen, which then shifted to the right lower quadrant of the abdomen. Prior to the onset of the abdominal pain, he reported experiencing nausea and anorexia for three days. His last bowel movement was two days prior to admission. The patient was ~~also~~ diagnosed with CF~~Cystic Fibrosis~~ at the age of four, and the~~his~~ disease progressed to exocrine pancreatics insufficiency, which was being treated with pancreatic enzymes. Upon reviewing the patient's past history, it was noted that he had several episodes of pneumo~~-~~nia, for which he was appropriately treated with antibiotics.~~;~~ N~~n~~otably, no history of constipation or recurrent abdominal discomfort was reported prior to this. At home, the patient was prescribed a~~A~~lbuterol inhalation~~er~~ as needed, d~~D~~ornase a~~A~~lfa inhalation~~er~~, a~~A~~ztreonam lysine nebulization, 500 mg a~~A~~zithromycin three times a week, l~~L~~ansoprazole, l~~L~~umacaftor-ivacaftor twice a day, l~~L~~ipase-protease-amylase capsule three times a day, and a multivitamin capsule once a day. ~~The patient was also diagnosed with Cystic Fibrosis at the age of four and his disease progressed to exocrine pancreas insufficiency, which was being treated with pancreatic enzymes.~~ On abdominal examination, he was found to have~~had~~ diminished bowel sounds and ~~tenderness on right lower quadrant with~~ equivocal rebound tenderness on the right lower quadrant. Lab~~-~~oratory analysis showed leukocytosis (white blood cell count,~~WBC~~ 13.0 mm/K3~~;~~, n~~N~~eutrophil~~s~~ count, 62%) with a normal differential. He had no electrolyte imbalances. Computed t~~T~~omography ~~(CT)~~ of the a~~A~~bdomen revealed thickening, ~~and~~ edema around the termi~~-~~nal ileum, inflammatory changes in the ~~a~~ colon ~~with inflammatory changes~~, free fluid in the right paracolic gutter adjacent to the cecum, ~~-~~an appendix measuring 5.3×4.6 mm, and reactive lymph nodes (Figures 1 and 2).

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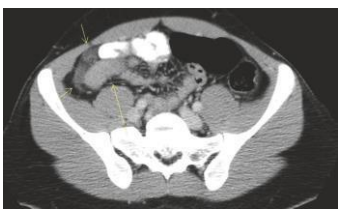

FIGURE 1: Axial abdominal computed tomography CAT scan depicting thickening around the terminal ileum and colon (y Yellow arrows) along with extralumi- nal fluid and reactive lymph nodes.

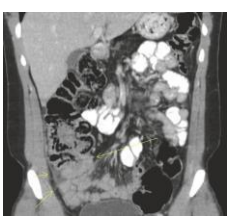

FIGURE 2: Coronal view computed tomography scan with showing thickening of the ileum with a distended appendix (yellow arrows).

measuring 5.8 4.6 mm, and reactive lymph nodes (Figures 1 and 2). Due to extraluminal fluid and cecal wall edema with inflammation, early acute appendicitis could not be excluded as a possible diagnosis. Surgical intervention was performed required, which revealed a ruptured microperforation of a cecal diverticulum and a distended appendix in chronic adhesions, for which he required an appendectomy and partial cecectomy with an intact ileocecal valve (IC valve) valve. Postoperatively, he was diagnosed with DIOS and was subsequently started on pPolyethylene gGly–col. The patient made an unremarkable recovery and was discharged. home to beHe was

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followed up in the outpatient clinic ~~without~~ and did not have ~~any~~ recurrence of any symptoms.

3. **Discussion**

4. Due to the improved life expectancy of CF patients, DIOS is now being increasingly diagnosed in adult patients with CF. ~~Distal Intestinal Obstruction Syndrome (DIOS) was called a Meconium Ileus equivalent in the past, described by the collection of viscid fecal material within the lumen combined with sticky mucoid intestinal content adherent to the intesti- nal wall of the terminal ileum and cecum [1].~~ Perez-Aguilar et al. reported ~~a~~ that the prevalence of DIOS was 19.5% (mean age 20.6 years) among 46 CF patients in a retrospective analysis, while Dray et al. ~~conducted a cross-sectional study~~ reporte~~ding~~ a 15.8% (mean age 28.9 years) prevalence ~~in~~ among 171 CF patients in a cross-sectional study [2, 3]. Despite the~~Though there continues to be a~~ limited assessment ~~of~~ on the prevalence of DIOS in adult CF patients, DIOS is ~~considered~~more common among adults ~~compared to~~than among children ~~due to~~because of ~~increased~~ disease progression.

~~5.~~ ~~Distal Intestinal Obstruction Syndrome (DIOS), previously known as was called a Meconium Ileus-~~ equivalent~~, in the past, described~~is characterized by the collection of viscid fecal material within the lumen combined with sticky mucoid intestinal content adherent to the intesti-nal wall of the terminal ileum and cecum [1].~~ Defective intestinal chloride and water secretions into the gut, luminal acidity, and loss of bile salt all contribute to the development of DIOS [1]. These patients characteristically present with right lower quadrant pain, nausea, abdominal distension, and failure to pass stools or flatus [1, 3]. In some patients, a palpable right lower quadrant mass ~~can~~ may be ~~appreciated~~ present that may be confirmed on abdominal radiography~~X-ray~~ [1]. Though abdominal ~~X-rays are~~radiography is recommended to aid in the diagnosis of DIOS, ~~they are~~it is inadequate in differentiating ileus from other causes of abdominal pathologies that may present in ~~Cystic Fibrosis~~CF patients [4]. Due to ~~the proximity of~~ the anatomical ~~locations~~proximity, as well as the overlapping clinical presentations, appendicitis and intussusception may mimic DIOS. This ~~which~~ further leads to diagnostic uncertainty~~. Overlap of several intra-abdominal pathologies in CF increases the risk of misdiagnosis, especially with acute appendicitis,~~ as these ~~patient's~~ underlying pathologies may be masked in CF patients with pulmonary infections~~'~~ using antibiotics, as seen in our case [5, 6].

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Commented [A21]: I believe this sentence would be more appropriate here as this paragraph describes the characteristics and diagnosis of DIOS.

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Osmotic laxatives are the cornerstone of bowel regimens for the treatment of DIOS. The most commonly prescribed laxative is pPolyethylene Gglycol, (PEG) administerrated at a dose of 20--40 ml/kKg/hH, up twitho a maximum of 1 lL/kg/h for a total of 8 hours, resulting in aachieving fecal effluent consisting of clear fluid, along with the resolution of abdominal pain and constipation [1, 6]. If the diagnosis remains unclear, and thus, requires surgical intervention, ICileocecal valve resection should be considered to prevent the development and recurrence of intestinal obstruction sequalae and growth, especially in adolescents [7].

With the increase in immigration of foreigners intothrough America, inner-city and community hospitals may not be sufficiently equipped with a Cystic FibrosisCF care center; moreover,nor may these hospitals may not have programs in provision, with expertise available to other clinicians involved in patient care. Therefore, our case highlights the significance of considering DIOS as a differential diagnosis in patients with a history of CF presenting with right lower quadrant abdominal pain and constipation, particularly in hospitals without a CF care program available.

