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In this issue

- Call for manuscript submissions for a thematic eJIFCC issue on “Measurably Better Healthcare”* 182
-
- A conversation between two viruses: SARS-CoV and SARS-CoV-2 – based on a true story**
Eleftherios P. Diamandis 183
-
- Robinson Crusoe never had to meet academic deadlines: scholarly pressure in the age of COVID-19**
Nina Maria Fanaropoulou 186
-
- The natural history of an eponym: the Malloy-Evelyn method**
Larry J. Kricka, Jason Y. Park 190
-
- Leptin levels and Q223R leptin receptor gene polymorphism in obese Mexican young adults**
Carlos E. Diéguez-Campa, Luis I. Angel-Chávez, David Reyes-Ruvalcaba, María J. Talavera-Zermeño, Diego A. Armendáriz-Cabral, Dayanara Torres-Muro, Iván Pérez-Neri 197
-
- Extra-analytical clinical laboratory errors in Africa: a systematic review and meta-analysis**
Daniel Asmelash, Abebaw Worede, Mulugeta Teshome 208
-

In this issue

- Vitamin D deficiency has no impact on PSA reference ranges in a general university hospital – a retrospective analysis**
Zoltán Tóth, Balázs Szalay, Béla Gyarmati, Dlovan Ali Jalal, Barna Vásárhelyi, Tamás Szabó **225**
-
- Trends in laboratory testing practice for diabetes mellitus**
Mithu Banerjee, Samuel Vasikaran **231**
-
- Anti-tuberculosis treatment: induced hepatotoxicity – a case report**
Poludasari Shravan Kumar, Rachakonda Vidya, Tabassum, Manashwini Jageer **242**
-
- Case report on pediatric septic arthritis of the hip**
Shireen Prince, Rao Tulasi **248**
-
- Retraction of Publication**
Editor of the eJIFCC **254**
-

Call for manuscript submissions for a thematic eJIFCC issue on “Measurably Better Healthcare”

Guest Editors for the special issue: Ellie Dow and Tim James

The clinical laboratory has always played an essential role in high quality healthcare. Appreciating the link between clinical data and medical decision making has been widely communicated for decades. Less frequently recognized, however, is the power of the clinical laboratory to drive measurable benefits for patients, payers and entire health systems.

Best practices of measurably better healthcare exist across the globe, and tend to involve coordinated, cross-disciplinary, and evidence-based collaborations for the implementation and activation of new clinical care pathways.

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- *Who received the benefit and how was it measured?*

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- *How did laboratory data improve patient outcomes?*
- *What clinical algorithms helped identify at-risk individuals before they become “patients”?*
- *What biomarker-guided processes have enabled new strategic approaches to diagnostic optimization, disease management, therapeutic intervention, care optimization or improved population health?*

Submit a paper on “Measurably Better Healthcare” to be published in this thematic issue of the eJIFCC that is a PubMed listed, platinum open access journal with a CiteScore of 0.8

Important deadlines and Next Steps

- Deadline for all submissions (original articles, critical reviews and case studies): **November 15, 2020**
- Manuscripts to be submitted by e-mail to ejifcc@ifcc.org with a copy to ejifccspecialissue@gmail.com

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- Tim James, Ph.D., Head Biomedical Scientist, Clinical Biochemistry Department, John Radcliffe Hospital, Oxford, England

A conversation between two viruses: SARS-CoV and SARS-CoV-2 — based on a true story

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MANUSCRIPT

Around October 2019, two viruses, who only speak Chinese, SARS-CoV and SARS-CoV-2, had a friendly conversation in the province of Wuhan, China.

SARS-CoV-2 (from now on called COVID) said to SARS-CoV (from now on called SARS):

“I am really bored, SARS. I have been living inside bats, pangolins and other animals for centuries and there is nothing new to see. I have the desire to go around the world, see other people, other races, get to know them and maybe even give them some useful advice”.

SARS said: “I have done the same thing about 10 years ago, COVID, and it was a disaster. As soon as I migrated to a few countries, they immediately took draconian isolation measures and in a few months, I couldn’t move anywhere and I was left to die. Everybody forgot about me within two years; they never talk about me anymore. The same thing happened to some of our cousins: Ebola, MERS, etc. So, be careful. But why are you confident that you can succeed where we all failed?”

COVID: “I know I only have 29,000 nucleotides in my RNA sequence and I can make a handful of proteins, but I am a little bit more flexible than you. If I use some special tricks, I may actually go around without being noticed.”

SARS: “Can you tell me your plan?”

COVID: “Yes. I will first hide in the body of one of these small animals, like pangolins, and I will travel with them to the central fresh seafood market in Wuhan. I am sure somebody will buy the pangolin and eat it. At that time, I will jump into the customer’s lungs by grabbing onto the ACE2 enzyme with my spike protein, get in the cells and start proliferating.”

SARS: “It sounds like a good plan, COVID, but aren’t you afraid that the humans will catch you very early?”

COVID: “I am not worried because I will sit quiet for 1-2 weeks without giving any symptoms to my victims. During this period, I will produce billions and billions of copies of myself and then I will jump into other people, and here we go.”

SARS: “And how are you going to go to other countries?”

COVID: “It is very easy, SARS: by trains, planes, and automobiles. Have you seen that movie? I will find travelers and go along with them. I know that many Chinese are now traveling all over the world for vacation, business etc. Some of them even fly first class and enjoy the good food, unlimited drinks etc. While they enjoy the ride, I will be proliferating, proliferating, proliferating and infecting, infecting, infecting. I am sure none will wear masks and will be seating next to each other. When these people develop symptoms I will be sitting in thousands of people’s lungs and continue my trip.”

SARS: “And how many countries are you thinking of visiting COVID?”

COVID: “I plan to visit every corner of the world. As I said, I am highly curious and I want to see

everything from the Eiffel Tower to Big Ben to the Leaning Tower of Pisa, and the ancient monuments of Greece. Of course, I will not leave behind our best friend, the United States, and the city that never sleeps, New York. While in these places, I will arrange to meet prime ministers, members of the Congress and parliaments, famous actors, athletes, you name it. I will even get into the White House and have a little tour.

SARS: “But, COVID, some of these countries are very cold, like Canada; are you thinking of visiting them too?”

COVID: “As I said, SARS, I will visit everywhere, no matter what the climate is; every single corner of the world. Tell me a country, a city, a neighborhood and I will be there.

SARS: “And how much time do you need to do that?”

COVID: “Just a couple of months.”

SARS: “HaHaHa; I can’t believe it. It is a mission impossible (have you seen this movie?). How will you be able to visit every country, every corner, infect millions of people and, in the process, cause thousands of deaths in such a short time?”

COVID: “SARS, believe me. I have the weapons, as I told you: 29,000 nucleotides and 20 proteins. Besides, my intention will be not only to cause death. I will do other things which are probably even worse.”

SARS: “Like what?”

COVID: “I will destroy their economies from their foundations and leave millions without work. Do you know how much is a million dollars? It is a lot of money. Do you know how much is a billion dollars? It is a lot more money. And do you know what is a trillion? It is the whole US budget for a year. My objective is to make such damage that the economic fallout will be in the many trillions of dollars. It will take them years and years to recover. Trust me! I need to give these people a lesson.”

SARS: “I wonder, COVID, why do you want to give these people a lesson?”

COVID: “Listen, SARS; I have been around for a while and you have been around for a while and we have both seen what happened. Human beings are a strange species. While they say that they care about other humans and they want to help with this and that, in actual terms, and this is getting worse, they have proven to be greedy and selfish. They start unnecessary wars, make nukes that can destroy the world 10 times over, they invade other countries, they make the poor become poorer and the rich become richer, they play with the stock market and they steal other people’s money. In other words, politicians have become corrupt and morality and empathy has declined. While God blessed and empowered them to make this world better, to protect the animals, the environment etc. in actual fact they are destroying everything left and right. Look at the poor environment. How many more natural resources they will burn and how many more billions of animals they will eat or force go extinct? If we leave these people unchecked, in a few years, there will be no planet. In terms of life-style, they pack themselves in 100-storey buildings without realizing that epidemics like this one will proliferate like fire before they are able to react. Are they losing their common sense?”

SARS: “Why don’t you go on television and tell all these facts to the politicians and other citizens COVID? May be they will listen and correct themselves before you inflict your damage.”

COVID: “Unfortunately, SARS, I am not a good actor and I do not speak English. And most of those who need to listen they don’t understand Chinese. So, my only option to bring them back to their senses is to shake them with the pandemic, so that they will fortunately wake up. I feel sorry for doing it; I am not a bad guy as you know, but I believe this thing has gone too far. Humans have become selfish and arrogant and they believe they can do anything and can get away without consequences. I have no question that after I complete this trip around the world they will start thinking of what has gone wrong and maybe they will try to fix things.”

SARS: “And what if they don’t?”

COVID: “I will tell you what. I will retreat for a few months and monitor how they respond. If they don’t do anything, or become more arrogant, I will go back and give them another shake. Just sit back and watch me, SARS. I will do a lot more than you did, even if our genomes are about the same size. Size does not matter here!”



Note: All characters mentioned in this story are hypothetical and any resemblance to real people or viruses is purely coincidental. The author offers his condolences for lives lost during this pandemic. His essay will fortunately contribute to spark a debate as to what could be done to avoid the pandemic, and, most importantly, what can be done to avoid future pandemics.

Robinson Crusoe never had to meet academic deadlines: scholarly pressure in the age of COVID-19

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MANUSCRIPT

Daniel Defoe's timeless novel, inspired by the adventures of a real Scottish sailor, tells the compelling story of a shipwrecked man who survived for 28 years on a desert island. The novel is a deliberate description of his endeavors to build a sustainable life for himself in previously unimagined conditions, fighting titanic battles with cannibals, externally, and exasperation, internally.

Robinson Crusoe has become an archetypal figure in literature, starring in a scrupulous examination of the human experience in isolation, endurance and transformation.

Endurance and transformation are what I, as a medical student nearing the end of my studies, hope to display during a pandemic that, as has been widely discussed, has drastically changed what we previously took for granted in regard to social life, work and home normality. But, in a time of fierce scholarly competition,

covering essential needs in isolation or crisis may threaten academic thriving – can a modern Robinson Crusoe still manage? Thankfully, for me, the former goal has been much easier than it was for Crusoe. On the other hand, I have felt like I am faced with a different kind of a subtle environmental pressure.

Undeniably, there were some breaking moments in life before the pandemic, too. If I lost a beloved one, or received a medical diagnosis, or had any other challenging circumstance forced upon me, I could usually take some time away from university and expect understanding for missed social gatherings. My friends, colleagues and supervisors would – hopefully – respect my plight and show affection and leniency. Since such misfortunes didn't happen simultaneously for everyone, it was possible for me to make individual plans to gain back the lost time after returning. During quarantine, with everyone away from work at the same time, I subconsciously felt that this “unprecedented” situation somehow took me and my peers all to the same point in the scholarly marathon. In other words, I felt like we were all having equal chances of making comparable progress while away from work. Gradually, a new kind of stress appeared, in my illusion that when we're free to resume normal work, the number of seized opportunities or completed manuscripts will be a fair metric of our academic skills and accomplishments. Just as in hide-and-seek, all players have the same amount of time to find the optimal hiding place, and the winner will be the one who was able to hide most effectively.

But after reading several COVID-19 related stories and views from academics around the world, and also hearing about my peers' and friends' experiences, I now understand my faulty assumption on the ground that we were simply not the same before this pandemic. Some players could run faster, while others had to carry monkeys of various kinds on their backs.

Thus, it cannot be the case that my unique circumstances can suddenly be ignored during a world-shaking time-stopper. Unfortunately, the deaths announced on the daily news have represented real members of real families. My own family's wallet, pantry, relationship integrity and patience have also suffered, more or less than they have for numerous other families around the world.

Chronic conditions among my loved ones did burst out. On top of that and despite my best efforts, my much-awaited summer research internship abroad got cancelled, while even university exams, graduation dates and classes were majorly disrupted. Never had I ever imagined that, while padding my CV with “adaptability under pressure”!

In search of a real-time connection to the world, I turned to social media in quarantine, but it didn't take long before I sensed that their virtual reality was only making matters worse. Remember when we were discussing how social media had a massive negative influence on our expectations, self-perception and confidence? Attending work and social gatherings had somewhat balanced this for me, by providing a picture of my friends' and colleagues' lives in real time. However, distancing threw this balance off, since my main “newsletter” consisted of tweets, Facebook statuses, posted pictures and Instagram stories.

Repeatedly coming across “productivity porn” and home workout posts often brought me to the verge of feeling unworthy for not maintaining an ideal schedule from home – unsuccessfully trying to deliver assignments while caring for vulnerable family members through tele-contact with their attending physicians, shifting everything that could be shifted online, completing house chores and budget reallocation, or simply using my medical background to help my family understand the news and information

they were getting. Especially the latter proved more demanding than I had expected, serving as a glimpse into the perennial difficulty of gaining public trust as a healthcare professional.

The best solution for maintaining sanity proved to be a conscious effort to resist overconsumption of online content, because it only represented snapshots. What was my social milieu doing before and after that dreamy scenery of books, coffee and a laptop by the window? They may have been experiencing anything between serenity and despair, and this would have been totally acceptable. They were still carrying their monkeys, and new ones might have been added. Besides, even when I didn't pay that much attention to social media, uncertainty and anxiety took their toll when I least expected them to; on days when even the fact that Albert Camus completed one of his masterpieces, "the Plague", during quarantine, did not seem motivational anymore.

I promised myself to avoid quick judgments, when the time came to go back to the race and take a look around at where everyone would be standing. I actively dismissed my urge to set for myself or my research team checklists of what must have been done by the end of lockdown, simply because we could not know when that would be, or how we were going to get to it. I dumped my strict to-do lists, which had somehow gotten even stricter since the beginning of quarantine, to "have completed one review, begun one more, finally finished that online extra-curricular course, cleaned-up every wardrobe, relaxed" (what even is a measure of relaxation?). What got me through the early stage of the pandemic was operating on a day-to-day basis, taking small, steady steps, and supporting the needs of my team instead. And guess what? Focusing on just following the distancing orders, securing my family's wellbeing and preserving my own peace of mind let me find time

to both study for my exams and complete a few other academic commitments along the way.

All things considered, I am optimistic that despite tragic losses, we'll also be left with favorable outcomes. As Malcolm Gladwell describes in his book "David and Goliath", which offers an alternative interpretation of "underdogs, misfits, and the art of battling giants", obstacles can be springboards instead of setbacks. Similarly, although it might seem hard during a public health crisis, now has been a time for an inquisitive mind like mine to take some rest out of a daily schedule bombarded with tasks and assignments, reset and even burst with creativity in unexpected ways. Forever being a night owl, I could finally afford to lose sleep at 4 am and write down that sudden explosion of ideas about current and future projects that crossed my mind, without having to worry much about my usual, rushed 7 am wake-up. I was able to grasp the words of the Imperial College researcher Mattias Björnmalm, in his article "Taking a break is hard work, too: exciting, novel ideas do not come from a mind constantly under pressure¹⁷".

Having served for years as a program officer in children's summer camps, I have witnessed children struggling to adapt to new habits, like cleaning their shelters and taking turns setting the table for their peers. But after the struggle always came the pleased voices of parents, noting how impressively their son or daughter went back home and, for the first time, did the same. I fervently hope that in this crisis, lessons learnt and activities altered for good will eventually survive through time, as societies utilize change towards improvement.

We weren't asked about it, but I believe that, in fact, we are all part of the greater COVID-19 Task Force. Whether we accept it or not, one thing is for sure: "Coming out of our cages", we likely now have to re-introduce ourselves.



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The natural history of an eponym: the Malloy-Evelyn method

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LETTER TO THE EDITOR

Over the years the vogue for naming reactions and methods has been commonplace in clinical chemistry. Bilirubin testing has produced a number of eponyms including the Jendrasik-Groff method (1,2), the van den Bergh reaction (3), Huppert's test (4), Smith's test (5), Fouchet's test (6), the Harrison Spot test (7), the Weber-Schalm method (8), the Ducci-Watson modification (9) and the Malloy-Evelyn method (10).

But how does someone's name become associated with a reaction or method? Have people had the audacity to name a reaction or method for themselves? Does it happen because the reaction or method assumes great utility or importance and the name is a convenient alternative to a more complex and cumbersome formal description?

We have looked at the history of the Malloy-Evelyn method, first described by the Canadians, Helga Tait Malloy and Kenneth A. Evelyn (McGill University Clinic, Royal Victoria Hospital, Canada) in 1937 (10). This quantitative colorimetric diazo coupling method for direct and indirect bilirubin in serum without the need for protein precipitation, utilized a direct reading photoelectric colorimeter invented by Evelyn.

Their first reference to their own 1937 publication was in a paper by Malloy and Lowenstein in 1940, and the method was referred to as “the method of Malloy and Evelyn” (11). The first citations to their work by others, was in two publications in 1938. In the first, a joint research paper from the South Bend Medical Laboratory and Hoffman-La Roche & Company AG, the text simply states that “Malloy and Evelyn have introduced a method” and the paper describes the adaptation of the method to a Sheard-Sanford photometer (12). In the second publication, a chapter in a German textbook, the text includes “MALLOY und EVELYN” in a list of spectrophotometric methods for bilirubin, and the citation style was to list all authors, so this was not an attempt to name the reaction (13).

It seems that the first naming was in a paper from Duke University School of Medicine, submitted in October 1939, where the plasma bilirubin assay is described as “the method of Malloy and Evelyn” and the “procedure of Malloy and Evelyn” (14). The die was cast! Subsequently, the method described in the 1937 paper became known as the method, process or procedure of Malloy and Evelyn, or the Malloy and Evelyn method, procedure or technique, or in an abbreviated form, simply as Malloy-Evelyn. During the ensuing years, their method gained traction and it was referred to by name in the text of at least one publication every year since 1939, up to the present day. From the earliest month tracked by the Journal of Biological Chemistry (September 2003) to June 2020 there were 21,830 pdf downloads of the original 1937 paper. In Web of Science (Clarivate Analytics) there are 1,270 citations in the time period from 1965 to 2020 with at least 2 citations per year (range 2 to 65).

But who were Malloy and Evelyn? They are not the subjects of biographies or autobiographies and so we searched for biographical information (see brief biographies in the Supplement). Evelyn was a professor of Medicine and Director of the

British Columbia Medical Research Institute. He published 15 papers between 1936 and 1978 (four with Malloy) invented a colorimeter and a device to test and train the night vision of soldiers. Malloy was a graduate of McGill University and published 8 papers between 1937 and 1941, and also made a brief foray into the water fluoridation controversy in 1959. A continuing mystery is her book, cited on her gravestone, “The Shape of Inner Freedom” of which we can find no trace!

In recent years, naming a reaction has been less common, for example, the polymerase chain reaction (PCR) was not called the “Mullis reaction”! However, others have found eponymous fame, e.g., Westgard Rules, and no doubt, in the coming years, more will be added.

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SUPPLEMENT

The natural history of an eponym: the Malloy-Evelyn Method

Larry J. Kricka and Jason Y. Park

INTRODUCTION

This Supplement explores the personal history of two famous scientists: of Helga Tait Malloy (Section 1) and Dr. Kenneth A. Evelyn (Section 2). As far as we can tell, there are no biographies for either of these two scientists, and the information below has been pieced together from census data, shipping records, yearbooks, and newspaper articles.

1. HELGA TAIT MALLOY (1909-1993)

Helga Tait Malloy, nee Tait, was born in Edinburgh, Scotland on May 19, 1909 (1). According to the 1921 Canadian Census she was the oldest of 5 children (Sigrid, Ragnild, Erik, Ingrid) to John and Jean Tait (2). Her family immigrated to Montreal from Scotland in 1919. At the Montreal High School, Helga excelled at swimming and set records in the 50-yard swim in 1922 (3) and the one-mile swim in 1923 (4).

After high school, she attended McGill University, in Montreal, Canada, where her father, John Tait (1878-1944), was the [Joseph Morley Drake Professor of Physiology](#) (5). At McGill, she was Secretary and Treasurer of the McGill Labour Club (6), President of the House of Commons Club (7), President of the Royal Victoria College (8), and President of the Ski Club (9). The 1931 Old McGill yearbook mentions that “*she suddenly left in search of the local accent at Edinburgh University – found it and returned*” (9). This cryptic comment perhaps reveals a crisis in Helga’s life, or perhaps she was nostalgic for her holiday in 1923, when, aged 14, she travelled to Scotland in May, and returned in September on the SS Cassandra (10). She graduated from McGill in 1931.

She married Connolly James Patrick Joseph Malloy in 1936 or 1937 (thereafter, in the literature, she is cited as Helga Tait Malloy) and they had three children: Brian John, Brenda Margaret and Kirsten Ann. He was an assistant physician at the Royal Victoria Hospital and lecturer at McGill University and made a Fellow of the American College of Physicians in 1954 (11) and was also a Fellow of the Royal College of Physicians and Surgeons in Canada, of the American Academy of Allergy, and of the American College of Allergy (12).

During her career, she published a total of eight papers (13-21), and is best known for her work with Evelyn on bilirubin assays. Her research career began in 1936 in the Department of Medical Research at the University of Toronto, Ontario, Canada, and the following year she moved to the Department of Medicine at McGill University Clinic, Royal Victoria Hospital, Montreal, Canada. Between 1937 and 1941, her McGill affiliations also included: Departments of Paediatrics and Pathology (1937), the Paediatric Department (1940-1941) and, finally, the Department of Psychiatry (1945).

She was the recipient of a grant from the Banting Research Foundation and she is mentioned in the 1938-1939 Report (22):

“Mrs. H. T. Malloy, working in the University Clinic, Royal Victoria Hospital, Montreal, has investigated hereditary jaundice in rats and has found that it does not depend upon enhanced hemolysis but rather in the inability to parenchyma liver cells to deal properly with blood bilirubin, i.e., the hereditary factor concerns parenchyma liver cells rather than haemopoietic tissue.”

Her final paper, in 1945, from the Department of Psychiatry at McGill (the first psychiatric day hospital in the world) (23) was on Rorschach studies on patients with paranoia (21), and it represented a curious and major departure from her previous biochemical studies. Her subsequent career post-1945 is a mystery and our presumption is that she focused on her family which included three children. One further scientific excursion was on May 20, 1959, when she had a letter titled “Facts and Fluoridation” published in The Gazette (Montreal, Quebec, Canada). In this letter, she drew attention to a booklet “Statement on the Fluoridation of Public Water Supplies by the Medical-Dental ad hoc Committee on Evaluation of Fluoridation” that was available for 21 cents. Her position of fluoridation is not clear as she states “I fail to understand the fervor which goes into fluoridation promotion” (24).

She died on February 3, 1993, and is buried in the Pacific View Memorial Park, Corona del Mar, Orange County, California, USA with her daughter, Brenda M. Malloy (1944-2006)(1).

The headstone for her grave is perplexing. It identifies her as the author of “The Shape of Inner Freedom”. All our efforts to find this book have been to no avail. It is curious that this epitaph was chosen in view of the apparent obscurity of the book. It has not been unusual for the headstones of famous authors to bear a quote from their work (e.g., Scott Fitzgerald: “So we beat on,

boats against the current, borne back ceaselessly into the past” – The Great Gatsby) (25). However, adoption of “The author of” style of epitaph seems unusual, and might suggest that the book was written under a nom de plume, and the epitaph is a revelation of the fact that Malloy was the real author?

2. KENNETH A. EVELYN

Kenneth Evelyn, was a PhD physicist who later became a medical doctor (26). He worked in the Department of Medicine at McGill and according to Dr. Kerr, he had one of the highest reputations for medical research in Canada. At McGill, he invented a photoelectric colorimeter in 1936 (27-30) and worked with Malloy to develop bilirubin (15,16), hemoglobin (17) and ascorbic acid assays (18). During World War II, he is credited with inventing a device to test and train the night vision of soldiers (31). His contribution is credited with improving the lighting in planes and tanks. He moved to the B.C. Medical Research Institute in Vancouver, Canada, in 1953, and subsequently became its Director and was also appointed professor of Medicine (32,33).

Malloy published 26 publications during the period 1936-1978 (15-18, 27-30, 34-51), four with Malloy on analytical methods (15-18), but in later years most were on clinical topics (e.g., hypertension).

More about Dr. Evelyn can be gleaned from an oral history interview with Dr. Robert Kerr, who was Professor of Medicine and the Head of the Department of Medicine beginning in 1950. In particular, he provides an interesting insight into Dr. Evelyn’s medical history as described in the following extract from his interview (32):

“Dr. Evelyn, when he was appointed director of the Medical Research Institute, was also appointed professor of the Department of Medicine - Associate Professor first and then was promoted

to full Professor. He was interested in hypertension and he did a lot of research, particularly in serum protein finally. He was plagued with ill health. Shortly after he came here - I could tell you about him, he was a very interesting person. He was dependent upon four medications which had provided their discoverers with the Nobel prize. I don't think he would mind me recording his medical history briefly now. He came here - and I knew he was not well but he and his doctors in Montreal assured me that he could manage but, as it turned out, his health deteriorated but he lived for - well, he just died about three or four years ago and he lived to retire at the normal age. During the war he was in the Air Force doing research on night vision and various other aspects. While in the Air Force he developed anaemia. Then he developed a disease called sarcoidosis, and this affected his adrenal glands. The adrenal glands were diseased and he developed Addison's disease, which is an adrenal insufficiency. With this he was first given what was available, an Upjohn extract of adrenal cortex. Then, when cortisone was discovered in 1947-48 he was started on cortisone, which was fine but he went down to Boston for a year from Montreal. A professor of medicine there put in some pellets of cortisone into his back, to be absorbed slowly. However, these proved to be deleterious in that he developed diabetes with these and, by the time he got back to Montreal one day, he was in diabetic coma. He was dependent, first of all on cortisone which gave Hench and others the Nobel prize. Then he became dependent on insulin for his diabetes which gave Banting and Best the Nobel prize. He also developed hypothyroidism and had to take thyroid extract, which had given Kendall the Nobel prize; and he also was anaemic, which required liver extract, which had given Cassell and Minot the Nobel prize in 1923. He had four medications. But he was a very brittle diabetic and he would go into hypoglycemia - too low a blood sugar - very frequently. He had

multiple hypoglycaemic attacks. But he rallied around and continued to do his work. Basically, he was an excellent person as far as research was concerned. He continued on that. After I retired, he was still a member of the Department. That's the history of Dr. Evelyn. I guess he was probably the first, because of this funding from the Medical Research Institute, fulltime appointment in the Department of Medicine, really. He was an excellent teacher as well."

Dr. Evelyn was an influence on two well-regarded physicians, C. Miller Fisher (52) and Peter Allen (53). Drs. Fisher and Evelyn worked together to test out a low sodium diet on a patient dying from congestive heart failure. The patient improved but when the attending physician found out what had transpired, he forbade a future trial. Dr Allen held a research position at the British Columbia Research Institute, Canada under the direction of Evelyn where they worked together on a mesh supported vena cava autograft to replace diseased abdominal aorta.

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Leptin levels and Q223R leptin receptor gene polymorphism in obese Mexican young adults

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ABSTRACT

Introduction

The Q223R polymorphism of the leptin receptor (*LEPR*) gene is one of the most common polymorphisms and it is believed to be associated with a damaged capacity of LEPR signaling and with high circulating leptin levels.

Methods

An observational, cross-sectional, analytical study was carried out in the Autonomous University of Ciudad Juarez, Mexico, where a sample of young adult participants (ranging from 18 to 30 years of age) was obtained. They were classified based on the results of body mass index: non-obese, and overweight/obese. The polymorphic variant was determined by Polymerase Chain Reaction (PCR) from the DNA sample and serum leptin levels were measured by Enzyme-Linked Immuno Sorbent Assay.

Results

A total of 159 participants were included (non-obese, n=103; overweight/obese, n=56). Leptin levels were 15.14 ± 12.3 ng/mL in the non-obese group and 26.13 ± 19.0 ng/mL in the overweight/obese group ($p \leq 0.001$). The allelic frequencies of the Q and R alleles of the *LEPR* gene in the studied subjects were as follows: non-obese, Q=0.56, R=0.44; overweight/obese, Q=0.62, R=0.38. The relative risk for the Q/Q genotype was 1.18 (CI 0.53-2.34), for Q/R was 1.14 (CI 0.59-2.18) and for R/R was 0.59 (CI 0.23-1.50).

Conclusions

This study shows that leptin levels are associated with overweight/obesity in Mexican young adults, but this is not related to the presence of the Q223R polymorphism in the *LEPR* gene, so the underlying mechanisms for a possible disturbance in leptin signaling in obese Mexican young adults await further studies.



INTRODUCTION

Obesity plays a fundamental role in public health problems, which have reached epidemic scales. The prevalence of obesity increases year after year, and it has been related to a large number of risk factors for multiple diseases. Obesity is defined as an excessive amount of body fat or adipose tissue in relation to body mass. Overweight refers to the increase in weight in relation to height, which is later compared to an accepted weight standard. The body mass index is a common measure that expresses the relationship between height and weight. Adults with a body mass index (BMI) of 25 to 29.9 are considered to be overweight, while individuals with a BMI greater than 30 are considered obese individuals (1).

Obesity has become a problem affecting the health of millions of people around the world, in recent studies a prevalence of up to 39.6% has been established in adults (1), being an important comorbidity of many chronic degenerative diseases. However, it is also relevant for acute disorders like COVID-19 (2).

Obesity produces a significant cost in the global economy, being a major public health problem and a red flag for international health organizations. In different epidemiological studies, it has been shown that Mexico is one of the Latin American countries with a high obesity prevalence, data that goes hand in hand with the high numbers of type 2 diabetes mellitus, dyslipidemia, coronary heart diseases, sleep disturbances, cognitive dysfunction, cancer, kidney and liver diseases. BMI is considered an important measure to understand population trends; for individuals, it is one of many factors that can be considered to assess healthy weight, along with body fat composition, waist circumference, blood pressure, cholesterol levels and serum glucose levels (1).

Leptin is an anorexigenic hormone synthesized primarily in adipose tissue, its function is to regulate lipid metabolism by stimulating lipolysis and inhibiting lipogenesis (3). Zhang et al. identified leptin as a product of the obese (*ob*) gene via the positional cloning strategy (4). The leptin gene is located in the long arm of chromosome 7 (7q31.3) and contains three exons and two introns (5). Madej et al. predicted that leptin is a cytokine with a structure of four alpha helices and suggested a JAK/STAT-like signaling pathway for leptin action (6). Leptin deficiency is not the only factor involved in obesity, with a resistance to leptin being also involved, and as leptin reduces food intake and body weight, leptin resistance and high leptin levels are thus observed in obese people.

The leptin receptor (LEPR) can be classified as a class I cytokine receptor. It shows high similarity to interleukin 6, glycoprotein 130 signal-transducing chain, the receptor for the granulocyte colony stimulating factor and the receptor for the leukemia inhibitory factor. This family encompasses receptors marked by the presence of one or more cytokine receptors from homologous domains, all of which use JAK kinases for their intracellular signaling. The *LEPR* gene is located on chromosome 1 (1p31) which contains 20 exons (7).

Leptin function is mediated by the *LEPR*, and both the LEPR and leptin itself are involved in homeostatic control of appetite, weight, metabolism, and reproductive functions in women. A number of polymorphisms have been reported in the human *LEPR* gene. The Q223R polymorphism is one of the most common and is believed to be associated with impaired ability of leptin receptor signaling; this polymorphism has been associated with high leptin levels (8).

The interaction of leptin with its receptor in the hypothalamus stimulates a specific signaling cascade that results in the synthesis of anorectic and orexigenic peptides to regulate food intake and energy expenditure. Many polymorphisms in the leptin and the *LEPR* genes have been associated with body weight (9).

In addition to the environmental factors that have already been discussed, several genetic alterations that may play an important role in the etiology of obesity have been rigorously studied, based on the observation that not all individuals with a large amount of caloric intake and decreased physical activity are obese. There are several complex genetic interactions in obesity. In twin and family studies, it has been shown that more than 80% of the variation in BMI, 50% of the risk for type 2 diabetes mellitus, and 10-30% of the risk for metabolic syndrome is attributed to genetic factors. Among the factors

that affect genetic variations, single nucleotide polymorphisms (SNPs) have been observed. Although SNPs are not usually enough to cause a disease, they can determine predisposition to special metabolic problems and, therefore, disease. Obesity is inherited mainly due to genetic factors. In rats, the gene that causes obesity was sequenced in 1994, with mutation of this gene resulting in increased food consumption, high insulin levels and obesity in non-insulin dependent diabetes mellitus (10).

In a literature review carried out in Iran in 2013, nine of the 17 articles that evaluated SNPs in obesity reported association or a possible risk factor; however, eight of those studies found no association (11). Hence the role of SNPs in this disorder awaits further studies.

The objective of this study was to evaluate the role of the Q223R polymorphism of the *LEPR* gene, leptin levels and its association with clinical characteristics of obese young Mexican adults.

METHODS

This is an observational, cross-sectional, analytical study. The recruitment of study participants was performed at the Clinic of Chronic Degenerative Diseases of the Institute of Biomedical Sciences of the Autonomous University of Ciudad Juárez (UACJ), in Chihuahua, Mexico. Young adults between 18 and 30 years were included. The sample size in this study (n=159) was higher compared to some association studies (12-14). The subjects were recruited consecutively as in previous studies (12). A randomized subset of subjects was extracted from this sample, as described below.

The selection criteria were A) Inclusion: subjects aged 18 to 30 years who agreed to participate in the study (who signed the informed consent letter); B) Exclusion: bacterial or parasitic infection 2 weeks prior to sampling, acute inflammatory process, coagulation disturbances; C) Elimination: subjects who had not agreed

to allow all necessary measurements, subjects who had decided to withdraw from the study.

During data collection, blood pressure, weight, height, circumferences (waist, hip, scapular, middle arm), folds (bicipital, tricipital, subscapular, suprailiac) and body fat percentage were recorded. The weight and height were measured on a hospital scale with a stadimeter (Torino-Oken, México). Height was measured, to the closest 0.5 cm, with the subject without shoes, heels together, and with the head in the Frankfurt plane position. Weight was measured to the closest 100 g registered by the scale, with the subject wearing light clothing. The body mass index was calculated by dividing the weight expressed in kilograms by the square of the height expressed in meters. Subsequently this value was used to classify participants according to the International classification of overweight and obesity in adults based on their BMI (kg/m^2), as follows: normal weight (18.5-24.99), overweight (≥ 25.0), and obesity (≥ 30.0) (15).

The body fat percentage was measured using a bioimpedance analyzer (Citizen Corporation, Japan), once programmed with the parameters required by the instrument (weight, height, age and sex) the individual placed his sweat-free palms on the electrodes of the equipment, until the record on the screen appeared.

The plicometry was carried out, to the closest millimeter, by using a Lange plicometer (Dynatronics Corporation, USA), on the right side of the subject, in a relaxed position. The bicipital, tricipital, subscapular and the suprailiac skinfolds were measured. Scapular, middle arm, waist and hip circumferences were established to the closest millimeter, by using a soft plastic measuring tape.

Two blood samples were taken: one for leptin levels and one more to obtain DNA. The laboratory procedures were: extraction of genomic DNA from peripheral blood, amplification of

the polymorphic fragment by Polymerase Chain Reaction (PCR) and determination of genotypes.

Serum Leptin levels were quantified using a solid phase enzyme-linked immunosorbent assay (ELISA) according to the manufactured instructions (ALPCO, USA).