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Consent

Informed consent was obtained from the patient for this case report.

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Conflicts of Interest

Authors' Contributions

All authors contributed to the revision and approval of the manuscript.

Acknowledgements

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References

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1. C. Colombo, H. Ellemunter, R. Houwen, A. Munck, C. Taylor, and M. Wilschanski, "Guidelines for the

diagnosis and manage- ment of distal intestinal obstruction syndrome in cystic fibrosis patients," Journal of Cystic Fibrosis, vol. 10, pp. S24–S28, 2011.

2. F. Perez-Aguilar F, J. Ferrer-Calvete J, D. Nicolas D, Berenguer J, and J. Ponce, J. "Digestive alterations in cystic fibrosis. Retrospective study of a series of 46 adult patients,". Gastroenterología y Hepatología Gastroenterologia y hepatologia,. 1999 Feb;vol. 22, no. (2,); pp. 72–78, 1999.

2.

3. X. Dray, T. Bienvenu, N. Desmazes-Dufeu, et al, “Distal intestinal obstruction syn- drome in adults with cystic fibrosis,” Clinical Gastroenterology and HepatologyClin Gastroenterol Hepatol, vol. 2, no. 6, pp. 498–503, 2004.

3.

4. K. Nassenstein, B. Schwerger, M. Kammer, J. Status, T. Lauen- stein, and J. Barkhausen, “Distal intestinal obstruction syn- drome in the early postoperative period after lung transplan- tation in a patient with cystic fibrosis: morphological findings on computed tomography,” Gut, vol. 54, no. 11, pp. 1662–1663, 2005.

4.

5. Y. Al Abed, Y., W. Hameed, J. W., Roy, J., and A.P.S.& Kumar, A. P. S. (2007). “Appendicitis in an adult patient with cystic fibrosis: a diagnostic challenge,”. Gut, vol. 56, no. (12), pp. 1799–1800, 2007.

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7.6. J. M. Abraham and C. J. Taylor, “Cystic Fibrosis & disorders of the large intestine: DIOS, constipation, and colorectal cancer,” Journal of Cystic Fibrosis, supplement 2, pp. S40–S49, 2017.

7. A. Mentessidou, I. Loukou, G. Kampouroglou, A. et al, “Long-term intestinal obstruc- tion sequelae and growth in children with cystic fibrosis oper- ated for meconium ileus: expectancies and surprises,” Journal of Pediatric Surgery, vol. 2018; 53, no. (8, pp.) 1504–1508 2018.

Title: Acute Appendicitis Masquerading Distal Intestinal Obstruction Syndrome in Adult Cystic Fibrosis

Thank you for submitting the above manuscript.
Below please find a list of comments and changes to be made to the manuscript. Please make all necessary revisions and email us the revised manuscript.
We encourage you to send your revision within 45 days.
When submitting your revision please include the following items:

- A rebuttal letter that responds to each point brought up by the academic editor and reviewer(s) as a 'Response to Reviewers' file.
- A clean revised manuscript as your 'Manuscript' file.
- A marked-up copy of the changes made from the previous article file as a 'Revised Manuscript with Track Changes' file. This can be done using 'track changes' in programs such as MS Word and/or highlighting any changes in the new document.

Thank you for your thoughtful suggestions and insights, which have enriched the manuscript and produced a better and more balanced account of the research.
The manuscript has been rechecked and appropriate changes have been made in accordance with the suggestions. The responses to the comments have been prepared and are given below. We hope that the revised manuscript is now suitable for publication in your journal.

Major points:

1. The title of the case is misleading. Since the patient had both appendicitis and DIOS, there was no 'masquerading'.

Response: Thank you for your comment. We have revised the title to "Symptoms of Acute Appendicitis Overlapping with Distal Intestinal Obstruction Syndrome in Adult Cystic Fibrosis"

2. The novelty of the case is not apparent from the Introduction. If DIOS is already associated with acute abdominal pain and abdominal emergency, what is the novelty here?

Response: Thank you for your comment. Our case is novel because we used the patient's cystic fibrosis (CF) history to diagnose distal intestinal obstruction syndrome (DIOS), despite the initial diagnosis of appendicitis. Therefore, we were able to successfully treat the patient accordingly. We would like to highlight that physicians should consider a diagnosis of DIOS for abdominal pain in CF patients.

3. There is no clear conclusion to the case. Please add a concluding sentence to the manuscript.

Response: Thank you for your suggestion. We have added the following sentence at the end of the Discussion of the revised manuscript.
"Therefore, our case highlights the importance of considering DIOS as a differential diagnosis in patients with a history of CF presenting with abdominal pain and constipation."

4. Was consent obtained from the patient for publishing this case?

Response: Thank you for your query. We have included a section on patient consent in the manuscript.

5. The Introduction doesn't flow well into the Case Report. End of the Introduction section should have introduction of case.

Response: Thank you for your suggestion. We have added the following sentence at the end of the

Commented [A30]: Thank you for sending in your responses to the reviewer comments for editing. I have edited the responses for language, checked that they adequately answer the reviewers' questions, and included comments where additional information is needed. I have also checked the manuscript to ensure that the required changes have been made to the manuscript. I have also prepared a response to the general comments for you to use during resubmission.

Commented [A31]: I have changed the title of the manuscript accordingly and included "symptoms of" at the beginning of the title for better clarity.

Commented [A32]: Is this the first case to report on this? If so then please consider stating this, as this would highlight its novelty.

Commented [A33]: I have included this in the revised manuscript.

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Introduction of the revised manuscript: "We report the case of a 22-year-old man with a history of CF who presented to our hospital with right lower quadrant abdominal pain. Despite the initial suspicion of acute appendicitis, he was subsequently diagnosed with DIOS."

Commented [A35]: I have included this in the revised manuscript.

Minor points:

1. The yellow arrows in the figure are not visible. Please use thicker arrows to point to the relevant sections.

Response: Thank you for this suggestion. We have updated the figures.

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Life Sciences Sample

Case Report

Methylmalonic Acidemia with Novel *MUT* Gene Mutations

Inusha Panigrahi, Savita Bhunwal, Harish Varma, and Simranjeet Singh

Department of Pediatrics, Advanced Pediatric Centre, PGIMER, Chandigarh, India

Correspondence: Inusha Panigrahi; inupan@yahoo.com

Abstract

Methylmalonic acidemia (MMA) is a rare inherited metabolic disorder caused by deficiency of the enzyme methylmalonyl-CoA mutase. The disease is characterized ~~presented~~ in early infancy by lethargy, vomiting, failure to thrive, and encephalopathy and is fatal ~~deadly~~ if left untreated. A 5-year~~s~~-old boy presented with poor weight gain and recurrent episodes of fever, feeding problems, and lethargy, since~~from~~ the age of 11 months~~, and poor weight gain~~. He was admitted to our hospital and evaluated for metabolic disorders; he was ~~causes and~~ diagnosed with~~as~~ methylmalonic acidemia (MMA). He was treated with vitamin B12 and carnitine supplements and has been~~on~~ followed ~~-~~up for the last three~~3~~ years. Mutation analysis by next generation sequencing ~~(NGS),~~ and ~~supplemented with~~ Sanger sequencing~~,~~ revealed two novel variants in exon 5 and 3 of the *MUT* gene responsible for the MMA ~~in exon 5 and exon 3~~. Recently, he developed dystonic movements including orofacial dyskinesia. Our results indicate the necessity of genetic testing if MMA is suspected; it ~~would~~can confirm the diagnosis, aid in selecting treatment options, and may enrich the panel of responsible gene variants.