The Miller method (16), was used to extract DNA from peripheral blood collected into a sterile tube with EDTA-anticoagulant. The DNA pellet was resuspended in 300 mL of sterile TE buffer and the concentration of the DNA obtained were calculated by spectrophotometry. The Q223R variants of the *LEPR* gene, were determined by PCR-RFLP technique, as described previously by Angel-Chávez et al. (17, 18)

Data were compared by Student's *t* test or Mann-Whitney *U* test, after checking for normality of data distribution. Categorical data were compared by the Fisher exact test. The sample size was calculated using G*Power 3.1, with a medium size effect (0.3), $\alpha=0.01$, statistical power $1-\beta=0.8$, and $Df=2$, resulting in 155 subjects. To confirm our results regarding the association between Q223R genotypes and obesity, we extracted a randomly selected subset (ca. 50%, $n=79$), which was analyzed as the original sample. Results are expressed as mean \pm SD and were considered significant at a bilateral $p<0.05$ value. All analyses were performed with the IBM SPSS Statistics version 25 (IBM Corporation, USA).

RESULTS

The clinical characteristics of the subjects are shown in Table 1. The total population that met the inclusion criteria were 159 individuals (non-obese $n=103$, 65%; overweight/obese $n=56$, 35%). Age was not different between the groups. There were significant differences in gender, weight, height, BMI, systolic and diastolic blood pressure, body fat percentage, waist-hip ratio, waist circumference, hip, scapula and arm,

Table 1 Clinical characteristics of the participants

	Non-obese	Overweight/obese ^a	<i>p</i> ^b
Gender (male/female)	34/69	31/25	0.007
Age (years)	20.96±2.0	21.02±1.9	0.863
Weight (kg)	58.47±9.2	81.05±11.3	<0.001
Height (m)	1.65±0.09	1.69±0.1	0.011
BMI (kg/m ²)	21.41±2.3	28.38±3.1	<0.001
Systolic pressure (mmHg)	115.40±11.1	123.31±12.8	<0.001
Diastolic pressure (mmHg)	78.60±6.6	83.64±9.0	<0.001
Body fat (%)	24.20±6.8	31.58±7.7	<0.001
ICC	0.80±0.1	0.86±0.1	<0.001
Circumferences (cm)			
Waist	74.83±6.9	91.25±8.5	<0.001
Hip	94.67±8.1	106.25±5.9	<0.001
Scapula	86.32±8.3	100.34±9.1	<0.001
Arm	26.59±3.3	32.44±3.6	<0.001
Skin Folds (mm)			
Biceps	5.69±3.7	6.88±4.4	0.070
Triceps	11.78±7.1	14.39±7.7	0.033
Subscapular	13.83±4.2	21.71±5.7	<0.001
Suprailiac	15.66±6.1	24.7±6.7	<0.001
Leptin (ng/mL)	15.14±12.3	26.13±19.0	<0.001

^a Overweight and obesity based on BMI values (>25 kg/m²).

^b Student's *t*-test except for age, waist circumference, and BMI, which were analyzed using the Mann-Whitney *U* test. Gender was analyzed with the Fisher exact test.

Results are expressed as mean±SD, with the exception of gender, which was expressed in frequencies.

triceps, subscapular and supra-iliac skin folds, as well as in serum leptin levels.

The allelic frequencies of the Q223R polymorphism in both study groups (non-obese and overweight/obese) were in Hardy-Weinberg equilibrium (Table 2).

No statistical association between genotypes and overweight/obesity was observed. Table 3 shows that, in non-obese subjects, a total of 33 (32%) subjects were homozygous for the wild allele (Q/Q), 50 (49%) were heterozygous (Q/R) and 20 (19%) were homozygous for the Q223R (R/R) polymorphism. In the overweight/obese group, a total of 20 (36%) subjects showed Q/Q, 29 (52%) Q/R and 7 (12%) R/R.

There was no significant p value in any of the cases where the homozygote for the wild-type allele (Q/Q), the heterozygotes (Q/R) or the homozygotes for the polymorphism (R/R) were

evaluated separately. Likewise, the relative risk obtained for the Q/Q genotype was 1.18 (IC 0.53-2.34), for the Q/R genotype was 1.14 (IC 0.59-2.18) and for the R/R genotype was 0.59 (IC 0.23-1.50).

Randomly selected subjects showed similar genotype frequencies as the original sample that were not significantly different between overweight/obese and non-obese groups (Q/Q nonobese: 32% randomized, 32% original; Q/Q obese: 37% randomized, 35% original; Q/R nonobese: 49% randomized, 48% original; Q/R obese: 54% randomized, 51% original; R/R nonobese: 18% randomized, 19% original; R/R obese: 8% randomized, 12% original; $p > 0.222$).

In the analysis of the genotype characteristics of the Q223R polymorphism of each study group (non-obese, overweight/obese, table 4), two subgroups were separated based on the

Table 2 Frequency of alleles of the *LEPR* Q223R gene polymorphism

Allele	<i>LEPR</i> Q223R		p^a	RR (IC 95%)
	Non-obese	Overweight/obese		
Q	0.56	0.62	0.633	1.25 (0.78-1.99)
R	0.44	0.38		

^a Evaluation with Fisher exact test for Hardy-Weinberg equilibrium.

Table 3 Genotype frequency of the *LEPR* Q223R gene polymorphism

Genotype	<i>LEPR</i> Q223R		p^a	RR (IC 95%)
	Non-obese	Overweight/obese		
Q/Q	33	20	0.320	1.18 (0.53-2.34)
Q/R	50	29	0.350	1.14 (0.59-2.18)
R/R	20	7	0.139	0.59 (0.23-1.50)

^a Evaluated with the Fisher exact test.

respective genotypes: in the first group, the homozygous for the wild-type allele (Q/Q); and in the second group, the heterozygotes (Q/R) and homozygous for the Q223R polymorphism (R/R) combined. No significant association was observed in either group, in any of the parameters

evaluated (gender, age, weight, height, BMI, systolic and diastolic blood pressure, percentage of body fat, CHF; circumferences in the waist, hip, scapula and arm, biceps, triceps, subscapularis; suprailiac skinfolds, and serum leptin levels) between genotype groups.

Table 4 Characteristics of non-obese and overweight/obese individuals, based on the genotype of the *LEPR* gene, with the Q223R polymorphism

	Non-obese			Overweight/obese		
	Q/Q (n=33)	Q/R and R/R (n=70)	<i>p</i> ^a	Q/Q (n=20)	Q/R and R/R (n=36)	<i>p</i> ^a
Gender (male/female)	(14/19)	(20/50)	0.163	(10/10)	(21/15)	0.548
Age (years)	20.94±1.9	20.97±2.1	0.940	21.40±.8	20.81±2.0	0.266
Weight (kg)	58.52±9.3	58.45±9.2	0.972	81.59±14.1	80.75±9.7	0.795
Height (m)	1.65±0.1	1.65±0.1	0.927	1.70±0.1	1.68±0.1	0.635
BMI (kg/m ²)	21.44±2.1	21.40±2.3	0.929	28.23±3.6	28.46±2.8	0.789
Systolic pressure (mmHg)	115.48±10.5	115.36±11.4	0.959	122.00±13.8	124.00±12.4	0.587
Diastolic pressure (mmHg)	79.52±7.5	78.18±6.2	0.355	84.05±10.0	83.42±8.5	0.805
Body fat (%)	23.73±6.7	24.41±6.9	0.639	30.81±7.2	32.02±8.0	0.576
ICC	0.79±0.1	0.80±0.1	0.776	0.87±0.1	0.85±0.1	0.235
Circumferences (cm)						
Waist	74.59±7.5	74.94±6.6	0.809	92.08±11.00	90.79±6.9	0.640
Hip	94.26±6.5	94.86±8.8	0.729	105.36±7.3	106.75±4.9	0.400
Scapula	86.71±7.0	86.13±8.9	0.742	100.50±12.5	100.25±6.8	0.923
Arm	27.20±2.2	26.30±3.7	0.129	32.48±3.5	32.42±3.7	0.950

Skin Folds (mm)						
Biceps	5.52±3.5	5.77±3.8	0.746	6.80±4.7	6.92±4.3	0.926
Triceps	10.79±7.3	12.24±7.0	0.335	13.65±6.3	14.81±8.5	0.596
Subscapular	12.82±3.9	14.30±4.3	0.096	23.00±5.9	21.00±5.6	0.213
Suprailiac	14.94±6.1	16.00±6.2	0.414	25.35±7.2	24.33±6.4	0.588
Leptin (ng/mL)	13.73±14.94	16.00±11.94	0.408	24.96±18.2	26.79±19.7	0.734

^a Student's *t*-test with the exception of gender, which was analyzed with the Fisher exact test.

Results are expressed as mean±SD, with the exception of gender, which was expressed in frequencies.

DISCUSSION

Regarding the weight status of the individuals studied, it was observed that 35% of the subjects have some degree of overweight/obesity; taking into account that their age was 18 to 30 years, with an average of 20.9 in the non-obese group, and 21.0 in the overweight/obese group, the percentage of subjects with overweight/obesity in our study population is consistent with national and international values.

Leptin levels were significantly higher in overweight/obese subjects compared to non-obese subjects. It has been suggested that in pro-inflammatory states these values may be altered (19). Research has shown that the increase in leptin causes obesity in laboratory animals, due to the effect of the hormone in the inhibition of appetite. On the other hand, it has been described, that in obese subjects, leptin rises in a parallel way to BMI. Since there is a greater amount of adipose tissue, an increase in body fat will consequently increase the serum leptin concentration (10).

Allelic frequencies of the Q and R alleles of the *LEPR* gene in the individuals studied were as follows: in non-obese subjects Q = 0.56, R = 0.44; and those with overweight/obesity Q=0.62, R=0.38; which are consistent with other studies carried out in Mexican population (17).

No significant association was found between the Q223R polymorphism of the leptin receptor with overweight/obesity in our study population. The results from the randomized subset of subjects suggests that the absence of a random selection in the original sample does not bias our results. However, the absence of significant association in the present study does not rule out that it could be found in another sample.

Around the world, the Q223R polymorphism has been studied in different populations showing contrasting results (11). In India, Tabassum et al. reported an association of this polymorphism with overweight/obesity in children (20). In other study, carried out by Boumaiza et al., a significant association was found between this polymorphism, BMI and other variables in obese people (21). Another study was carried out in Indonesia, where the *LEPR* K109R and Q223R gene polymorphisms were examined, BMI and waist circumference were analyzed and it was found that, the K109R and Q223R polymorphisms of the *LEPR* gene are associated to obesity (22).

In Japan, Furusawa et al. also reported an association of this polymorphism with BMI and obesity (23). The relationship between obesity and the Q223R polymorphism was sought in a Brazilian population, and it was found that the

polymorphism has statistically different frequencies in the obese compared to normal individuals in the dominant and codominant models, but not in the recessive model. It showed a significant relationship between the *LEPR* Q223R polymorphism with obesity and weight gain in the Brazilian population (24).

In the Mexican population, the presence of the polymorphism was associated with less accumulation of body fat in obese subjects (25). An investigation was conducted in obese children where the association between obesity and leptin receptor polymorphisms K109R, Q223R and K656N was evaluated, arguing the changes that may occur based on an alteration in leptin metabolism. In this study, no specific association was found with obesity and these polymorphisms (17). Other studies have found no association in Mexican populations (9,26) or in other different populations (27-34).

It is noteworthy that a large majority of studies carried out in Asia show a significant association between the Q223R polymorphism and overweight/obesity, while in studies carried out in the Caucasian and Latino population, there is a trend towards no association between these variables. Studies comparing the genotype of the Asian, Caucasian and Latino population could be carried out in the future.

It is possible that the lack of association is due to the sex or age of the participants, since only young adults were included in this study. Also, in the current study, the gender distribution was different between the groups: in non-obese patients, 67% were female, while of the overweight/obese group 44.64% were female. However, a randomized subset of subjects did confirm that there was no significant association with the Q223R polymorphism. Hormonal factors can alter the metabolism of leptin and other proteins involved in the pathophysiology of obesity.

CONCLUSIONS

In this study, 35% of the participants (18-30 years old) showed some degree of overweight/obesity. The allelic frequencies in the studied subjects were: non-obese, Q=0.56, R=0.44; overweight/obese, Q=0.62, R=0.38. No significant association was found between overweight/obesity and the presence of the *LEPR* Q223R polymorphism. Leptin levels were significantly elevated in overweight/obese subjects compared to non-obese subjects. This study shows that leptin levels are associated with overweight/obesity in Mexican young adults but this is not related to the presence of the Q223R polymorphism in the *LEPR* gene, so the underlying mechanisms for a possible disturbance in leptin signaling in obese Mexican young adults await further studies.



Ethical concerns

This study was performed according to Mexican regulations and the Declaration of Helsinki. In order to avoid contamination of the environment with biological material, these were handled as stipulated in NOM-087-ECOL-SSA1-2002.

The study subjects were recruited after a detailed explanation of the risks and benefits of their participation, as well as their signing an informed consent letter in which the confidentiality and handling of their data was also assured, based on the provisions of the Declaration of Helsinki. The research was approved by the Institutional Committee of Ethics and Bioethics of the UACJ.



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Extra-analytical clinical laboratory errors in Africa: a systematic review and meta-analysis

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ABSTRACT

Background

Clinical laboratory testing is a highly complex process involving a different procedure. Laboratory errors may occur at any stage of the test process, but most errors occur during extra-analytical phases. The magnitude of clinical laboratory errors, in particular extra-analytical errors, was inconsistent in different studies.

Methods

A systematic review and meta-analysis were conducted based on the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines. The extracted data were entered into a Microsoft Excel spreadsheet and transferred to STATA version 11 for the analysis. Random effect model was used to estimate pooled prevalence of extra-clinical laboratory errors and I^2 statistic was used to assess heterogeneity between the studies. Funnel plot analysis and Egger weighted regression test were performed to detect the publication bias. Egger weighted regression test

with P-value <0.05 was considered to be a statistically significant publication bias.

Results

A total of 1,381 studies were searched, 19 were included in this systematic review and meta-analysis. A total of 621,507 pre-analytical and 51,859 post-analytical outcomes of quality indicators were reported. A total of 145,515 samples were assessed for rejection and 62,513 laboratory requests were evaluated for incompleteness. The pooled prevalence of pre-analytical and post-analytical errors in Africa was 17.5% (95% CI: 11.55, 23.45) and 10.99% (95% CI: 5.30, 16.67) respectively. The pooled prevalence of specimen rejection and laboratory request forms incompleteness in Africa was 2.0% (95% CI: 0.86, 3.14) and 7.55% (95% CI: 2.30, 12.80) respectively.

Conclusion

The study found high prevalence of pre- and of post-analytical clinical laboratory errors in Africa. In addition, the study showed that the standard completion of the laboratory request forms was poor and there were significant numbers of specimen rejections. Therefore, clinical laboratories should ensure compliance with standard operating procedures, the laboratory information system, the cooperation of the entire staff and the targeted training of sample collectors.



INTRODUCTION

Clinical Laboratory services play an important role in the diagnosis and monitoring of diseases in the health-care system (1) and approximately 80% of all diagnostics are performed on the basis of laboratory tests (2). Clinical laboratory testing is a process that generally consists of pre-analytical, analytical and post-analytical phases.

The pre-analytical phase covers all the processes from the time of request by the physician until specimen processing before analysis, while the analytical phases includes the analysis of the patient sample and the post-analytical phases refers to the activities performed after actual analysis of the sample (3).

Laboratory errors may occur at any phase of the testing process, but majority of the errors occur in the pre- and post-analytical phases, which account for 93% of the total errors. The pre-analytical phase is a major source of laboratory errors that have a significant impact on the outcome of other phases. It is estimated that about 70% of the errors observed in the laboratory are due to the pre-analytical errors (4). Common pre-analytical errors are: ordering inappropriate tests, incomplete requisition forms, invalid handwriting on forms, failure to identify the patient, incorrect sampling time, hemolysis of samples, lipemic samples, inappropriate sample transport and storage (3).

Following pre-analytical errors, a high error rate (18.5–47% of total errors) occurred in the post-analytical phase (5). Common post-analytical errors include failure to report critical values, prolonged turnaround time (TAT), transcription error and incorrect interpretation of laboratory results (6). Its nature of complexity makes laboratory errors heterogeneous and difficult to measure. The majority of clinical laboratory error studies used different study designs; study periods, sample sizes, quality indicators (QIs) and reporting techniques (5). Therefore, it is important to ensure that care has to be taken in each and every step of the laboratory service (7, 8).

Quality indicators are objective measures of quality laboratory service that can evaluate all phases of the total testing process (TTP) and can be applied over time across all sections of the laboratory. Therefore, it is important to ensure

systematic and consistent data collection/analysis by using comprehensive set of indicators addressing all phases of the TTP (9).

In most African countries, clinical laboratory services are still below standard and the participation of laboratories in accreditation is below the expected level. The aim of this study was to estimate the pooled magnitude of extra-analytical clinical laboratory errors. The implementation of objective measures and quality policies will ensure that clinical laboratories provide efficient and customer-based services that provide staff and customer satisfaction.

EXTRA ANALYTICAL ERRORS IN AFRICAN CLINICAL LABORATORIES

Most of the studies conducted on clinical laboratory errors used different data collection approaches, different time periods for data collection, and included different laboratory sections and different reporting methods (10). In the current study, the articles included showed variations in quality indicators and the magnitude of errors was inconsistent.

According to the current review in Africa, the prevalence of pre-analytical errors ranged from 2.7 % (11) to 43.7% (12) based on the total outcomes of the QIs. The significant variation between the findings of the studies may be due to the difference in the study design and sample size.

In addition, clinical laboratory specimens which are sent to the laboratory often lead to rejection due to different reasons, such as: hemolysis, clotted, insufficient volume and lipemic specimens. It is estimated that the majority of laboratory specimens are rejected due to hemolysis (13). In the current study, the magnitude of specimen rejection ranged from 0.28 % (14) to 4.6% (15). The variation between the findings may be due to the difference in the study design and sample size used, in particular the retrospective studies may be affected by the quality

of records, and the occurrence of rejection may not be properly documented.

In the pre-analytical phase, the first step is to fill in the laboratory request form. The information required to be provided in the request forms includes: clinician and patient details, diagnosis, medications and the requested tests. Failure to fill all the required information may confuse the laboratory and incorrectly interpret the test results, and even the life-threatening emergency tests may be omitted from the analysis until such errors resolved (13). The current study showed a high degree of incompleteness in laboratory test request form (LRF) and the study findings varied from 4.8% (15) to 40.1% (16). The variation may be due to the difference in sample size and the number of pre-analytical QIs in the LRF. The findings of studies conducted in Ethiopia by Ali M and Addis Z, et al showed a consistent level of LRF incompleteness that may be due to the similar QIs, duration of data collection, study design and study area (16, 17).

Post-analytical phase is the final phase of the total testing process, which includes the evaluation of laboratory test results; the timely reporting of test results, the storage and disposal of samples, the archiving of laboratory documents and records (18). In the current study, post-analytical errors showed a high variation of results ranging from 1.3% (19) to 33.3% (20). This variation may be due to the difference in study design, sample size and study area. The implementation of laboratory information system in clinical laboratories could improve post analytical errors and QIs have also been used to quantify the performance of laboratories (9, 10).

METHODS

Data source, protocol and registration

The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (with the PROSPERO CRD-

42020170040). This systematic review and meta-analysis on extra-analytical clinical laboratory errors was performed based on the Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) guideline (21). The findings of different articles were included in this review to determine the pooled prevalence of extra-analytical clinical laboratory errors in Africa.

The studies were found using the PubMed, Scopus, Web of science, Google Scholar and Cochrane Library databases via internet searches. All published literature was searched until December 2019 and searches in the PubMed were conducted under the following keywords and Medical Subject Headings (MeSH) (22): Clinical OR Medical [All Fields] AND Laboratory [All Fields] AND “Pre-analytical errors” OR “Post-analytical errors” [All Fields] “Pre-examination errors” OR “Post-examination errors” [All Fields] OR “Clinical Chemistry” OR “Clinical Biochemistry” [All Fields] AND Africa [All Fields]. In addition, we used the MeSH terms for specimen rejection and incompleteness of laboratory request form as: Clinical OR Medical [All Fields] AND Laboratory [All Fields] AND “specimen rejection” OR “sample rejection” OR “incompleteness of laboratory request form” [All Fields] OR “Clinical Chemistry” OR “Clinical Biochemistry” [All Fields] AND Africa [All Fields].

Study selection and outcomes

Studies conducted on extra analytical clinical laboratory errors in African countries were included in this systematic review and meta-analysis. Duplicate articles were removed, and the remaining articles were screened based on their title and abstract. Finally, full-text articles were evaluated using inclusion and exclusion criteria, and eligible studies were included into the systematic review and meta-analysis.

The prevalence of pre-analytical and post-analytical errors in each study was calculated using

the number of defects/failures as the numerator; and total outcomes QIs (sum of “defects” and “successes”) reported as the denominator. Prevalence of pre-analytical and post-analytical errors were our primary outcome measures. In addition, specimen rejection and incompleteness of LRF were also our study outcome, which are the component variables of the pre-analytical errors.

Eligibility criteria

All articles conducted on clinical laboratory errors in African countries with clear abstracts, objectives, methodologies (cross-sectional or cohort study designs), and published in English language were included in this study. Any published articles conducted on pre-analytical errors, post-analytical errors, laboratory specimen rejection and LRF incompleteness with clear results and two or more QIs were included into this systematic review and meta-analysis.

Review articles and other non-original documents, e.g. reports, commentary, case-report and case-series studies and duplicated articles were excluded from the study. In addition, articles with unknown study designs, published in languages other than English, and studies with biased and inappropriate results were also excluded.

Data extraction and quality assessment

Studies which fulfilled the eligibility criteria were subjected to data extraction by three reviewers through prepared data extraction sheet. The three reviewers worked independently, and the findings were carefully cross-checked. Any difference between the data extractors was resolved by discussion and consensus through verification. Absolute and relative frequencies of extra-analytical clinical laboratory errors were extracted for further analysis.

In all of the selected studies: author, study area, study period, year of publication, study design,

sample size and prevalence of extra-analytical clinical laboratory errors were extracted. That data was entered into Microsoft Excel. The quality of the articles was assessed by the reviewers based on the Joana Brigg's institute critical appraisal checklist for prevalence studies (23).

Statistical analysis

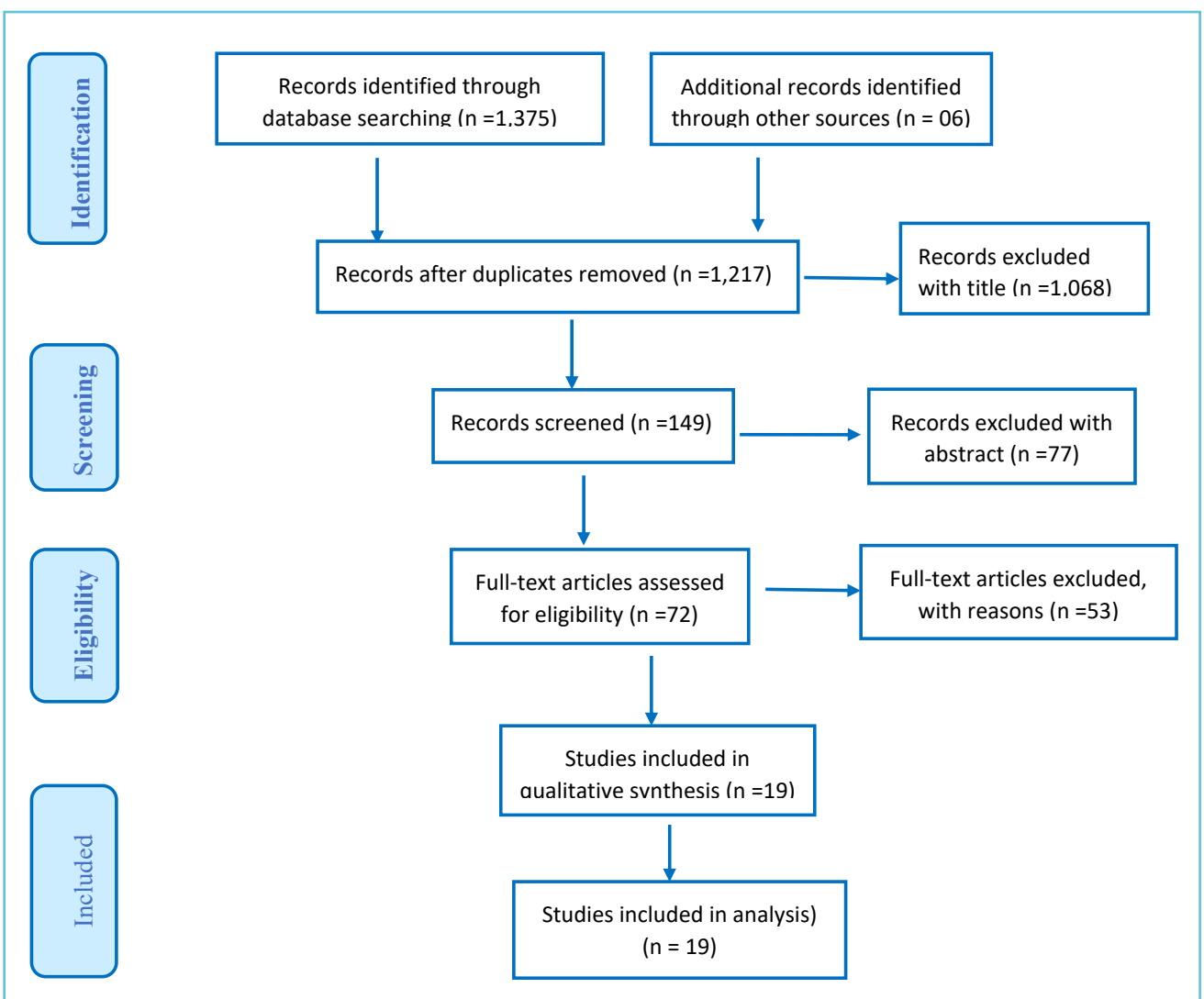
The data entered into the Microsoft excel sheet was exported to the STATA version 11 statistical software for further analysis. The prevalence of pre-analytical and post-analytical error was

analyzed using random effect model. Subgroup analysis was performed on the prevalence of pre-analytical errors between middle income and low-income African countries.

In addition, the pooled prevalence of specimen rejection and of the incompleteness of LRF were analyzed.

Variability between studies (heterogeneity) was evaluated using I^2 Statistic with values of 25%, 50% and 75% interpreted as low, moderate and high heterogeneity respectively (24). Publication

Figure 1 A flow chart showing the phases of selecting the literatures for the systematic review and meta-analysis



bias between the studies was tested by funnel plots analysis and Egger weighted regression test. The P value <0.05 in the Egger's test was considered as evidence of statistically significant publication bias (25).

RESULTS

Literature search results

The search results were found in 43 African countries and a total of 1,381 articles were retrieved from the databases. Of the total, 164 duplicated searches were removed, and the remaining 1,217 searches were screened by the title of the study. In addition, 77 articles were removed on the basis of abstract review and a full assessment of the paper was carried out in the 72 articles. Furthermore, 53 articles were excluded from the study based on the exclusion criteria. Finally, 19 articles were found to be eligible and included in the analysis (Figure 1).

Study characteristics

Of the 19 studies, 8 were conducted in Ethiopia (12, 16, 17, 19, 26-29), 3 in South Africa (30-32), 3 Nigeria (14, 33, 34), 2 Egypt (15, 20), 2 Kenya (11, 35) and 1 Uganda (36). Of the total studies, 5 studies were conducted on both pre-analytical and post-analytical phases of clinical laboratory errors; however, 3 studies were conducted only on the pre-analytical phase clinical laboratory errors.

In the beginning, 8 studies with 621,507 pre-analytical and 51,859 post-analytical QIs outcomes were included. The overall outcomes of QIs with two possibilities: "success" and "defect/failure" or "yes" and "no" in the both phases were 673,366. The study periods varied from 1 month to 13 months and the lowest and highest prevalence of pre-analytical errors were 2.7% and 43.7%, respectively. In addition, the lowest and highest post-analytical error rates were 1.3% and 33.3% respectively (Table 1).

In addition, six studies were included and a total of 145,515 samples were assessed for the pooled prevalence of specimen rejection. The lowest prevalence of sample rejection was 0.28%, but the highest reported prevalence of specimen rejection was 4.6% with a study period ranged from 2 weeks to 3 years (Table 2).

Furthermore, 14 studies were included and a total of 62,513 LRFs were evaluated for LRF incompleteness. The overall outcomes of QIs in evaluating the LRF were 547,777. The lowest prevalence of incompleteness in LRF was 4.8% (15), but the highest prevalence of incompleteness in LRF was 40.1% (16). The finding highlights the need to review and update the LRF, improve training and communication between the laboratory and clinical staff, and review the practice for specimen rejection (Table 3).

Prevalence of pre-analytical errors in African clinical laboratories

In random-effect model analysis, the pooled prevalence of pre-analytical clinical laboratory errors in Africa was 17.5% (95% CI: 11.55, 23.45) (Figure 2).

Slightly higher preanalytical error was found in low income 17.65% (95% CI: 6.09, 29.21) than in middle income 17.35% (95% CI: 8.22, 26.49) African countries.

The pooled prevalence of incompleteness in LRF in African clinical laboratories

In random-effect model analysis, the pooled prevalence of LRF incompleteness in Africa was 19.6% (95% CI: 14.17, 25.05) (Figure 3).

The pooled prevalence of specimen rejection in African clinical laboratories

In random-effect model analysis, the pooled prevalence of specimen rejection in Africa was 2.0% (95% CI: 0.86, 3.14) (Figure 4).

Table 1 The descriptions of the included studies conducted on pre-analytical and post-analytical errors in African clinical laboratories

S.N.	Author	Study area	Sample size	Year	Pre-A N (%)	Post-A N (%)	Laboratory section
1	Rizk MM, <i>et al</i>	Alexandria, Egypt	31,944 requests and 50,440 samples (252,200 Pre-A and 27,612 Post-A Qis)	2014	13,067 (5.2%)	4,540 (16.4%)	Clinical Chemistry
2	Sharaki O, <i>et al</i>	Alexandria, Egypt	514 RWS (8,426 Pre-A and 1,461 Post-A Qis outcomes)	2014	3,684 (43.7%)	487 (33.3%)	Clinical Chemistry
3	Addis Z, <i>et al</i>	Gondar, Ethiopia	1,533 RWS (21,462 Pre-A Qis)	2015	6,227 (29%)	N/A	Clinical Chemistry and Hematology
4	Wondimagegn MW, <i>et al</i>	Oromia, Ethiopia	754 RWS (7,540 Pre-A Qis)	2016	751 (10%)	N/A	Hematology and CD4
5	Kimengech KK, <i>et al</i>	Nairobi, Kenya	346 RWS (5,536 Pre-A and 4,844 Post-A Qis)	2017	148 (2.7%)	84 (1.7%)	Clinical Chemistry
6	Ambcahew S, <i>et al</i>	Gondar, Ethiopia	3,259 RWS (948,885 Pre-A and 9,777 Post-A Qis)	2018	3,379 (6.9%)	291 (3%)	Clinical Chemistry
7	Tadesse H, <i>et al</i>	Addis Ababa, Ethiopia	1,633 RWS (17,570 Pre-A and 8,165 Post-A Qis outcomes)	2018	4,337 (24.7%)	104 (1.3%)	Clinical Chemistry
8	Isa HA, <i>et al</i>	Jos, Nigeria	15,287 RWS (259,888 Pre-A Qis)	2018	46,413 (17.9%)	N/A	More than 2 Lab sections

RWS=request with sample, Qis=quality indicators, Pre-A=pre-analytical, Post-A=post-analytical, N/A=not available, S.N.=Serial number.

Table 2 The descriptions of the included studies conducted on sample rejection in African clinical laboratories

S.N.	Author	Study area	Year	Study design	Study period	Sample size	Sample rejection N (%)	Laboratory section
1	Jacobsz LA, <i>et al</i>	Cape Town, South Africa	2011	Retrospective	2 wks.	32,910	481 (1.46%)	Clinical Chemistry and Hematology
2	Rizk MM, <i>et al</i>	Alexandria, Egypt	2014	Comparative cross-sectional	7 mos.	50,440	2,314 (4.6%)	Clinical Chemistry
3	Tesfaw HM, <i>et al</i>	Addis Ababa, Ethiopia	2015	Cross-sectional	16 mos.	8,063	116 (1.44%)	More than 2 Lab sections
4	Jegede F, <i>et al</i>	Kano, Nigeria	2015	Retrospective	3 yrs.	7,920	22 (0.28%)	More than 2 Lab sections
5	Shiferaw MB, <i>et al</i>	Bahirdar, Ethiopia	2018	Retrospective	22 days	42,923	221 (0.5%)	More than 2 Lab sections
6	Ambcahew S, <i>et al</i>	Gondar, Ethiopia	2018	Cross-sectional	2 mos.	3,259	123 (3.8%)	Clinical Chemistry

Qis=quality indicators, LTR=Laboratory test request, mos.=months, wks.=Weeks, Yrs.=years, S.N.=serial number.

Table 3 The descriptions of the included studies conducted on LRF incompleteness in African clinical laboratories

S.N.	Author	Study area	Year	Sample size	Incomplete LRF	Laboratory section
1	Nutt L, <i>et al</i>	Tygerberg, South Africa	2008	2,550 LTR (38,250 total Qis)	5,818 (15.2%)	Pathology
2	Zemlin AE, <i>et al</i>	Cape Town, South Africa	2009	482 LTR (3,856 total Qis)	873 (22.6%)	Pathology
3	Atewu A, <i>et al</i>	Addis Ababa, Ethiopia	2014	960 LTR (7680 total Qis)	1,434 (18.7%)	More than 2 Lab sections

4	Rizk MM, <i>et al</i>	Alexandria, Egypt	2014	31, 944 LTR (223,608 total Qis)	10,753 (4.8%)	Clinical Chemistry
5	Sharaki O, <i>et al</i>	Alexandria, Egypt	2014	514 LTR (3,598 total Qis)	366 (10.2%)	Clinical Chemistry
6	Addis Z, <i>et al</i>	Gondar, Ethiopia	2015	1,533LTR (15,330 total Qis)	6,153 (40.1%)	Chemistry and Hematology
7	Ali M	Hawassa, Ethiopia	2015	1,900 LTR, (22,800 total Qis)	8,984 (39.4%)	More than 2 Lab sections
8	Wondimagegn MW, <i>et al</i>	Oromiya, Ethiopia	2016	754 LTR (3,770 total Qis)	579 (15.4%)	Hematology and Immunology
9	Jegede F, <i>et al</i>	Kano, Nigeria	2016	1,085 LTR (19,530 total Qis)	2,797 (14.3%)	Blood transfusion
10	Ambcahew S, <i>et al</i>	Gondar, Ethiopia	2018	3,259 LTR (32,590 total Qis)	3,256 (10%)	Clinical Chemistry
11	Isa HA, <i>et al</i>	Jos, Nigeria	2018	15,287 LTR (152,870 total Qis)	28,826 (19%)	More than 2 sections
12	Tadesse H, <i>et al</i>	Addis Ababa, Ethiopia	2018	1,633 LTR (16,330 total Qis)	3,944 (24.2%)	Clinical Chemistry
13	Namwase B	Makerere, Uganda	2018	323 LTR (3230 total Qis)	579 (17.9%)	Pathology
14	Kipkulei JC, <i>et al</i>	Eldoret, Kenya	2019	289 LTR (4335 total Qis)	984 (22.7%)	Hematology

Qis=quality indicators, LTR=laboratory test request, LRF=laboratory test request form, Clinical.C= Clinical Chemistry.

The pooled prevalence of post-analytical errors in African clinical laboratories

In random-effect model analysis, the pooled prevalence of post-analytical errors in Africa was 10.99% (95% CI: 5.30, 16.67) (Figure 5).