1. Introduction

Methylmalonic acidemia (MMA) is a rare inherited metabolic disorder caused by deficiency of the enzyme methylmalonyl-CoA mutase. MMA presents with lethargy, acidosis, hypoglycemia/ hyperglycemia, ketosis, and recurrent episodes. MMA due to mutations in the *MUT* gene ~~mutations~~, which encodes the enzyme methylmalonyl-CoA mutase, usually leads to severe phenotypes, and around 35–40% of the cases are due to novel~~new~~ mutations [1, 2]. ~~There can be~~ M~~m~~issense or nonsense mutations, deletions, insertions, and so on in the *MUT* gene can ~~and so on~~ lead~~ing~~ to a clinical phenotype, and these~~.~~

v~~V~~ariants ~~of the *MUT* gene encoding the enzyme methylmalonyl-CoA mutase~~ are responsible for about 60% of MMA cases; mut0 and mut- phenotypes are caused by complete and partial enzyme deficiency, respectively. Variations in the *MMAA*, *MMAB*, *MMADHC*, and *MCEE* genes are required for normal functioning of methylmalonyl CoA mutase.

Here, we report the case of a 5-year~~s~~-old boy with MMA who presented with poor weight gain and recurrent episodes of fever, feeding problems, and~~,~~ lethargy~~,~~ since~~from~~ the age of 11 months~~, and poor weight gain~~. This case is novel because we ~~who was~~ found that the patient had ~~to have~~ two previously unreported mutations in the *MUT* gene.

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Thank you for submitting your revised manuscript for editing. I have made changes to this manuscript for grammar, clarity, and readability. Do go through all m changes and comments carefully to ensure that I have retained your intended meaning, and do get back to m if you would like to clarify your intended meaning at any point. I have also checked the manuscript for conformance with the formatting guidelines provided and have checked the response letter. As always, pleas let me know if you have any further questions or concerns.

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1.As per the journal, units of measurement should b presented simply and concisely using System International (SI) units; please ensure that units are presented using the SI system at all instances.
2.References for some previously published data is missing. Please ensure that references are provided wherever required.
3.Informed consent from the patient is required and should be mentioned in the manuscript.
For more information, please refer to the guidelines at https://www.hindawi.com/journals/crig/guidelines/.

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2. Case Presentation

A 5-year-old boy ~~The child~~ presented ~~for the first time at the age of 11 months,~~ with complaints of fever, vomiting, poor feeding, and lethargy since the age of 11 months. We observed that the patient ~~On examination he~~ had pallor and tachypnea and was drowsy. Laboratory tests suggested that the patient had ~~Further evaluation was suggestive of~~ high anion-gap metabolic acidosis with ketonuria (urine ketones 3+) and ~~with~~ normal electrolytes, blood sugar (94 mg/dL~~l~~), vitamin B12, and homocysteine levels. Plasma ammonia and plasma lactate were ~~was~~ 118 units~~,~~ and ~~plasma lactate was~~ 2.9 units, respectively. Transcranial magnetic simulation ~~TMS~~results were~~as~~ normal, but ~~urine~~ gas chromatography mass spectrometry (GC-MS) analysis of urine revealed elevated 3-OH propionic acid [12.39 retention time (RT)] and ~~as well as elevated~~ methyl malonic acid levels [16.92 RT, Suppl Figure 1, in Supplementary Material available online at https://doi.org/10.1155/2017/8984951]. Since then, the patient~~this child~~ was on a low--protein diet, carnitine, biotin, thiamine, and vitamin B12 injections; he ~~. Child~~ was thereafter admitted to the hospital on seven~~multiple~~ occasions ~~(7 times)~~ with acute decompensation and managed as per protocol. Mutational analysis ~~was sent~~ for MMA ~~which~~ showed a single heterozygous missense variant c.976 A>G (p.Arg326Gly) in exon 5 of the *MUT* gene (genomic coordinates: chr 6: 49421405)~~:~~ ~~as~~ a variant of uncertain significance. Chromosomal microarray analysis ~~done~~ did not reveal any major deletion or duplication that~~which~~ could disrupt the gene. Since exon 3 and exon 6 were not adequately covered by next generation sequencing (NGS), further evaluation by Sanger sequencing for targeted exons was performed~~done~~, and a second~~2nd~~ mutation in exon 3 c.753 G>A (p.=) was identified. The variants were predicted ~~found to be~~ as damaging by the ~~on~~ SIFT database ~~score~~ (Suppl data) and as ~~. They were also predicted to be~~ deleterious by ~~on~~ Polyphen-2 and Mutation Taster, but they were absent ~~and not found~~ in the ExAC database. Brain ~~MRI~~magnetic resonance image ~~brain~~ of the patient (done at the age of four~~4~~ years) ~~was~~ showed~~ing~~ multifocal cystic encephalomalacic changes with surrounding gliosis in deep white matter predominantly in frontoparietal regions (Figure 1). During~~In~~ the latest admission of the patient to the hospital, we observed~~child was found to have~~ fresh neurological findings in the form of perioral tremors, generalized~~s~~ hypertonia, and generalized~~s~~ dystonia with clonus with exaggerated deep tendon reflexes. The patient~~He~~ was treated with intravenous dextrose and sodium bicarbonate and was continued on carnitine and ~~injection of~~ vitamin B12 injections. Plasma ammonia and plasma lactate levels were~~as~~ 18 units and ~~lactate level was~~ 4.9 units, respectively. Brain magnetic resonance image ~~MRI brain~~ of the patient ~~was repeated and~~ revealed bilateral basal ganglia hyperintensities, suggestive of metabolic stroke. After the subsidence of acute crisis, he was discharged on carnitine, ~~injection of~~ vitamin B12 injections, and trihexyphenidyl. The p~~P~~arents were counseled regarding the patient's prognosis and possible~~for~~ prenatal diagnosis for subsequent~~next~~ pregnancies~~y~~.

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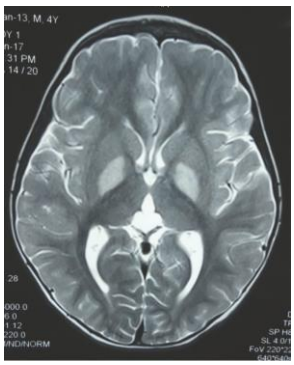

FIGURE 1: The MRI Brain magnetic resonance image of the 5-year-old boy with *MUT*-related methylmalonic acidemia showing predominant frontoparietal abnormalities in the form of encephalomalacia and gliosis.