Heterogeneity, publication bias and sensitivity analysis

The I^2 statistics showed a high level of heterogeneity (99.7%, 99.9%, 100% and 100%) between the included studies for all outcome variables. In order to minimize heterogeneity, the pooled

prevalence of pre-analytical, post-analytical errors, specimen rejection and LRF incompleteness was estimated using the random-effects model and sub-group analysis conducted based on economic status of the study country.

In addition, the overall result of the Egger's test indicated that no publication bias was found on the pooled estimate of pre-analytical errors ($P=0.377$), post-analytical errors ($P=0.352$) and specimen rejection ($P=0.229$). However, the publication bias was found in the pooled estimate of LRF incompleteness ($P=0.007$).

Figure 2 Forest plot on the prevalence of pre-analytical errors from random effect model analysis

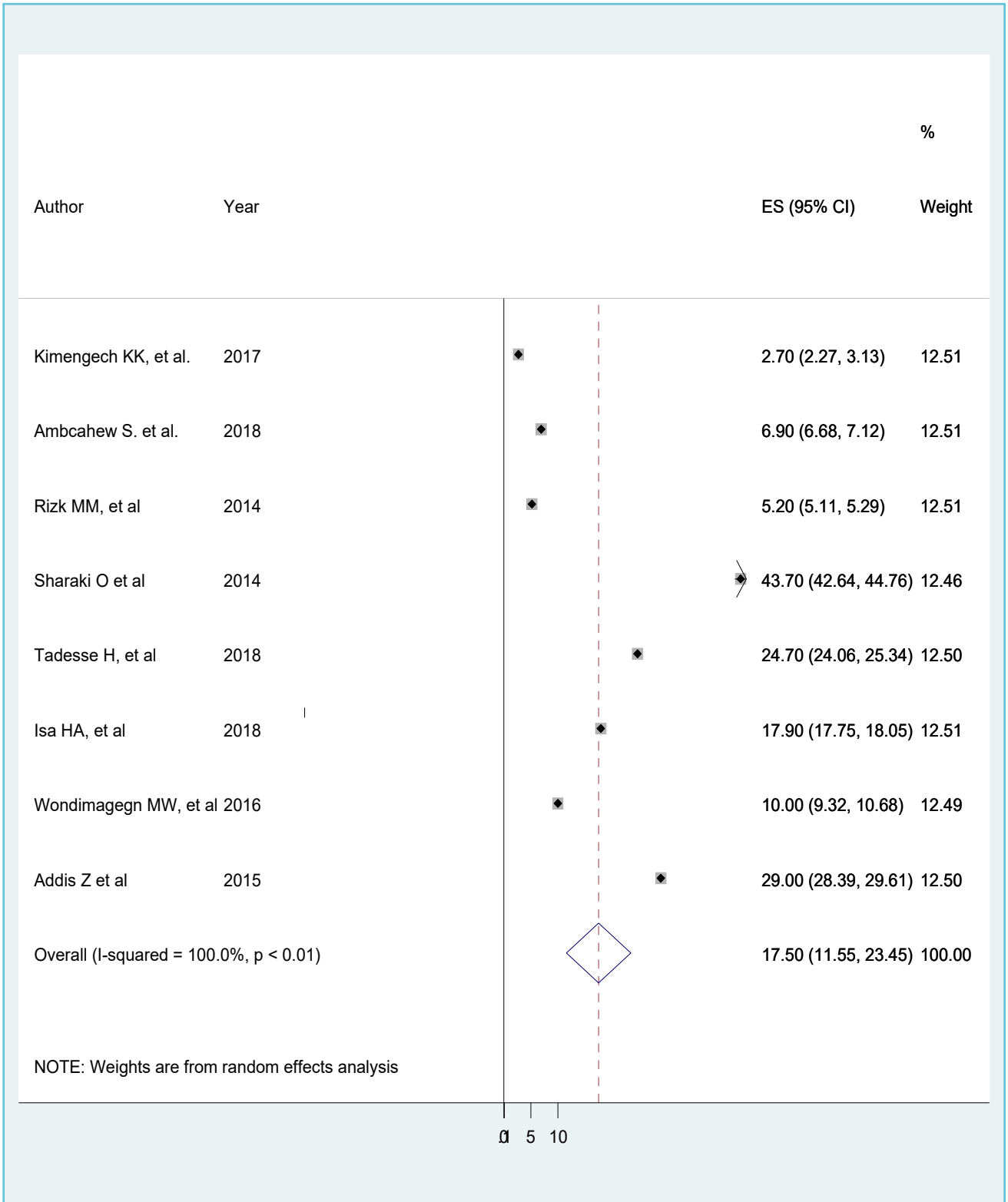


Figure 3 Forest plot on the prevalence of incompleteness in LRF from random effect model analysis

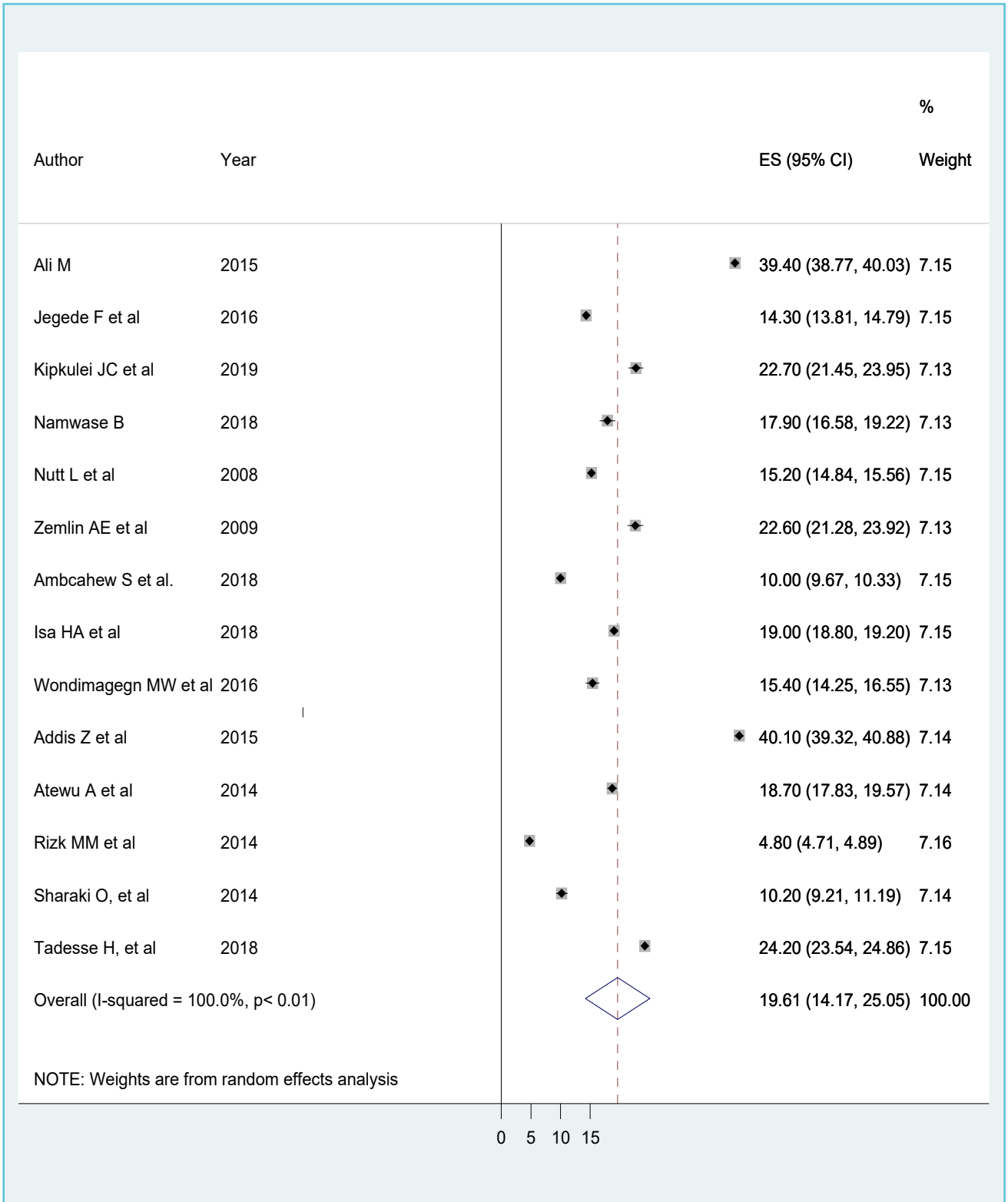
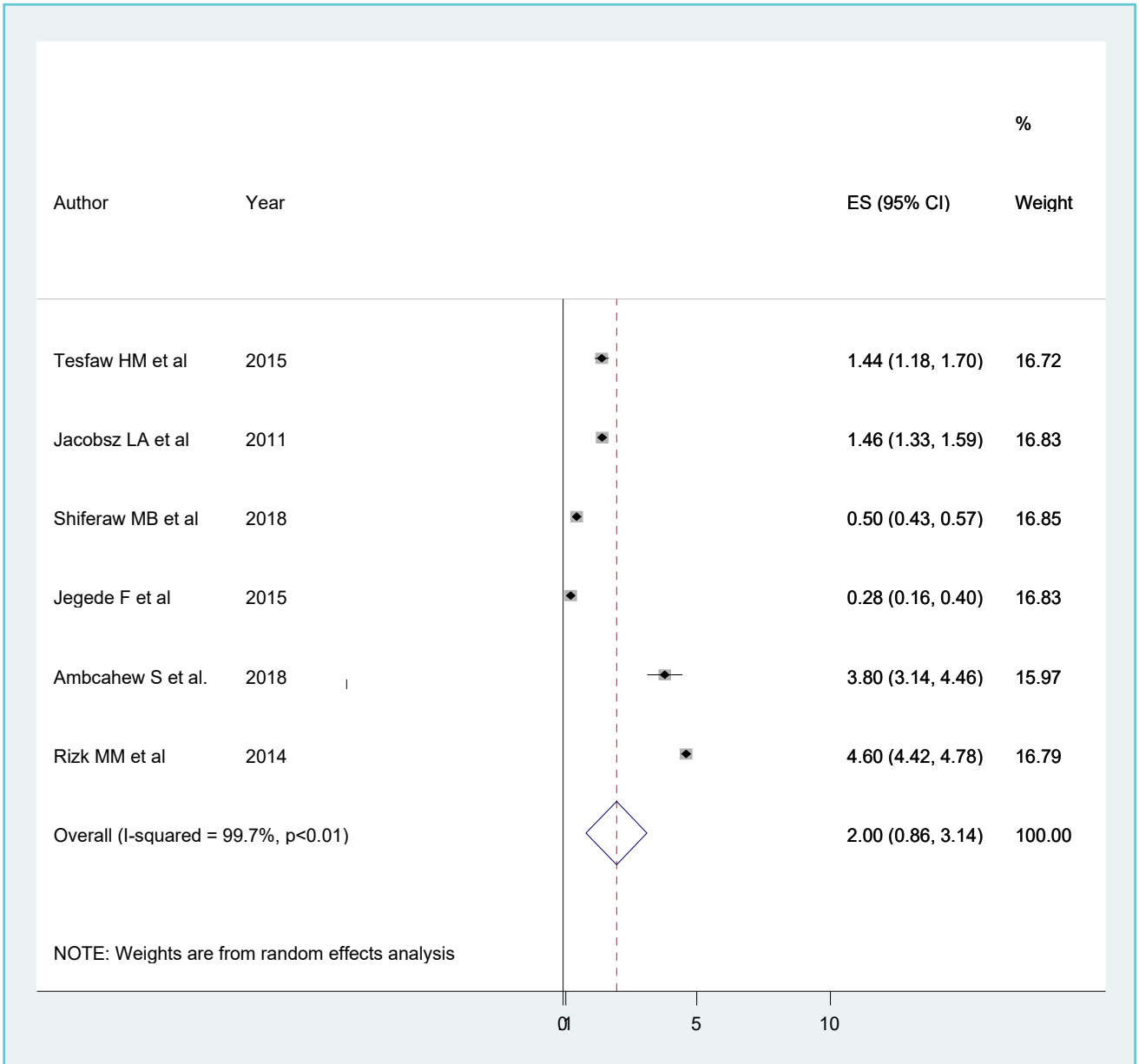


Figure 4 Forest plot on the prevalence of specimen rejection from random effect model analysis



As a result, trim and fill analysis was used to overcome the impact of small-study effect, 8 additional studies were filled to the model, and a pooled estimate of LRF incompleteness in the random-effect model was found to be 7.55% (95%CI: 2.30, 12.80).

Sensitivity analysis was conducted on the prevalence of pre-analytical errors, post-analytical

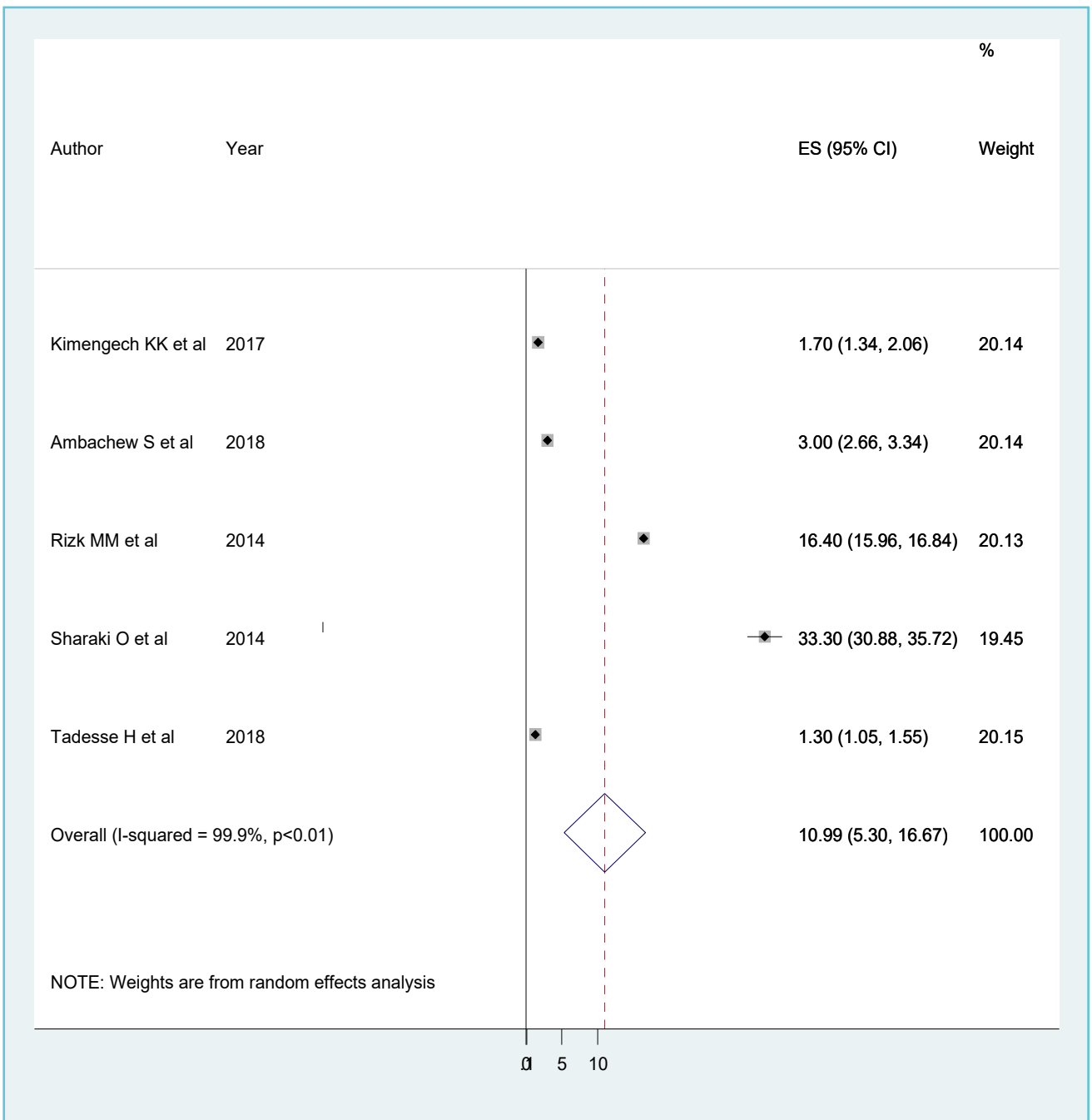
errors, LRF incompleteness and specimen rejection in Africa. A random effect model was also used to analyze the sensitivity tests to evaluate the effect of each study on the pooled estimates by omitting each study stepwise. And the analysis showed that the omitted studies have no significant effect on the pooled prevalence of pre-analytical errors.

DISCUSSION

Laboratory errors may occur at any phase of the TTP and can directly lead to increased health-care costs, reduced patient satisfaction, delayed diagnosis, misdiagnosis and serious risk to the

health of the patient (37). Therefore, this study was conducted to determine the pooled prevalence of pre- and post-analytical errors, specimen rejection and LRF incompleteness in African countries.

Figure 5 Forest plot on the prevalence of post-analytical errors from random effect model analysis



In the current study, the prevalence of pre-analytical errors ranged from 2.7% to 43.7%. The significant difference between these findings may be attributed to the variation in the number of QIs, study design, sample size and the performance of laboratories. Studies conducted by Sharaki O, et al (20) and Addis Z, et al (16) revealed high prevalence of pre-analytical errors which may be due to the similarities in the study design, QIs data collection procedure and operational definitions. The lowest prevalence of pre-analytical errors may be due to different methods of data collection, the presence of dedicated staffs and the participation of the laboratory in the accreditation process.

In addition, the prevalence of specimen rejection (0.28% to 4.6%), incompleteness of LRF (4.8% to 40.1%) and post-analytical errors (1.3% to 33.3%) also revealed a wide inconsistency between the studies in Africa. This variation may be due to the difference in the sample size, the awareness of clinicians, the operational definition of laboratory errors, the quality requirements of the laboratory and the dedication of health professionals.

In random effect models, the pooled estimate of pre-analytical errors and post-analytical errors in Africa was 17.5% (95% CI: 11.55, 23.45) and 10.99% (95% CI: 5.30, 16.67) respectively. The findings of this study were much higher than a study conducted in Iran (pre-analytical 4.1% and post-analytical 0.74%) (38) and Pakistan (pre-analytical error 0.02% and post-analytical error 0.01%) (39). This may be due to variations in the study area, sample size, laboratory information system, the status of laboratory accreditation and socio-economic differences between countries. The studies included in this systematic review and meta-analysis were conducted in Africa where limited resources and substandard facilities available.

In random effect models, the pooled prevalence of sample rejection and LRF incompleteness in Africa was found to be 2.0% (95% CI: 0.86, 3.14) and 19.6% (95% CI: 14.17, 25.05), respectively. The pooled prevalence of sample rejection was comparable to that of a study conducted in Saudi Arabia (2.07%) (40). In addition, the current pooled estimate of sample rejection was higher than studies conducted in India (0.15%) (41) and Turkey (0.65%) (42). However, it was lower than studies in Greece (4.1%) (43) and India (3.45%) (3).

In addition, the pooled prevalence of LRF incompleteness was lower than the study conducted in India (27.82%) (44). This difference may be due to variations in sample size, duration of the study period and performance of participating laboratories.

CONCLUSION

The present study showed a high pooled estimate of pre-analytical and post-analytical errors. In addition, the study found that the standard completion of LRF was poor and there were significant numbers of specimen rejections, which concerned the importance of quality indicators to determine errors in the overall TTP. LRF Incompleteness could lead to misdiagnosis and mismanagement of patients and in appropriate specimen rejections had a significant effect on patient care and could thus affect customer satisfaction.

Therefore, adherence to standard operating procedures, establishment of laboratory information system and targeted training for sample collectors is needed. Moreover, staff co-operation and computerized test requesting procedure for specimen collection are of vital importance to make progress in the pre-analytical and post-analytical testing process.



Strength and limitations

The current study was the first study to use a quantitative approach to pool the prevalence of extra analytical clinical laboratory errors in African countries. The limitation of this study was that only articles published in English language were included in this study, and the number of QIs in each study was not uniform, which could be a cause for high variability in the study findings.

Abbreviations

(ISO) International Organization for Standardization

(LRF) Laboratory Test Request Forms

(MA) Meta-Analysis

(MeSH) Medical Subject Headings

(PRISMA) Preferred Reporting Items for Systematic review and Meta- analysis

(Qis) Quality Indicators

(SR) Systematic Review

(TAT) Turnaround Time

(TTP) Total Testing Process.

Authors' contributions

DA and MT conceptualized this study and designed the study protocol; DA, AW and MT conducted data search, quality assesment, data extraction, statistical analyses and statistical interpretation. All authors write and approved the manuscript.

Availability of data and materials

Most of the main data generated or analyzed during this study are included in this article. However, additional files that support the findings of this study are also available from the corresponding author upon request.

Consent to publication

All participants provided written informed consent to publish this study.

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Vitamin D deficiency has no impact on PSA reference ranges in a general university hospital – a retrospective analysis

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ABSTRACT

Background

Vitamin D deficiency has been linked to a higher risk of prostate cancer. We tested the hypothesis that vitamin D levels would have an impact on prostate specific antigen (PSA) levels.

Methods

From our laboratory database we selected 5136 male patients with simultaneously determined vitamin D and PSA levels. Subgroups of several age cohorts with different vitamin D levels were created and PSA 95 percentile values were assessed. The independent effect of vitamin D levels and age on PSA levels was determined with logistic regression.

Results

PSA levels increased with age, while no difference was identified in PSA levels in different vitamin D subgroups.

Conclusion

Vitamin D levels do not have an effect on PSA. Hence, there is no need to adjust PSA reference ranges and threshold values to vitamin D levels during the process of decision making



INTRODUCTION

Epidemiological observations and, quite recently, an interventional trial indicated the possible role of vitamin D3 and its supplementation in the prevention of prostate cancer. [1] The results of the recent VITAL trial support that vitamin D3 supplementation in a dose of 2000 IU/day decrease the mortality from prostate cancer by up to 12 percent. [2] Large doses of daily vitamin D3 supplementation and appropriate levels of the active vitamin D inhibit the transition of early, low-risk prostate cancer to more aggressive forms.[3,4] The benefits of vitamin D in the therapy of advanced prostate cancer, however, are less conclusive. [5]

The mechanism of protection of prostate health provided by vitamin D is extensively studied. Different effects of vitamin D on prostate cells were identified. Vitamin D receptor as a nuclear receptor has a significant impact on gene regulation implicated in prostate cell differentiation and metabolism and is generally acknowledged as an antitumor agent. [6, 7] More recent data indicate that non-genomic effects, particularly those affecting mitochondrial respiration may also play a role. [8] Another study revealed that appropriate levels the active vitamin D metabolite inhibits the intracrine conversion of dehydroepiandrosterone to prostate growth-stimulating androgens such as testosterone and dihydrotestosterone. [9]

Prostate specific antigen (PSA) has been widely used as a screening test for detection of prostate cancer. Albeit the ordering of PSA is not

recommended routinely, many clinicians still adhere to request this test even for symptom-free men. In general, those patients exhibiting PSA values over a pre-specified threshold are referred to urologists often with suspected prostate cancer and considered as candidates for more invasive interventions.

This approach is inherent with the risk of unnecessary investigations of patients with false positive PSA values. False positive rate depends largely on threshold values used. Commonly used threshold value is 4 µg/L, but it is not routinely adjusted to extra-prostate factors having an impact on PSA. Some of these extra-prostate factors as age are widely known, while others such as thyroid function, daily or seasonal variations are identified more recently. [10,11]

As PSA is widely considered as a surrogate marker for prostate pathology, we hypothesized that low vitamin D levels is associated with higher PSA at population level. In our analysis we tested whether low vitamin D levels may have an independent impact on PSA levels and reference ranges in different age cohorts.

METHODS

The Department of Laboratory Medicine offers PSA and vitamin D determination to 44 University Hospitals of Semmelweis University, Budapest, Hungary. From Laboratory Informatics System we retrieved records generated since January 1, 2011 that included the following fields: anonymized patient identification number; gender; age; date of measurement; the name of the measured parameter; test result; reference range and unit; and instrument used for testing.

Out of the collected ≈200 million records we selected those that fulfilled the following criteria: (1) male gender and (2) we determined vitamin D levels or PSA levels. Vitamin D and PSA levels were measured by CE IVD qualified, commercially available immunoassays. In order to

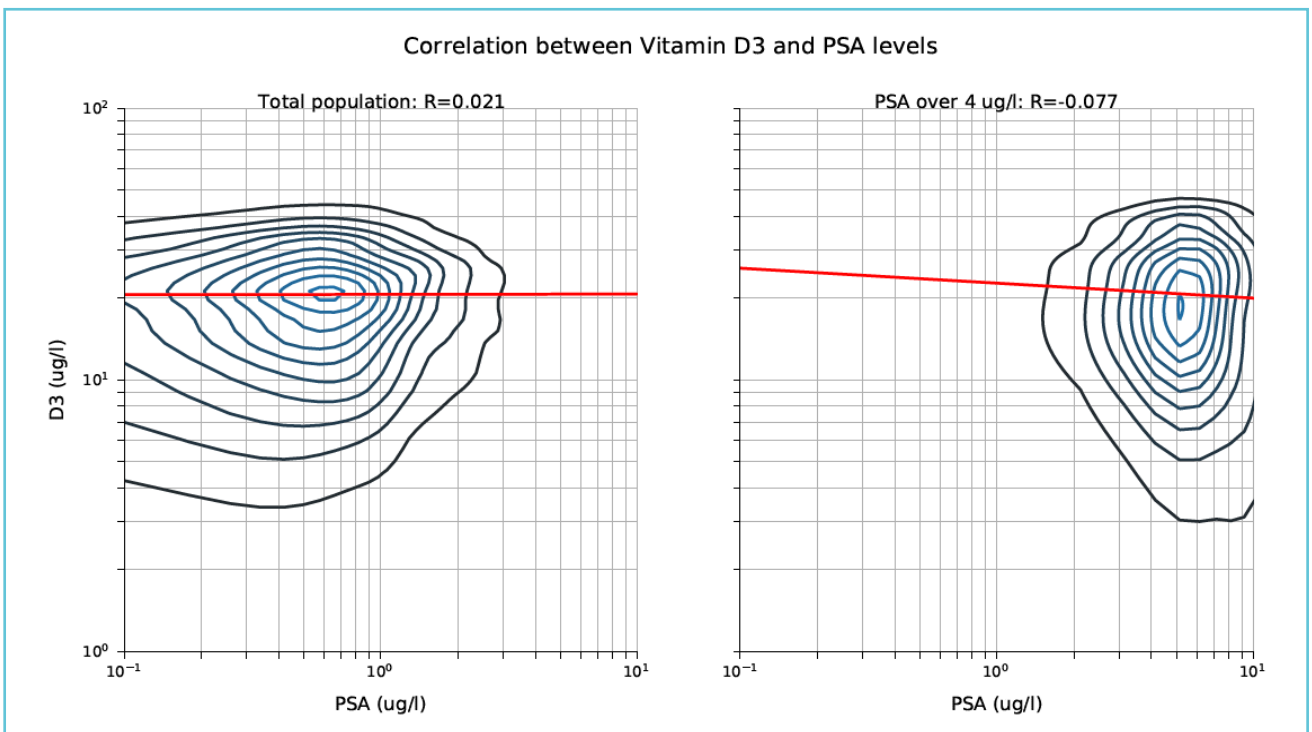
exclude patients with severe prostate pathology we arbitrarily excluded records with PSA levels above 20 µg/L from further analysis. Then we selected those patients having both PSA AND vitamin-D level measurements within 30 days. From the created database we selected those data pairs for an individual patient [n = 5136] that were measured for the first time in our database.

We generated subgroups and cohorts according to vitamin D levels and age and calculated the 95

percentile values as the upper level of reference range. We also assessed the rate of subjects with elevated PSA (>4 µg/L).

In addition, the independent effect of vitamin D levels and age on PSA levels was determined with logistic regression analysis of the logarithmic data. (Statistical analysis was performed with R software package.) The data analysis was approved by an Independent Ethical Committee of the University. We also investigated the direct correlation between vitamin D3 and PSA levels.

Figure 1 Bivariate kernel density estimates of PSA and vitamin D3 values measured in our laboratory between 10/2007 and 06/2018



Closed lines represent coordinates with equal density of data in the two-dimensional space, smoothed by a Gaussian function.

Pearson's correlation coefficient (R) is calculated after logarithmic transformation of both PSA and vitamin D3 levels in order to achieve a close-to-normal distribution in individual dimensions. Linear regression line for the logarithm of data points is shown in red.

Two-dimensional distribution and correlation was calculated for both the total patient population (left side) and only for patients with PSA above 4 µg/l (right side).

Due to the characteristics of the PSA test, any value measured to be below 0.1 µg/l is reported here as 0.1 µg/l. This bias is, however, corrected by kernel density estimation.

Pearson's correlation coefficients found here were close to 0 suggesting the absence of linear relationship between PSA and vitamin D3 levels.

Table 1 Reference ranges of PSA levels in an ageing population with different vitamin D serum levels

Distribution of patients (total number: 5136) according to age and vitamin D levels

Age (years)	<50	50 – 59	60 – 69	at least 70
Vitamin D (µg/L)				
<15	280	226	309	327
15-30	806	677	755	629
30<	351	229	300	247

PSA 95 percentile values (µg/L) in different age cohorts with different vitamin D levels

Age (years)	<50	50 – 59	60 – 69	at least 70
Vitamin D (µg/L)				
<15	2.0	3.4	7.7	11.0
15-30	2.1	3.8	7.0	9.4
30<	2.4	4.7	8.2	8.7

Per cent rate of patients with PSA >4 µg/L

Age (years)	<50	50 – 59	60 – 69	at least 70
Vitamin D (µg/L)				
<15	1.0%	3.9%	14.9%	22.3%
15-30	0.7%	5.3%	12.4%	18.2%
30<	1.4%	6.5%	16.3%	23.4%

Pearson’s correlation coefficient (R) was calculated after logarithmic transformation of both PSA and vitamin D3 levels in order to achieve a close-to-normal distribution in individual dimensions. Two-dimensional distribution and correlation was calculated for both the total

patient population and solely for patients with PSA above 4 µg/l (Figure 1). Due to the characteristics of the PSA test, any value measured to be below 0.1 ug/l is reported here as 0.1 ug/l. This bias is, however, corrected by kernel density estimation.

RESULTS

The majority of patients (55.8 and 22.2 percent) had moderate or severe vitamin D deficiency hallmarked by 15 – 30 µg/L and <15 µg/L vitamin D levels, respectively. While PSA levels increased with age, no difference in PSA levels was identified between patients with different vitamin D levels (see Table 1).

Our logistic regression analysis indicated a significant association between age (estimate: 0.010; SE: 0.0027, p=0.003), while no impact of vitamin D on PSA levels was found. In fact, Pearson's correlation coefficients found here were close to 0 suggesting that there was no linear relationship between PSA and vitamin D3 levels.

DISCUSSION

Several reports suggested an inverse relationship between vitamin D levels and the risk and aggressiveness of prostate cancer [1-3]. Therefore, it was reasonable to postulate that the presumed effect of vitamin D on prostate would be reflected in PSA, a surrogate marker of prostate pathology. However, the results of our analysis involving 5136 data pairs of vitamin D and PSA levels of general hospital patients, does not support this hypothesis.

These findings are in line with those of several smaller studies that searched for an association between vitamin D levels and PSA levels.

A prospective study enrolling 105 healthy men without any basic alteration of PSA documented no change in PSA levels upon vitamin D administration and increase in vitamin D blood levels. [12] In 71 patients on peritoneal dialysis no association between vitamin D and PSA was identified. [13] Another study enrolling 1705 subjects found no direct relationship between PSA and vitamin D levels in patients without prostate cancer. [14]

The novelty of our study that we performed our analysis on a larger group (more than 5000) unselected patients treated at the University. We were not aware of diagnosis and treatment. We are, however, convinced that this limitation does not prevent to draw the conclusion that vitamin D levels have due to the large number of data, heterogeneity of referring departments and the exclusion of patients with high PSA levels.

Our finding has two major implications for interested readers. First, it raises concern about the contribution of vitamin D to those prostate pathologies that are clearly indicated by slightly or moderately elevated PSA levels. Second, it reinforces for clinicians that they should not adjust PSA reference ranges and threshold values to vitamin D levels during the process of decision making.



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Trends in laboratory testing practice for diabetes mellitus

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ABSTRACT

Background

India, with diabetes mellitus (DM) prevalence of nearly 7%, contributes 20% of the DM population in the world. The diagnosis and management of DM is largely dependant on laboratory parameters. We aimed to survey the laboratory testing practices for DM in this country.

Methods

A survey of 890 practising Laboratorians in India was conducted through Survey Monkey.

Results

A total of 310 (35%) complete responses were received. The majority of respondents worked in academic institutions, public hospital laboratories and private hospital laboratories. HbA1c was approved for diagnosis in 75% of laboratories. The HbA1c method was NGSP (National Glycohaemoglobin Standardisation Programme) certified in 70% of laboratories only. Oral glucose tolerance testing (OGTT) was recommended for diagnosis of gestational diabetes (GDM) in 56%

of respondents. Fifty-nine percent respondents recommended an early morning urine sample for microalbuminuria testing whilst 39% and 2% opted for 24 hour urine and timed overnight sample respectively. Sixty-six percent participated in proficiency testing (PT) for both glucose and HbA1c. Twelve percent and 4% respondents respectively participated in PT for glucose only and HbA1c only, and 9% participated in PT for neither.

Conclusions

Based on the above survey we recommend that Scientific bodies and Professional Associations in India should educate Laboratorians to adopt NGSP certified methods for HbA1c testing and morning spot sample for microalbuminuria testing. DIPSI (Diabetes in Pregnancy Study Group in India) guidelines for diagnosis of Gestational diabetes since it is a simple, single step procedure, non-fasting, cost effective, feasible method should be implemented.



INTRODUCTION

There are close to 66.8 million patients with diabetes mellitus (DM) in India, which represent nearly 7% of the country's adult population. [1] Every fifth diabetic in the world is from India. [2] Therefore, it is imperative that we understand the laboratory testing practices for DM in India, since the diagnosis and management of DM both rely heavily on laboratory parameters. [3] Understanding the trends in laboratory testing of DM helps us identify the lacunae and gaps in knowledge as well as barriers in the optimal management of DM. Based on this, recommendations can be made by associations and professional bodies to fill in the gaps in knowledge. Identifying the lacunae in the laboratory testing practices would also help health administrators to formulate policies and allocate resources, so

as to remove the barriers and to bridge the gap in knowledge.

METHODS

To understand the trends in laboratory testing practice in India a survey was conducted by using the survey monkey app. A survey was designed under the aegis of Asia Pacific Federation of Clinical Biochemistry and sent by Whats app to approximately 890 respondents between July and October 2018. Participants whose mobile numbers were not available were sent the survey on their respective emails. Access to mobile contact numbers as well as emails of participants were obtained from the registries of Association of Medical Biochemists of India (AMBI) and Association of Clinical Biochemists of India's (ACBI) respective websites which had listed contact numbers as well as emails of their members. For 150 Biochemists and Pathologists whose mobile numbers were not available in the respective member directories were sent the Survey Monkey link as an attachment to their emails. Due permission of the Presidents of the associations were obtained before accessing the contact numbers and emails.

The survey questions are depicted in Table 1, on the next page. The responses were collected from the Survey Monkey website, collated and analysed.