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3. Discussion

The advent of NGS technology has enabled better characterization of mutations in several populations. In this study, we used NGS and Sanger sequencing to identify mutations in the genes linked to MMA development and revealed two novel variants of the *MUT* gene. In a Saudi study on 60 MMA patients of MMA, nonsense, missense, and frameshift mutations were detected across the *MUT* gene [3]. Another study in 43 Chinese patients identified 8 recurrent mutations and 10 novel mutations in the *MUT* gene [4]. A previous Indian study in 15 patients with of clinically diagnosed MMA identified one novel exon 12 mutation in the *MUT* gene with predicted pathogenicity. Here, we identified two novel variants, one in exon 3 and another in exon 5 of the *MUT* gene, and both were labelled as variants of unknown significance (VUS). The exon 3 variant is a synonymous variant, and a different nucleotide change c.753 G>C (p.Lys251Asn) has been reported earlier in ClinVar. Some synonymous variants can also affect the splicing or protein function and lead to clinical phenotypes. The identified exon 5 variant is novel, but another close variant c.977 G>A (p.Arg326Lys) has been reported in ClinVar. The variants were found to be deleterious on bioinformatic analysis and were absent not found in the ExAC database. Both variants identified in the present case could be responsible for possibly explain the MMA phenotype of MMA in the child. *MUT*-related MMA has poor prognosis in most cases. Specialized diet and supplements may not improve the outcomes, even if MMA is diagnosed early. Early recognition and appropriate treatment of acute crises are necessary. Metabolic stroke can sometimes occur in the absence of acute metabolic decompensation; thus, so meticulous neurological examination at each visit is important useful. The treatment options for MMA include therapy include early liver transplantation [5]; and possibly gene therapy could also be used in the future. Genetic counseling and prenatal diagnosis could help these families of the patients in making informed reproductive decisions.

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Conflicts of Interest

The authors declare that there is ~~they have~~ no conflict~~s~~ of interest regarding the publication of this article.

Acknowledgments

The authors would like to acknowledge Dhiti Omics Tech–nologies Pvt Ltd for help in mutation analysis.

References

[1] K. Splinter, A.-K. Niemi, R. Cox et al., "Impaired health-related quality of life in children and families affected by methyl- malonic acidemia," *Journal of Genetic Counseling*, vol. 25, no. 5, pp. 936–944, 2016.

[2] A. R. R. Devi and S. M. Naushad, "Targeted exome sequencing for the identification of complementation groups in methyl- malonic aciduria: a south Indian experience," *Clinical Biochem–istry*, vol. 50, no. 1-2, pp. 68–72, 2017.

[3] F. Imtiaz, B. M. Al-Mubarak, A. Al-Mostafa et al., "Spectrum of mutations in 60 saudi patients with mut methylmalonic acidemia," *JIMD Reports*, vol. 29, pp. 39–46, 2016.

[4] L. S. Han ~~LS~~, Z. Huang ~~Z~~, F. Han ~~F~~, et al., ~~Ye J, Qiu WJ, Zhang HW, Wang Y, Gong ZW, Gu XF.~~ "Clinical features and MUT gene mutation spectrum in Chinese patients with isolated methylmalonic acidemia: identification of ten novel allelic variants," ~~,~~ *World Journal of Pediatrics*, ~~,~~ vol. ~~2015 Nov~~ 1 ~~,~~ no. 11(4), pp. ~~:~~358–~~-~~365, 2015.

[5] M. Spada, P. L. Calvo, A. Brunati et al., "Liver transplantation in severe methylmalonic acidemia: the sooner, the better," *Journal of Pediatrics*, vol. ~~2015;~~167, pp. ~~:~~1173, 2015.

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The report presents an interesting case of methylmalonic acidemia (MMA) potentially caused by two newly identified mutations in the *MUT* gene. The study contributes to the array of mutations relevant to MMA and deserves publication. However, the manuscript has several flaws, which should be addressed before re-submission.

Major issues

Abstract: the background and clinical implications are missing.

In the background, the subject of the study should be introduced in 1-2 short sentences explaining what MMA is, including disease causes and clinical symptoms. The Abstract should end with the take-home message indicating the importance of genetic testing in cases suggestive of MMA; it should be patient-oriented rather than method-oriented. The statement that "With advent of NGS, judicious use of NGS with Sanger sequencing can help identify causative possibly pathogenic mutations" is trivial because it is obvious that without sequencing (either NGS or conventional Sanger), mutations cannot be identified; rather, it should be concluded that if MMA is suspected, genetic testing is necessary to identify the type of the disease, which would aid in applying the most appropriate treatment.

Response: As per the reviewer's suggestion, we added the following introductory sentences: "Methylmalonic acidemia (MMA) is a rare inherited metabolic disorder caused by deficiency of the enzyme methylmalonyl-CoA mutase. The disease is characterized in early infancy by lethargy, vomiting, failure to thrive, and encephalopathy and is fatal if left untreated."

The conclusion of the abstract was modified as follows: "Our results indicate the necessity of genetic testing if MMA is suspected; it can confirm the diagnosis, aid in selecting treatment options, and may enrich the panel of responsible gene variants."

Introduction is totally missing.

Every case report must be preceded by a concise Introduction section (1-2 paragraphs) presenting the disease and stating the importance of the case. The Introduction should describe MMA as an inherited autosomal recessive disorder caused by deficiency in the conversion of methylmalonyl coenzyme A (CoA) to succinyl CoA, resulting in the accumulation of methylmalonic acid in the blood to toxic levels. The manifestations of the disease should be described, including symptoms, age at the onset, severity range, treatment algorithms, and prognosis. The frequency of this disorder in the general population should also be indicated.

In the next paragraph, the genetic spectrum of the disease should be characterized, including the affected genes and related metabolic changes. Thus, it should be mentioned that variants of the *MUT* gene encoding the enzyme methylmalonyl-CoA mutase are responsible for about 60% of MMA cases; mut0 and mut- phenotypes caused by complete and partial enzyme deficiency, respectively, should be mentioned considering that the described case probably presented the mut- phenotype responsive to B12 supplementation. Variations in the *MMAA*, *MMAB*, *MMADHC*, and *MCEE* genes required for normal functioning of methylmalonyl CoA mutase should also be mentioned as they account for the rest of MMA cases.

Finally, the novelty of the present MMA case should be introduced by stating that the patient had two previously unreported mutations in the *MUT* gene.

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Thank you for sending in your responses to the reviewer comments for editing. I have edited the responses for language, checked that they adequately answer the reviewers' questions, and included comments where additional information is needed. I have also checked the manuscript to ensure that the required changes have been made to the manuscript and ensured consistency between the quoted text in th response letter and the corresponding text in the manuscript; **however, at most instances, I found that the revisions mentioned in the responses were not made in the manuscript. I have included comments t bring these instances to your attention.** Please ensure that the revisions suggested by the reviewer and mentioned in the responses are incorporated in the manuscript before submission.
My best wishes for the submission of the revised manuscript!

Response: We thank the reviewer for the recommendation. Accordingly, in the revised manuscript, we have added the Introduction section wherein we have explained the genetic nature of MMA, mentioned the genes involved, and described the relevant biochemical mechanisms. Clinical manifestations, age at onset, incidence of the disease, and treatment regimens have also been described in this section. The presentation of the case has been justified by stating that our patient demonstrated MMA symptoms, which could have been caused by novel variants of the *MUT* gene.

Case presentation is incomplete.

The numerical value for high anion gap (mmol/L) should be shown.

Response: Thank you for pointing this out. In the revised manuscript, we have presented the results indicative of high anion gap.