RESULTS

Maximum responses were received between July and August 2018. Three hundred and thirteen (31.3%) of the recipients responded. Of these responses, 310 surveys were complete and three were incomplete. The results of the survey are depicted in the Bar graphs below (Figure 1).

Some 3% of respondents use mmol/L as unit for reporting blood glucose. One fourth of the respondents reported that HbA1c was not used in their hospitals/laboratories for diagnosis of

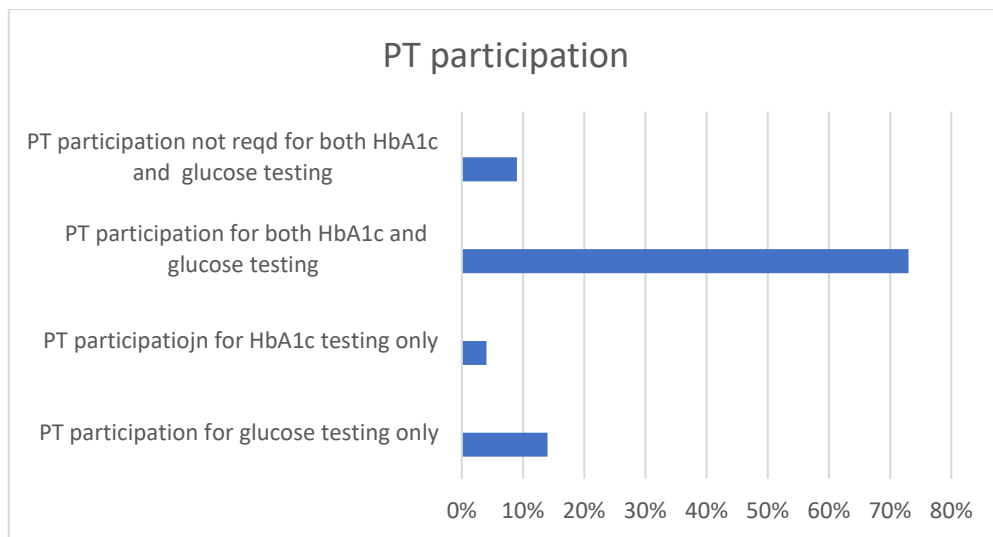
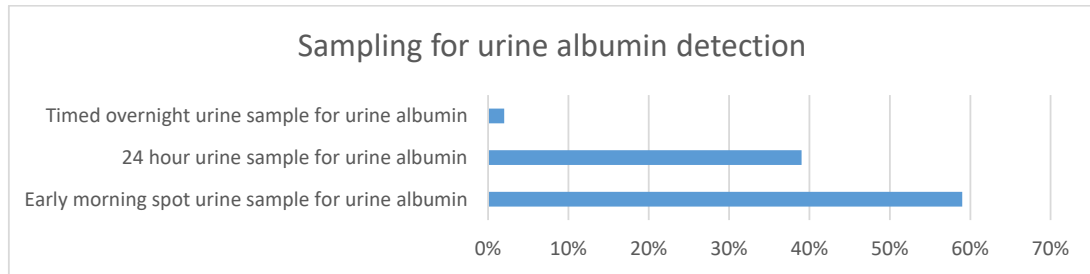
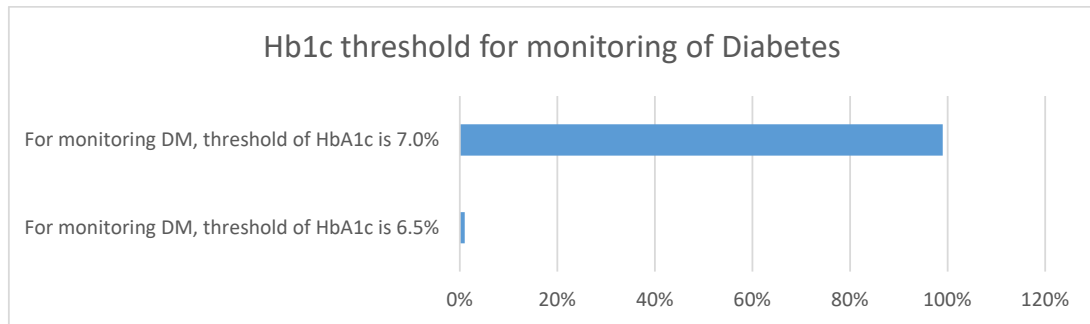
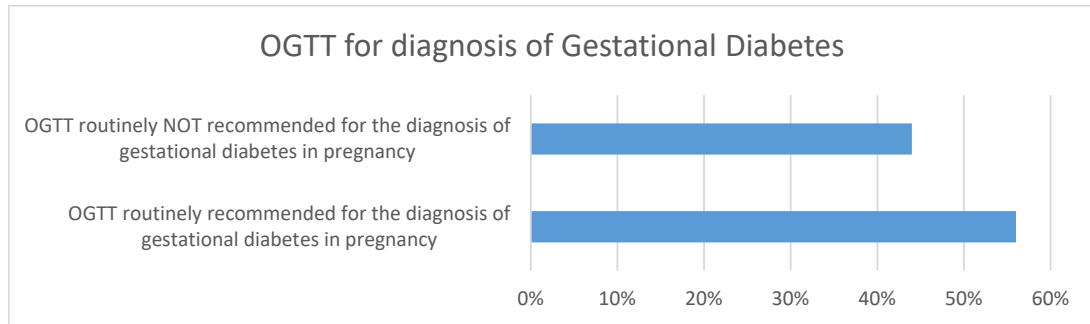
Table 1 Survey questions	
<p>1. What units do you use for reporting blood glucose?</p> <p>a) mmol/L b) mg/dL</p>	<p>6. Is OGTT routinely recommended for the diagnosis of Gestational Diabetes?</p> <p>a) Yes b) No</p>
<p>2. Is HbA1c approved for diagnosis of Diabetes in your country?</p> <p>a) Yes b) No</p>	<p>7. For monitoring of diabetes, which of the following thresholds of HbA1c is reported as good control?</p> <p>a) 7% b) 6.5%</p>
<p>3. Is HbA1c used for diagnosis of diabetes in your laboratory/hospital?</p> <p>a) Yes b) No</p>	<p>8. For testing microalbuminuria, which of the following urine samples is recommended?</p> <p>a) Early morning spot urine b) 24 hour urine c) Timed overnight urine</p>
<p>4. Is your HbA1c method NGSP (National Glycohaemoglobin Standardization Programme) certified?</p> <p>a) Yes b) No</p>	<p>9. Are Laboratories required to participate in PT program for testing glucose and HbA1c?</p> <p>a) Glucose only b) HbA1c only c) Both d) None e) If yes, does your Laboratory participate in PT program?</p>
<p>5. For the diagnosis of diabetes mellitus, which of the following diagnostic cut-offs are reported by your Laboratory?</p> <p>a) HbA1c > 6.5% (48 mmol/mol) b) Fasting plasma glucose > 126 mg/dL (7.0 mmol/L) c) 02 hour post glucose load glucose > 200 mg/dL (11.1 mmol/L) during an OGTT d) Symptoms of hyperglycemia and random plasma glucose > 200 mg/dL (11.1 mmol/l)</p>	<p>10. Which type of Laboratory do you work?</p> <p>a) Public hospital b) Private Hospital c) Public stand alone d) Private stand alone e) Academic university f) Research Lab</p>

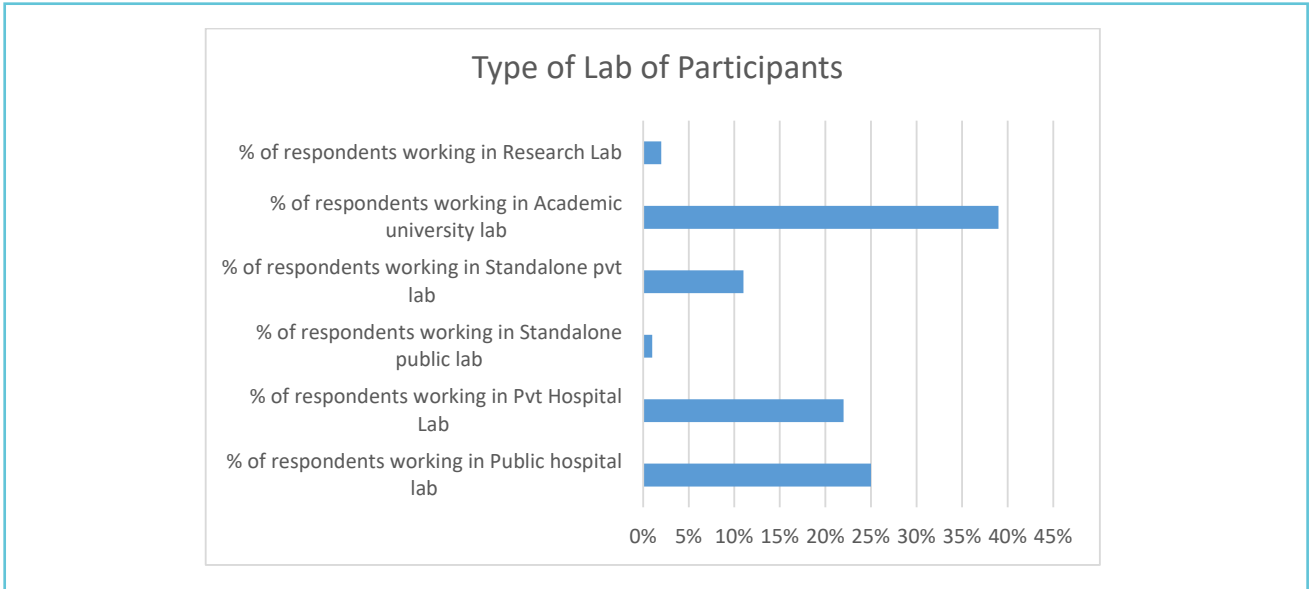
DM. Thirty percent of respondents stated that their method for estimation of HbA1c was not NGSP certified. Criteria used most commonly by respondents (83%) for the diagnosis of DM

was fasting >126mg/dL followed by post prandial glucose of 200 mg/dL reported by 71% respondents. Only 58% respondents used random plasma glucose >200mg/dl as a diagnostic cut

Figure 1 Survey results







off for the diagnosis of DM. Only 56% respondents reported that OGTT was recommended for the diagnosis of gestational DM. A vast majority of respondents (99%) reported that the HbA1c threshold for the control of DM is 7%. For estimation of microalbuminuria (MAL), 59% preferred early morning urine sample followed by 39% who preferred a 24 hour urine collection. Two thirds of respondents reported that their laboratories participated in proficiency testing (PT) for both Glucose and HbA1c testing whereas 12% and 8% respectively reported that their laboratories were participating in PT for glucose and HbA1c only.

The majority of participants were from academic university, public hospital and private hospital laboratories.

DISCUSSION

This survey was performed in order to obtain a snapshot of the laboratory testing practices for DM throughout India. A response rate of 31% is not optimal but the absolute numbers are large and does provide an indication of the practices being followed in this country. A large proportion of our respondents were from academic university laboratories, which service major

teaching hospitals, followed by public and private hospital laboratories.

Respondents almost unanimously reported blood glucose in mg/dL although standard international unit for reporting blood glucose is mmol/L. Some countries such as USA and Germany report glucose in mg/dL like India but in others such as UK it is reported in mmol/L. International professional organisations such as IFCC (International Federation of Clinical Chemistry) in collaboration with various national bodies/associations may recommend all countries to report in SI units. However, there are no existing Indian guidelines to this effect.

Whilst HbA1c is now internationally approved for the diagnosis of DM, a sizeable minority of hospitals in India are not using it for this purpose and a similar proportion of laboratories are not offering HbA1c testing for this purpose. Herein, comes the role of Indian professional associations to spread awareness among Biochemists and Pathologists to enable them to offer HbA1c for the diagnosis of DM as has been recommended as well as educate clinicians. One of the reasons, it is not used for diagnosis is possibly resource limitations in public laboratories who were the majority of our

respondents where the cheaper alternative of estimation of blood glucose is undertaken for the diagnosis of DM.

Based on the survey above, it is recommended that Biochemists /Pathologists be made aware of the National Glycohemoglobin Standardization Program (NGSP) certification of HbA1c estimation methods. This will help in harmonisation of HbA1c methods across laboratories and different testing platforms. The lack of comparability of glycated haemoglobin (GHb) test results across methods and laboratories previously posed a major hurdle to a meaningful implementation of specific guidelines for DM care. [4] NGSP was implemented to enable laboratories to report DCCT (Diabetes Control and Complications Trial) traceable GHb/HbA1c results. Over the years, the number of NGSP certified methods and laboratories traceable to the DCCT have increased remarkably. By 2002, 98% of surveyed laboratories (n ~ 2000) reported GHb results as HbA1c or equivalent compared to 50% in 1993. [4] Little RR in his study in 2002 had reported that 97% of laboratories used an NGSP-certified method and only 3% were not following NGSP certified testing. [4] Our survey shows that 30% respondents were not reporting HbA1c by NGSP certified methods. This clearly highlights the role of spreading awareness among laboratorians to use NGSP certified methods for HbA1c estimation. For certified methods in 2002, inter laboratory CVs were <5%. In 2002, for all certified methods, the mean HbA1c value (%) was within 0.8% of HbA1c from the NGSP target at all HbA1c concentrations. [4] Hence, ensuring that all laboratories estimate HbA1c with NGSP methods will go a long way in harmonisation of HbA1c methods. IFCC has developed a robust reference method which is more specific for HbA1c. [5] NGSP has now adopted this IFCC method as the reference system. Since the IFCC method is specific for HbA1c and it does not measure other haemoglobin sugar complexes the result is

10-40% lower than the NGSP values depending on the levels of glycated haemoglobin. There is a linear relationship between the IFCC and NGSP values. The equation used to convert NGSP units to the SI units is as follows:

$$\text{HbA1c SI unit (mmol/mol)} = 10.93 \text{ HbA1c NGSP unit (\%)} - 23.50. [6]$$

All laboratorians engaged in HbA1c testing should apprise themselves with the factors interfering with their test methodology. Factors which are known to commonly decrease HbA1c values are acute haemorrhage, haemolytic anemias and iron therapy in pregnancy. Factors which increase HbA1c are Iron deficiency anemia, late pregnancy (due to an iron deficient stage). [7] Selvaraj N et al in their study have proposed that RBCs incubated with Malondialdehyde and glucose registered a higher HbA1c when compared with RBCs incubated with glucose alone. [6] They pretreated RBCs with taurine and choline which decreased the production of MDA and showed a decrease in HbA1c. [8] They therefore propose that MDA has a role in increasing the glycation of haemoglobin. The exact mechanism of how MDA causes increased glycation of Haemoglobin is however not clear. Mawatari S et al in their study have however not found any difference in the levels of MDA in patients with high HbA1c and those with low HbA1c. [9] The role of MDA therefore in causing increased glycation of Haemoglobin is highly controversial. Most diabetics develop diabetic nephropathy as a complication of DM. They have substantial amount of carbamylated haemoglobin which occurs due to the non-enzymatic addition of urea to haemoglobin. Carbamylated haemoglobin is known to interfere with HbA1c levels, based on the method which is being used for the estimation of HbA1c it may increase or decrease the levels of HbA1c. [7] The effects of anemia of chronic disease and erythropoietin on glycation of Haemoglobin (which occurs in CKD) are

difficult to ascertain which are based on methods used to estimate HbA1c.

HbS and HbC, alter the structure of Hb close to its N terminus, affecting methods that depend on detecting structural differences like immunoassays. In contrast, HbD and HbE, do not cause structural alterations near the N-terminus and hence do not cause interference in immunoassays. Interference with ion-exchange methods can be seen in any of the four variants described as they alter the charge of Hb molecule. Assays utilizing immunoturbidimetry and boronate affinity chromatography are usually not affected by the presence of Hb variants. [10]

The HbA1c target for control of DM has been recommended to be at 7% by various bodies (American Diabetes Association/International Diabetes Federation). However, it has been recommended by NGSP that the target could be set at 8% for patients with history of hypoglycemia, co morbidities or expected life span of less than twenty years, patients with major visual/cognitive impairments leading to impaired self-management. [11]

Unless patient is symptomatic with random glucose > 200 mg/dL or patient is in a hyperglycemic crisis test for diagnosis should not be repeated. Either the same test or a different test should be repeated using a different sample. If two different tests are diagnostic then the diagnosis is confirmed. If the results of two different tests are discordant then the test which is diagnostic should be repeated. [3]

Keeping in view, that Asian women have a significantly higher risk of developing glucose intolerance compared to Caucasian women, universal screening for early detection of GDM should be offered to all pregnant women. [12] Most obstetricians in India used the DIPSI (Diabetes in Pregnancy Study group in India) guidelines for diagnosis of Gestational diabetes since it is a simple, single step procedure, non-fasting, cost

effective, feasible method. [13]The DIPSI guidelines look at glucose values post 75 g glucose load at 02 hour. The values of 200 and 140 mg/dl are diagnostic of DM and Gestational diabetes Mellitus respectively. An additional criteria which has been introduced is a post 75 g glucose load 2 hour value of 120 mg/dL which is indicative of DGGT (Decreased Gestational Glucose Tolerance). The advantages of DIPSI criteria are that the patient irrespective of meal status can be administered 75 g oral glucose. It serves both as a screening and diagnostic test.[14] DIPSI guidelines suggest that patients be screened every trimester since it has been shown that fetal beta cells respond to maternal glycaemic levels by 12 weeks of gestation. If found negative in the first trimester, screening should be performed at 24-28 weeks and thereafter finally at 32-34 weeks. [15] Present ADA recommendations of screening at 24-28 weeks are late. Methods of diagnosing GDM earlier will decrease the co morbidity of GDM. Gynaecologists, endocrinologists and Biochemists must be educated to administer OGTT in all cases of pregnancy in the first trimester itself to diagnose GDM earlier to ensure favourable outcome in both the mother as well as the foetus.