Units for plasma ammonia and lactate should be provided. Currently, it is difficult to assess the status of these parameters as they can be expressed in conventional units or SI units; accordingly, the numerical values can differ. I recommend the authors to convert all test results to SI units for consistency and provide reference ranges for all biochemical parameters.

Response: As per your suggestion, we have converted the levels of plasma ammonia and lactate into SI units and have indicated the reference ranges.

Actual concentrations of propionic acid and methylmalonic acid should be provided.

Response: In the revised manuscript, concentrations of propionic and methylmalonic are provided.

It should be explained how the diagnosis of MMA was made and whether alternative diagnoses such as propionic acidemia were considered, as both conditions are manifested by ketones in the urine and high blood ammonia levels; furthermore, a high level of propionic acid was detected.

Response: The symptoms observed in the child (tachypnea, recurrent vomiting, poor feeding, lethargy) were characteristic of MMA and were confirmed by biochemical testing. Propionic acidemia was ruled out as we did not find mutations in the *PCC* genes, which encode propionyl-CoA carboxylase.

There is no explanation of specific treatment decisions. In particular, B12 injections should be justified, considering that there are two types of MMA differing in the sensitivity to cobalamin supplementation, responsive and nonresponsive, and that B12 levels in the patients were normal, i.e., there was no B12 deficiency.

Response: Patients with all forms of MMA (mut0, mut-, and cbl types) are routinely treated with a low-protein diet and respond well to carnitine supplements, as they typically develop carnitine deficiency. Our patient also had severe lactic acidosis, most likely due to thiamine deficiency, and elevated levels of propionic acid, which is indicative of dysfunctional propionyl-CoA carboxylase requiring biotin; therefore, he was administered thiamine and biotin. Although not all forms of MMA are responsive to cobalamin, we prescribed B12 supplements to the patient, as it is considered the first-line treatment for MMA.

NGS results: MMA is a recessive condition, i.e., only homozygous variants result in the phenotype, whereas heterozygous variants are asymptomatic. Therefore, the reported heterozygous missense variant c.976 A>G (p.Arg326Gly) in exon 5 of the *MUT* gene cannot be responsible for MMA in this patient. This fact should be discussed.

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Commented [A 20]: I would suggest that you add this explanation in the manuscript to clearly communicate the difference between the two types of MMA to the reader.

Response: According to the recommendation of the reviewer, we have added the following sentences: "Since MMA is a recessive autosomal disorder, the newly found heterozygous missense variant in exon 5 of the *MUT* gene cannot be responsible for the symptoms of the patient. However, it is known that over 180 missense mutations in the *MUT* gene have been linked to the severe (mut0) MMA form; therefore, the newly identified missense mutation should be further investigated for its functional significance."

Line 45: The test results of plasma ammonia and lactate should be converted to the SI units and interpreted. Plasma ammonia levels significantly decreased but lactate levels increased in parallel with deterioration of neurological conditions of the patient.

Response: According to the recommendations of the reviewer, we have mentioned in the manuscript that plasma ammonia level significantly decreased, whereas plasma lactate level increased as compared to that in the original test results, and the overall condition of the patient worsened.

Lines 50-51: What was the prognosis for this patient? It should be indicated. Considering that the parents must be carriers of the MMA-causing mutation(s), were they asked to undergo genetic testing?

Response: Monitoring of the patient for several years – from 11 months to 5 years – revealed the progression of the disease. This was evident from the appearance of additional neurological symptoms at the age of 4 years; therefore, the prognosis was not favorable. We recommended the parents to undergo genetic testing and counseling before considering the next pregnancy. This information has been added to the revised manuscript.

Lines 52-55: This paragraph describing MMA should be moved to the Introduction.

Response: We thank the reviewer for the recommendation. The paragraph has been modified and moved to the Introduction.

Lines 61-64: The description of comparative advantages of NGS and Sanger sequencing is trivial and not directly relevant to the subject of the case report, which is identification of new mutations relevant to MMA. This paragraph should be reduced to the statement that the mutations were identified by NGS as well as by Sanger sequencing.

Response: According to the recommendation of the reviewer, this part was shortened as follows: "In this study, we used NGS and Sanger sequencing to identify mutations in the genes linked to MMA development and revealed two novel variants of the *MUT* gene."

Line 77: The variant in exon 5 is heterozygous and cannot be responsible for MMA symptoms as MMA is a recessive inheritance pattern and the mutation should be homozygous to cause the phenotype. The statement regarding the exon 5 variant should be corrected.

Response: We modified the sentence as follows: "Since MMA is a recessive autosomal disorder, the newly found heterozygous missense variant in exon 5 of the *MUT* gene cannot be responsible for the observed clinical manifestations. However, it is known that over 180 missense mutations in the *MUT* gene have been linked to the severe (mut0) MMA form (Keyfi et al., 2016); therefore, this variant should be further investigated in *in vitro* and *in vivo* models to determine its functional effects."

Implications of the study based on the specific findings of this case should be mentioned. How does the case contribute to the diagnosis and treatment options of MMA?

Response: Analysis of this case revealed that although the identified mutations do not seem

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Also, the revised manuscript does not mention that **"plasma ammonia level significantly decreased, whereas plasma lactate level increased as compared t that in the original test results, and the overall condition of the patient worsened."**
Please ensure that this information is added to the manuscript.

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to cause severe deficiency in the production of any functional enzyme linked to MMA pathogenesis, the symptoms presented by the patient are serious and the prognosis is not favorable, suggesting that the genotype and clinical phenotype associated with MMA are not always concordant. Nevertheless, in cases when MMA is suspected, genetic testing is important to confirm the diagnosis and the most appropriate treatment should be prescribed. These considerations have been added at the end of the Discussion.

5. The language is poor. There are multiple grammar and stylistic mistakes, which must be corrected. The spelling should be consistent either with the American or British English but not be the mixture of the two.

Response: The manuscript has been revised by a professional English-speaking editor and all language-related errors have been corrected.

Minor issues:

- All abbreviations must be defined at the first mention both in the Abstract and the main text.

Response: All abbreviations have been defined, as recommended.

- Gene symbols (*MUT*) should be italicized.

Response: In the revised manuscript, all gene symbols are italicized.

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Physical Science Sample

Structural ~~Prediction~~ prediction of ~~Bis~~bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) complex ~~Using~~ using ^{31}P NMR ~~Spectroscopy~~spectroscopy

Author Details

Abstract[1]

In this study, ~~The present paper reports the use of ^{31}P NMR spectroscopy to predict the isomer structures of~~ [bis-4-methoxy-phenyl-[3-(4-methoxy-phenyl)-allylidene]-amino]-bis[triphenylphosphine]ruthenium(II) complex, also known as bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II)~~.~~ complex, was synthesized using~~es. The complexation reaction was carried out (di-*p*-anisole)-1,4-azabutadiene (compound **1**), triphenylphosphine, and ruthenium chloride in 2:2:1 ratio~~ under reflux~~ing~~ conditions ~~of (di-p-anisole)-1,4-azabutadiene (compound 1), triphenylphosphine (PPh_3), and ruthenium chloride in the ratio of 2 : 2 : 1~~ for ~~five~~ 5 h~~hours~~. The formation of the ~~In addition,~~ ruthenium(II) complex ~~were~~ was confirmed by~~also characterized using~~ FTIR and UV–Vis spectroscopic~~ analyses to support the formation of ruthenium(II) complexes~~. ^{31}P NMR spectroscopy ~~pic study on ruthenium(II) complexes suggested~~ indicated the presence of ~~that there are~~ three isomers ~~present~~ after the complexation reaction. All the isomers of the ruthenium complex had ~~demonstrate~~ octahedral geometry.

Keywords: ^{31}P NMR spectroscopy; FTIR spectroscopy; UV–Vis spectroscopy; Ru complex; Isomers; Structure prediction

[1] NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared; UV–Vis, ultraviolet–visible

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In addition, I have crosschecked your responses to the reviewer comments with the changes in the manuscript. Please refer to my detailed comments in the response file. In the cases where additional information is required from you, I have added comments to bring them to your attention.
Should you have any questions, please feel free to get back to me.
My best wishes for your success with the manuscript.

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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an essential instrument analytical tool in the field of chemistry as it can helps determine elucidate the structure of a molecule, identify detect the presence of impurities in a sample, and determine the rates of formation and as well as degradation of a compound. Even in 1970s , NMR has was used as early as in the 1970s already been used to determine detect the cancer formation whichand was identified to be offered a simple, fast, and low–cost method to for this purpose identify cancer formation [1–3].

For Inorganic inorganic chemists commonly use, using of ^{31}P NMR spectroscopy to identify the structures of a complexes containing phosphine ligands is very common [4, 5]. ThFor example, the e well-known examples is the use of ^{31}P NMR spectroscopy to determine the mechanism of Wilkinson hydrogenation was determined by ^{31}P NMR spectroscopy, mechanism based on by identifying the coupling patterns among the phosphine ligands as well as those and also the coupling constants between the phosphine ligands as well asand the rhodium(I) metal centrecenter [6].

In As part of our long–term research interest on the synthesis of in ruthenium(II) complexes synthesis, we used the (di-*p*-anisole)-1,4-azabutadiene (**1**) and triphenylphosphine (PPh_3) as the ligands to for reaction react with ruthenium trichloride under reflux conditions. The structures of theProducts resulting complexes were formed, were checked identified by using ^{31}P NMR spectroscopy, FTIR spectroscopy, and UV–Vis spectroscopyand the results found in the spectra are worth to be discussed in the present communication.
For inorganic chemist, using of ^{31}P NMR to identify the structure of a complex containing phosphine ligands is very common [4, 5]. The well-known examples is the use of ^{31}P NMR spectroscopy to determine the Wilkinson hydrogenation mechanism by identifying the coupling patterns among phosphine ligands and also the coupling constants between phosphine ligands as well as rhodium(I) metal centre [6].

2. Methodology

The ruthenium complexes were characterized using UV/Vis, FTIR, and ^{31}P NMR spectroscopy. The IR spectra were recorded using on a Thermo Scientific Nicolet iS10 spectrophotometer in using KBr discs. The ^{1}H NMR spectrum for of compound **1** and ^{31}P NMR spectrum for of the ruthenium(II) complexes were recorded usingon a JEOL JNM-ECA 500 spectrometer with TMS as an the internal standard. The absorption spectra waswere recorded with on a Jasco V-630 UV–Vis spectrophotometer.

2.1. Preparation of (4-Methoxy-phenyl)-[3-(4-methoxy-phenyl)-allylidene]-amine or (di-*p*-Anisole)-1,4-azabutadiene (**1**)

4-Methoxycinnamaldehyde (1.62 g, 10.00 mmol) was dissolved in 10 mL of ethanol, and followed by the addition of 4-methoxyaniline (1.23 g, 10.00 mmol) which was then added to solution. The rReaction mixture was stirred for 4 hours h and to obtain a resulted in green-yellow solid, which. The solid was filtered, washed with 5 mL of ethanol, and dried *in vacuo*. The solid was purified by dissolving it in DCM and then layered with hexane via slow diffusion to yield compound **1**.: Yyield: 2.368 g (88.7%); IR (KBr, cm^{-1}) ν: 3036 (C-H stretching), 1627 (C=N- stretching), 1601 (C=C stretching, aliphatic), 1575 and 1468 (C=C stretching, aromatic), and 1110 (OCH_3 stretching)-; ^{1}H NMR (500 MHz, $CDCl_3$,) δ: 8.25 (d, 1H, Hz, -CH=N-), 7.47 (d, 2H, Hz,), 7.18 (d, 2H, Hz,), 7.05 (t, 1H, Hz, H-C_α), 6.99 (m, 1H, H-C_β), 6.90 (d, 4H, Hz,), 3.83 (s, 3H, OCH_3), and 3.81 (s, 3H, OCH_3); UV–Vis (DCM, /nm): 273, 373; Anal. Calc. for $C_{17}H_{17}O_2N$ (%): C, 76.38; H, 6.41; N, 5.24; Ffound (%): C, 76.75; H, 6.31; N, 5.05.

2.2. Preparation of [Bis4-methoxy-phenyl-[3-(4-methoxy-phenyl)-allylidene]-amino}]-bis-[triphenylphosphate]ruthenium(II) or Bis(di-*p*-anisole)-1,4-azabutadiene}-bis[triphenylphosphine]ruthenium(II) Complexes

For the synthesis of bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) complex, $RuCl_3 \cdot xH_2O$ (2.070 g, 1.0 mmol) and PPh_3 (0.525 g, 2.0 mmol) were added to a round–bottom flask containing 10 mL ethanol. and tThe mixture was then refluxed for 5 h. Compound **1** (0.316 g, 2.0 mmol) was then added to the round bottom flask, and the mixture was refluxed for another 5 h. The resulting pPale-maroon solids were wasformed , filtered and washed with hexane. and the p. Precipitate was dried *in vacuo*: IR (KBr, cm^{-1}) ν: 3034 (C-H stretching), 1661 (C=N), 1576 (-merged IR band of for aliphatic and aromatic C=C stretching from aliphatic and aromatic), 1469 (C=C stretching of aromatic ring), and 654 (Ru-C), and

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Commented [A16]: In the present study, a Ru(II) complex is synthesized, the complexation is confirmed by UV-vis and FTIR spectroscopies, and the isomers are identified by NMR spectroscopy. In general, this is a study on the synthesis and characterization of the complex by different techniques, and NMR is alone is not discussed exclusively (and neither in detail). Hence, suggest that the introduction be made more general. The focus should be on the importance of these complexes and the criticality of their structural elucidation rather than on NMR. Along with NMR, a brief description of UV–Vis and FTIR can also be included.

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577 (Ru-N); ; ^{31}P NMR (202.5 MHz, $CDCl_3$) δ: 49.7 (d, 1P, Hz), 47.4 (d, 1P, Hz), 41.7 (d, 1P, Hz), 39.7 (d, 1P, Hz), 35.1 (s, Ph_3P=O), and 29.9 (s, 1P); UV--Vis (DCM) (): 321 and 382.

On the other hand, theThe binding of compound **1** to the ruthenium(II) metal centrecenter wasis can be confirmed using FTIR and UV--Vis spectroscopyspectroscopies. The Comparing the IR spectra spectrum between of compound 1 and the ruthenium complexes (FigureFig. 41) reveals that the , the vibrations of C=N and C=C stretching bands are shifted with respect to those in **1**, thereby bands have been shifted confirming the after binding of **1** to the ruthenium(II) metal centrecenter. The For C=N stretching band is blue-, it shifteddd from 1627 cm^{-1} in the spectrum of compound **1** to 1661 cm^{-1} in the spectrum of the ruthenium complex [97, 108]. In contrast, whereas the for C=C stretching, the IR band appears at 1601 cm^{-1} in the spectrum of **compound 1** but it is notcannot be clearly shown detected in the spectrum of the complex, because the IR bands of aliphatic and aromatic C=C bands for aliphatic and aromatic were mergeing into one a single broard IR band centredcentered at 1576 cm^{-1}. Nevertheless, the two additional IR peaks are present at 577 and 654 cm^{-1} in the finger-print region of the spectrum at 577 and 654 cm^{-1} indicatingconfirm the formation of the respective Ru-N and Ru-C bonds [119].

FigureFig. 41: IR spectra of (a) compound **1** (a) and (b) ruthenium(II-) complexes (b).

The complexation of compound **1** to the ruthenium(II-) metal centrecenter is can be further supported by the UV--vVis data spectra as shown in FigureFig. 52. For In the case of compound **1**, two absorption bands were are observed at 273 and 372 nm, which are assigned to the transitions of in the benzene ring and transition of thei imine group [1210], respectively. After the complexation, both the absorption bands undergo significant bathochromic shifts to shifts to 321 and 382 nm, respectively, thereby ,confirming the Significant shifts of these two absorption bands have proven compound 1 was successfully bound binding of **1** to the ruthenium(II) metal centrecenter via the nitrogen atom from in the C=N group and the carbon atom from in the aliphatic C=C aliphatic group in of the C=C-C=N moiety. The bathochromic shifts of these two absorption bands was are due attributed to the backbonding of electrons from Ru to the antibonding orbitals of the C=C-C=N moiety in compound **1**. This backbonding, in turn, weakens has weakened the bonds in C=C-C=N [113].

FigureFig. 52: UV--Vis spectra of (a) compound **1** (a) and (b) ruthenium (II) complex (b).

3. Results and Discussion
Characterization of the ruthenium complexes was done using UV/Vis, FTIR, and ^{31}P NMR spectroscopy. The IR spectra was found by Thermo Scientific Nicolet iS10 in KBr disc. ^{1}H NMR spectrum for compound 1 and ^{31}P NMR spectrum for ruthenium(II) complexes obtained through JEOL JNM-ECA 500 spectrometer with TMS as an internal standard. The absorption spectra recorded with Jasco V-630 spectrophotometer.

Once the complexation was confirmed, as discussed above, The the ^{31}P NMR spectrum of the ruthenium complex (Fig. 3) was analyzed for its detailed structural elucidation. The ^{31}P NMR spectrum of the product shows appearance of two pairs of doublets and one singlet, indicating in the ^{31}P NMR spectrum for ruthenium complexes (Figure 1) indicatethe formation of that there are three isomers (1:1:1 ratio) present inas a result of the the complexation reaction with the ratio of 1 : 1 : 1.

FigureFig. 13: ^{31}P NMR spectrum for of ruthenium(II) complexes.

The singlet at 29.88 ppm reveals that the two PPh_3 units are magnetically equivalent in the ruthenium(II) complex. There can be three possible structures based on this singlet. In the first case, the In this case, the two PPh_3 units are either located at the axial positions of an octahedron and are, which is trans to each other (FigureFig. 24(a)) [127], while in the other two cases, they are located on the or located at equatorial plane, which is only trans only to either one of the C atoms from in the C=C bond (Fig. 4(b)) or the N atom from in the N=C bond (FigureFig. 24(bc)). The presence of the two PPh_3 units at the axial positions corresponds to the Apparently the one shown in Figure 2(a) is a trans -isomer (Fig. 4(a)), whereas the presence of these units at the equatorial positions corresponds to the the two isomers in Figures 2(b) and 2(c) are cis -isomers (Fig. 2(b) and 2(c)). Unfortunately, at this stage, we cannot are unable to identify which one is the correct structure represented based on by the singlet at 29.88 ppm at this stage.

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~~Figure~~Fig. ~~2~~4: Postulated structure~~s~~ of (a) *trans*- and ((b) and (c)) *cis*-[bis(di-*p*-anisole)-1,4-azabutadiene~~}~~]-bis[triphenylphosphine]ruthenium(II).

~~Meanwhile, a~~ The pair of doublets at 41.84 and 39.74 ppm with a ~~coupling constant of 21 Hz is assigned to a the cis -isomer of the ruthenium(II) complex as shown in Figure~~Fig. ~~3~~5(a). ~~Lastly~~ The other ~~, another~~ pair of doublets at 49.80 and 47.36 ppm with a coupling constant of 38 Hz is assigned to ~~a~~ the trans -ruthenium(II) complex shown in ~~(Figure~~Fig. ~~3~~5(b)~~)~~. It is evident that the ~~The~~ difference in- the coupling constants ~~between of the~~ ruthenium(II) complexes arises~~in Figures 3(a) and 3(b) is due~~ owing to the positions of the PPh_3 ligands. The doublet with a smaller coupling constant ~~(t, namely,~~ 21 Hz~~)~~ is ~~, was~~ assigned to the cis -isomer because both the PPh_3 ligands are in the equatorial plane. The ~~The presence of~~ doublets originate because the ~~for the~~ PPh_3 ligands in the complex ~~is shown in Figure 3(a) because both PPh_3 ligands~~ are trans to different atoms~~, that is,~~ (nitrogen and carbon~~)~~ ~~atoms~~. ~~For~~ In the ruthenium(II) complex shown in ~~as shown in Figure~~Fig. ~~3~~5(b), the two PPh_3 ligands are located at the axial positions and are trans to each other. ~~Unlike~~ As opposed to the case of the trans complex in ~~Figure~~Fig. ~~2~~4(a), the magnetic fields of the~~se~~ two PPh_3 units in the complex shown in ~~Figure~~Fig. ~~3~~5(b) ~~is~~ are different because the two ~~ligands of~~ (di-*p*-anisole)-1,4-azabutadiene ligands are trans to each other ~~at~~ on the equatorial plane ~~(Figure 3(b))~~. The ~~Lastly, the~~ single peak ~~observed~~ at 35.14 ppm is attributed to the ~~presence of the~~ triphenylphosphine oxide [~~1~~3~~8~~]. All the isomers of the ruthenium complex had octahedral geometry.

~~Figure~~Fig. ~~3~~5: Postulated structure~~s~~ of (a) *cis*- and (b) *trans*-[bis(di-*p*-anisole)-1,4-azabutadiene~~}~~]-bis[triphenylphosphine]ruthenium(II)~~}~~.

~~On the other hand, the binding of compound **1** to ruthenium(II) metal centre can be confirmed using FTIR and UV-Vis spectroscopy. Comparing the IR spectra between compound **1** and ruthenium complexes (Figure 4), the vibrations of C=N and C=C stretching bands have been shifted after binding to ruthenium(II) metal centre. For C=N stretching band, it shifted from 1627 cm^{-1} in compound **1** to 1661 cm^{-1} in ruthenium complex [9, 10], whereas for C=C stretching, the IR band appears at 1601 cm^{-1} in **compound 1** but it is not clearly shown in the complex because the IR bands of C=C bands for aliphatic and aromatic were merging into one board IR band centred at 1576 cm^{-1}. Nevertheless two additional IR peaks are present in the finger print region at 577 and 654 cm^{-1} indicating the formation of respective Ru-N and Ru-C bonds [11].~~
~~Figure 4: IR spectra of compound **1** (a) and ruthenium(II) complexes (b).~~
~~The complexation of compound **1** to ruthenium(II) metal centre can be further supported by the UV-vis data as shown in Figure 5. For compound **1**, two absorption bands were observed at 273 and 372 nm which are assigned to transition of the benzene ring and transition of the imine group [12], respectively. After the complexation, both absorption bands shifts to 321 and 382 nm, respectively. Significant shifts of these two absorption bands have proven compound 1 was successfully bound to ruthenium(II) metal centre via the nitrogen atom from C=N group and carbon atom from C=C aliphatic group in C=C-C=N moiety. The bathochromic shift of these two absorption bands was due to the backbonding of electrons from Ru to the antibonding orbitals of C=C-C=N moiety in compound **1**. This, in turn, has weakened the bond in C=C-C=N [13].~~
~~Figure 5: UV-Vis spectra of compound **1** (a) and ruthenium (II) complex (b).~~
~~In addition, the data from IR and UV-Vis revealed t~~The successful binding of ~~at~~ compound **1** ~~has bound~~ to the ruthenium(II) metal ~~centre~~center was confirmed from the IR and UV–Vis spectral data.
~~4. Conclusion~~
~~The~~ The ^{31}P NMR spectra revealed the ~~evidence from ^{31}P NMR spectrum has shown the~~ presence of three isomers of the bis(di-*p*-anisole)-1,4-azabutadiene~~}~~-bis[triphenylphosphine]ruthenium(II) complex in ~~the~~ 1:1:1 ratio ~~of 1 : 1 : 1~~. Two of the three isomers are those shown in Fig. 5, i.e., one cis and one trans isomer, while the third isomer could be any one of those shown in Fig. 4. ~~In addition, the data from IR and UV-Vis revealed that compound **1** has bound to ruthenium(II) metal centre.~~

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15.

HIGHLIGHTS

- Three isomers were detected for a phosphine-bearing Ru complex using ^{31}P NMR.
- Formation of Ru-N and Ru-C bonds were confirmed by FTIR spectroscopy.
- At least one cis isomer and one trans isomer of the complex were formed.

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Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

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RESPONSES TO REVIEWER COMMENTS

This is a relatively simpler and brief report on structure identification of an organometallic complex using ^{31}P NMR. There are certain missing gaps in this study. Though the topic of the study fits into the scope of the journal, I do not recommend its publication in the current form, however, the manuscript can be reconsidered for the same after major revisions.

Response: We thank you for your thoughtful suggestions and insights; the manuscript has benefited from these insightful suggestions. We have rechecked the manuscript and made the necessary changes in accordance with your suggestions (the revised portions have been marked in red font in the revised manuscript). Our responses to your comments are given below.

1. Some information is missing in experimental section describing the syntheses of compound 1 and the Ru complex. Details on the stirring of the reaction mixture are not complete. Particularly, the duration and temperature of stirring is critical is many syntheses, and this should be precisely mentioned for the easy reproducibility of experimental data. And, was the yield or nature of the product affected by the stirring speed/temperature/reaction time?

   Response:
   Thank you for noting this critical point. The reaction mixture for the synthesis of compound 1 was stirred for 4 h, while that for the synthesis of the Ru complex was stirred for 5 h. In both the cases, the temperature was maintained at 60 °C. Two different stirring speeds (250 and 150 rpm) were tested for both the reaction mixtures. However, no significant difference in the yield or nature of the products was observed.

2. Corresponding to the singlet at 29.88 ppm, the authors mention the existence of two probable structures, i.e., a structure in which phosphines are located at the two axial positions or a structure in which the phosphines are located on the equatorial plane and are trans to the carbon atom from C=C. I think there could be another probable structure corresponding to this peak: a structure in which phosphines are located on the equatorial plane but are trans to N=C bonds. Please discuss this aspect.

   Response:
   Thank you for your insightful comments. We analyzed the spectrum again and found that the peak at 29.88 ppm could also correspond to a structure in which the phosphines are on the equatorial plane and trans to N=C. This corresponds to a cis configuration of the complex. We have now included this in the manuscript and also in Fig. 2. However, we still cannot assign any specific structure based on this singlet.

3. In the structures shown in Figure2a and Figure 3b, the two phosphine ligands are trans to each other. Despite of this , only one peak is observed for the former, while a doublet is found for the latter. Please clarify this.

   Response:
   We thank you for this valuable comment. The magnetic fields of the two phosphine ligands shown in Fig. 3b are different because the two (di-*p*-anisole)-1,4-azabutadiene ligands are trans to each other on the equatorial plane. That is, the phosphine ligands shown in Fig. 2a are magnetically equivalent, while the ones shown in Fig. 3b are not. Thus, a singlet is observed for the former, while a doublet is observed for the latter. This aspect has now been discussed in the revised manuscript.

4. What solvent did the authors used for UV-vis spectroscopy? Although, bathochromic shift confirmed the bond formation to the metal center, authors have to mention probable reason for such a bathochromic shifting , particularly in the terms of electronic distribution. In addition, the shift of the first peak (273 nm to 321 nm) was significantly high than that of second peak (372 to 382 nm). Can authors give probable reason for this based on the bond formation to the metal center ?

   Response:
   All the samples for the UV–Vis spectroscopic measurement were prepared in water. After a careful survey of the literature, we have concluded that the bathochromic shift resulted from the backbonding of electrons from Ru to the antibonding orbitals of C=C-C=N in compound 1. That is, the addition of electrons to the antibonding orbitals weakens the C=C-C=N bonds, leading to the bathochromic shift. We have now discussed this aspect in the revised manuscript.

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I have flagged all such instances through comments for your consideration. Please do go through them and do get back to me, so that I can revise the text for you if needed.

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2. The comment regarding the higher shift of the first peak as compared with the second peak is not addressed. Please address this in the response and, if applicable, in the revised manuscript.

5. The authors may refer to the studies by Dharmaraj et al., Grushin et al., and Ahmed et al. on the metal complexes and cite them accordingly. In the title for the paper in the reference 13, "Complexation of bis-2-(benzylideneamino)phenol to cobalt(II) and zinc(II), and their spectroscopic **studie,**" studie should be studies.

   Response:
   We thank you for suggesting these valuable references. The information provided in these references has improved our understanding of the formation of metal complexes. We have now cited these references at relevant places in the manuscript. The title in reference 13 has also been corrected now.

6. The geometry of the complexes is not mentioned in the text.

   Response:
   We apologize for not mentioning the geometry of the isomers of the Ru complex. All the complexes were octahedral. We have mentioned this in the revised manuscript.

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