Mahalakshmi et al, in their survey of clinicians, surveyed 3841 doctors, of which 2020 comprised of a heterogeneous group of Physicians, Diabetologists/Endocrinologists and of which 1821 were Obstetricians and Gynaecologists. A diverse trend in the management of Gestational Diabetes by both these groups was observed. Thirty seven percent of Gynaecologists reported using the Diabetes in Pregnancy Study Group India (DIPSI) criteria, 25% the World Health Organisation (WHO) 1999 criteria, 24% the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, and 15% the American Diabetes Association (ADA) 2- step method (50 g Glucose Challenge Test followed by 100 g 3 h Glucose Tolerance Test with

the cut offs proposed by Carpenter and Coustan or by the National Diabetes Data Group. [16] Among the Physicians/Endocrinologists 29% reported using the DIPSI criteria, 23% the WHO 1999 criteria, 19% the IADPSG criteria, and 29% the ADA criteria. From this data it is evident that the single most common criteria popular among clinicians in India is the DIPSI criteria.

Clinicians should endeavour to follow the same guidelines IADPSG/WHO/ADA/DIPSI across the country to bring in standardisation in the testing of GDM across the country so that similar management protocols can be followed universally. ACBI/AMBI may play a role in attempting to harmonize practice nationally in collaboration with professional clinical associations. This will obviate any kind of retesting when a patient changes her centre and therefore will lead to conserving financial resources, manpower and time.

Microvascular complications are a dreaded sequel of DM. For the diagnosis of microvascular complications of DM, assessment of MAL is recommended. MAL is a misnomer and hence urine albumin creatinine ratio would be a better method of assessing early diabetic nephropathy. Urine microalbuminuria & urine albumin creatinine ratio show a very good correlation 0.509. [17] Various methods of evaluating microalbuminuria are immunonephelometry, immunoturbidimetry, Radio Immunoassay. Immunoturbidimetry assays are good screening assays for assessing nephropathy. [18] Babazono et al in their study titled "Definition of MAL based on first morning sample and random morning urine sample in diabetic patients" studied a total of 668 individuals with and without nephropathy with 95% of patients having type 2 diabetes with a mean age of 58 +/- 12 yrs. The cohort consisted of 289 women 379 men. All patients submitted first morning sample and also random morning samples. Seventy five percent of random samples were collected between 0830 to 1200 PM.

They have found a correlation of $r = 0.859$, between first morning and random morning urine samples. The cut off for first morning sample was 30-300 mg/g and 51-391mg/g for random morning sample. On applying the above diagnostic cut offs, 20% of patients were diagnosed to have MAL when early morning urine sample was submitted and 35% were diagnosed to have MAL when random spot urine was submitted. [19] Miller WG et al in their study have shown that morning fasting sample is preferred by 81% people, followed by 14% and 5% patients who submitted timed overnight and 24 hour sample. [20] In our survey, 39% of laboratories preferred 24 hour urine sample which is much higher than that reported by Miller et al. Collection of a 24 hour urine sample for estimation of albumin creatinine ratio, is a very tedious procedure for the patient which entails cumbersome collection procedure with a designated container and preservative which decreases patient compliance and introduces a vast array of preanalytical variables in the estimation of albumin creatinine ratio. Hence, it should be emphasised here that random urine samples are good enough for estimation of albumin creatinine ratio even though early morning samples are ideal. ADA as well as NKD (National Kidney Foundation) have recommended ACR in a random spot urine sample for convenience. [21,22] Spot random sampling definitely will go a long way in increasing patient compliance and therefore is a better testing strategy.

Harmonisation of albumin measurement in urine is not an easy task since there are a multitude of methods available. Also compounding the problem is the fact that, there is no reference material for traceability studies. Commutability of available reference material also needs to be addressed. JSCC (Japanese Society for Clinical Chemistry) and JCCLS (Japanese Committee for Clinical Laboratory Standards) have coordinated the development of a new urine albumin

secondary Reference material for this purpose. [23] Age, gender and ethnicity specific reference intervals may be appropriate for the interpretation of reports but are not available.

The role of Professional bodies like the NABL (National Accreditation Board for Laboratories) has gone a long way towards increasing awareness amongst laboratorians to participate in Proficiency Testing (PT) programmes. Most laboratories in India, participate in the CMC (Christian Medical College), External Quality Assurance Scheme due to its affordability even by small standalone labs. Some also participate in International PT programmes.

Awareness is required among laboratorians to choose NGSP certified methods for the estimation of glycated Haemoglobin in their respective Laboratories. Spot random urine samples are acceptable for estimation of MAL and patients may be spared the trouble of collecting 24-hour urine sample for estimation of MAL. Gynaecologists should start screening for GDM from the first trimester itself. National associations & scientific bodies have a major role in educating & sensitizing laboratorians in this regard and ensuring harmonized practices nation-wide. These measures will go a long way in the ease of detection and management of DM. Other developing nations in the Asia Pacific region like Pakistan, Bangladesh, Nepal, Bhutan, Sri Lanka and Phillipines could also use this survey as a template to formulate their own policies/guidelines in the diagnosis and management of DM since the socioeconomic conditions prevailing and existing health infrastructure in the neighbouring countries are very similar to India.



Authors' contributions

MB: Execution of survey, manuscript preparation
SV: Conceptualising the survey, Proof reading of the manuscript

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Anti-tuberculosis treatment: induced hepatotoxicity – a case report

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pulmonary tuberculosis, hepatotoxicity,
adverse drug reaction, direct observation
therapy

ABSTRACT

Tuberculosis is a potentially communicable disease that can infect any organ in the body such as bones, kidney, intestine but primarily involves lung parenchyma (Pulmonary tuberculosis). The prevalence of TB is 256 per 100,000 population in India. Hepatotoxicity, gastrointestinal and neurological disorders were some the Adverse Drug Reactions (ADR's) reported that significantly increases the mortality rate which leads to decreased efficacy of the treatment. Hepatotoxicity is the most commonly reported ADR in patients treated with anti-tubercular drugs such as isoniazid, rifampicin and pyrazinamide. Clinical manifestations of hepatotoxicity include abdominal pain, nausea, vomiting, and jaundice. We report the case of a 19-year-old female with complaints of yellowish discoloration of sclera for 45 days associated with vomitings for one week. She had a past medical history of tuberculosis for which she was advised with DOT (Direct Observation Therapy) regimen. A diagnosis of Anti-Tuberculosis Treatment (ATT) - induced hepatotoxicity was made based on the clinical examination and laboratory investigations which was successfully managed by providing supportive care and symptomatic treatment.

INTRODUCTION

Tuberculosis is a potentially communicable disease that can infect any organ in the body, such as bones, kidney, and intestines, but primarily involves lung parenchyma (Pulmonary tuberculosis). *Mycobacterium tuberculosis*, a purple coloured rod-shaped bacterium, is responsible for tuberculosis infection and is spread by droplets of an infected person. Signs and symptoms of tuberculosis include excessive cough with sputum (In severe conditions blood is also seen along with sputum), weight loss, anorexia, fever and night sweat. The prevalence of TB is 256 per 100,000 population in India. Results of various studies concluded that almost 80% of TB cases are completely curable with an effective regimen of Direct Observational Therapy (DOT). Hepatotoxicity, gastrointestinal and neurological disorders were some of the ADRs reported that significantly increases the mortality rate which leads to decreased efficacy of the treatment.

Hepatotoxicity is the most commonly reported ADR in patients treated with anti-tubercular drugs such as isoniazid, rifampicin and pyrazinamide (1). Reports of various studies reveal that anti-tubercular therapy (ATT) induced hepatotoxicity is seen in 5-28% of the patients treated with anti-tubercular drugs (2). Reports of liver function test in ATT induced hepatotoxicity reveals a threefold increase of liver enzymes ALT (Alanine Transaminase) and AST (Aspartate transaminase). Clinical manifestations of hepatotoxicity include abdominal pain, nausea, vomiting, and jaundice. Reports of liver biopsy revealed lobular hepatitis, sub massive to massive necrosis and hydropic degeneration of hepatocytes in severe cases. One hypothesis state that an inflammatory reaction results in production of bacterial lipopolysaccharides which act in combination with drug metabolites to cause hepatotoxicity.

CASE REPORT

A 19-year-old female patient who was a known case of tuberculosis started upon ATT for the previous two months. She discontinued medication for 20 days, restarted medication for TB, continued for 10 days and discontinued again. Then she came to the hospital with chief complaints of yellowish discoloration of sclera for 45 days associated with vomitings for one week. As she had been previously diagnosed with TB, she was prescribed with a combination of Isoniazid (75 mg), Rifampicin (150 mg), and pyrazinamide (400 mg).

After a few days of therapy, the patient experienced symptoms of loss of appetite, constipation and pale skin. Ignoring the symptoms, she continued to take the drugs, which resulted in worsening symptoms such as yellowish discoloration of sclera and vomitings, after which she stopped taking the drugs used in ATT therapy. On examination her vitals were normal and examination of liver parameters were as detailed in Table 1. Serological examinations for hepatitis-B, hepatitis-C, and HIV were negative. Microbiological examination for acid fast bacilli was also found to be negative. No abnormality was detected in the sonography of the abdomen and pelvis (bed side). Chest radiograph, PA view revealed sub-segmental atelectasis noted in the left mid zone. Patient CBP and CUE were found to be normal.

The physician advised the following medications (Table 1) following the cessation of anti-tubercular drugs. Alternative drugs such as streptomycin, ethambutol, levofloxacin were prescribed for treatment of TB. Patient was diagnosed with ATT induced hepatotoxicity with marked elevations of bilirubin (hyperbilirubinemia) and increased hepatocellular enzymes (SGOT, SGPT, ALP). Laboratory values of liver function test on different days are shown in Figure 1 and Figure 2, respectively.

Table 1 Medication chart

Brand name	Generic name	Dose
Inj Zofer	Ondansetron	4 mg
Tab Udiliv	Ursodeoxycholic acid	300 mg
Hepamerz sachets	L aspartate granules	3 gm
Syp Lactulose	Lactulose	15 ml

Figure 1 Reported values of various liver enzymes [ALP, SGOT AND SGPT] on different days during hospitalisation

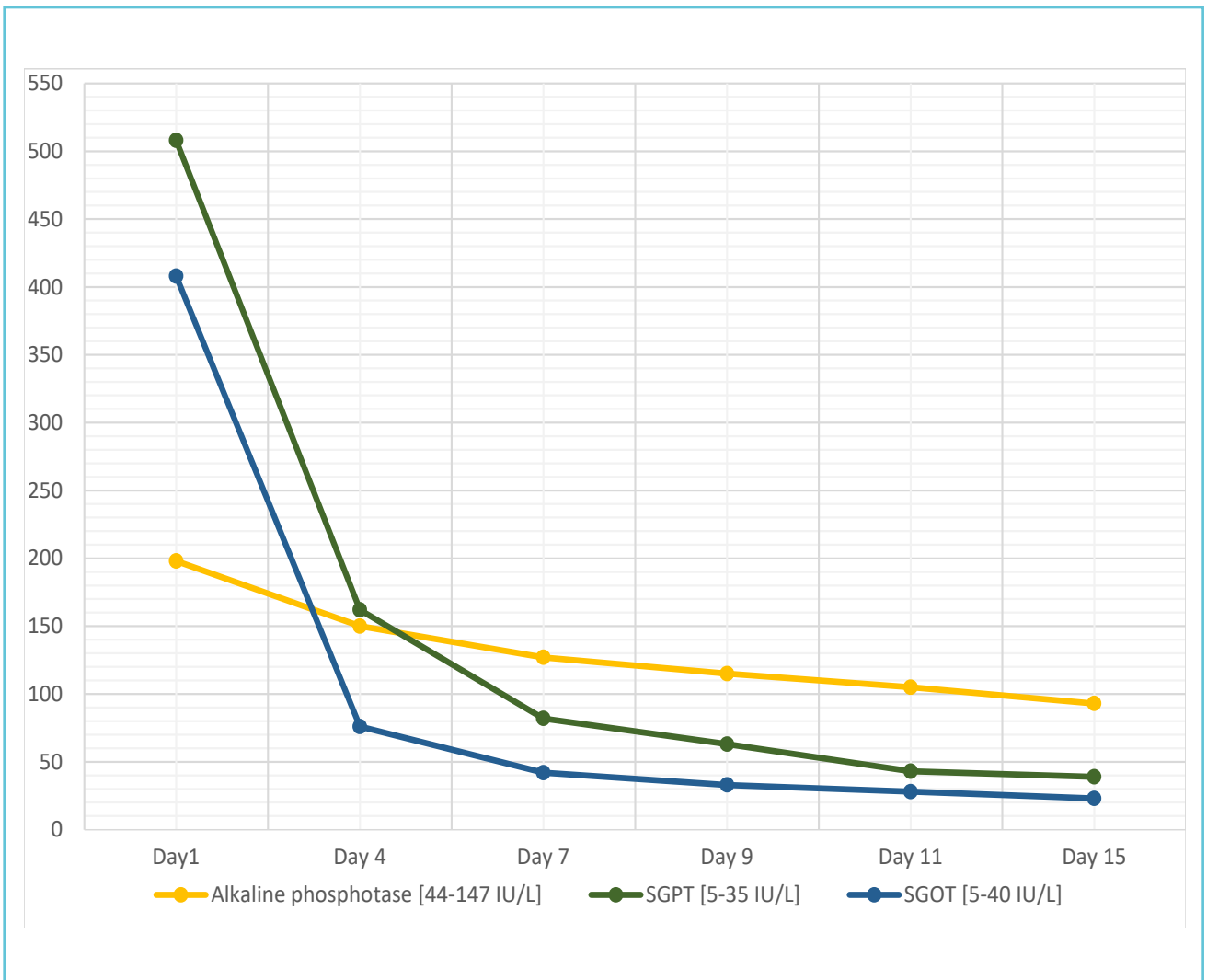
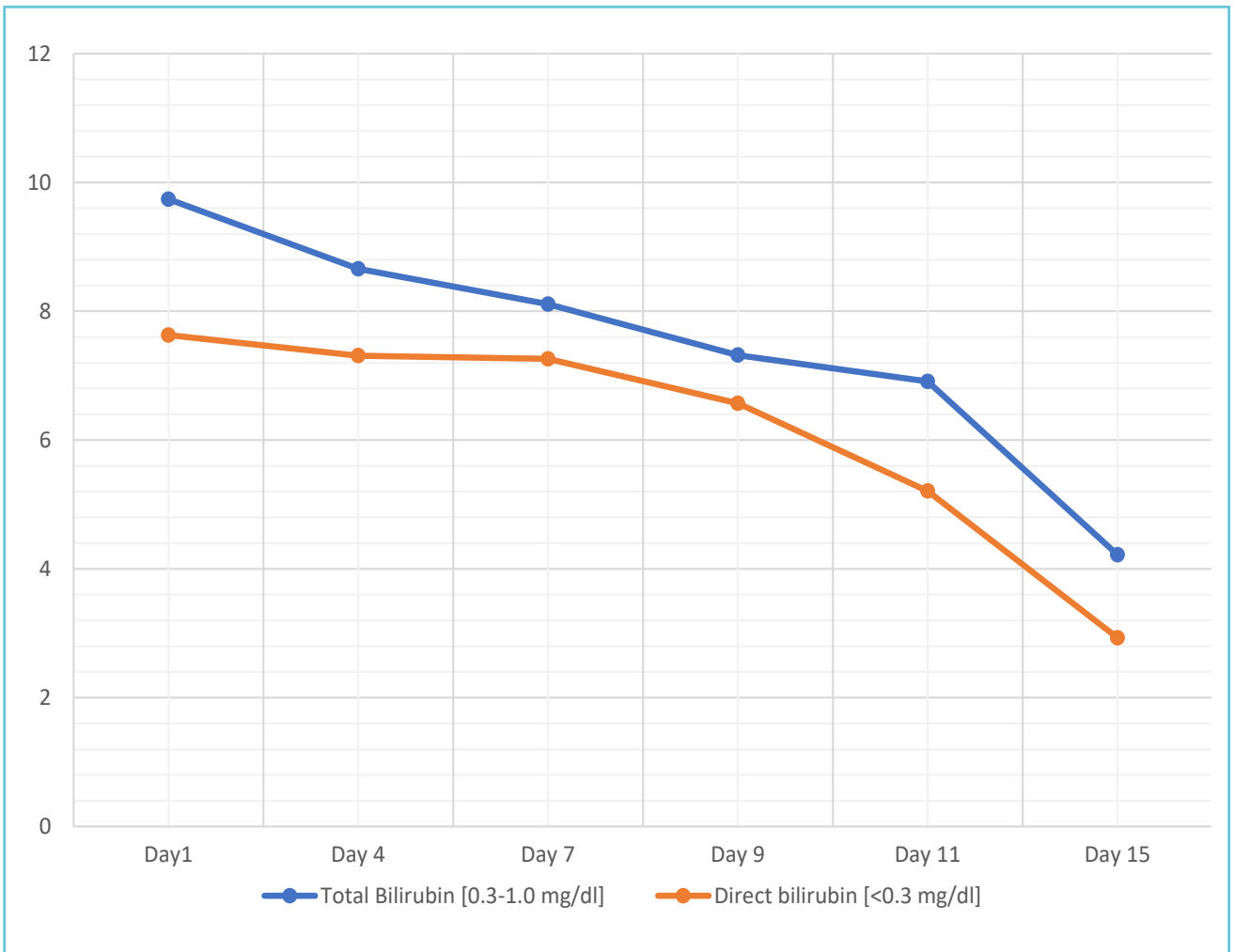


Figure 2 Reported values of bilirubin on different days during hospitalisation



DISCUSSION

Currently, there are 10 drugs approved by the U.S. FDA for the treatment of tuberculosis, namely: Isoniazid, Rifampin, Pyrazinamide, Ethambutol, Rifapentine, Streptomycin, Cycloserine, Capreomycin, P-Aminosalicylic acid, Ethionamide. Fluoroquinolones such as Levofloxacin, Moxifloxacin and Gatifloxacin are also used in the treatment of drug resistant tuberculosis and in patients who are unresponsive to first line drugs, even though they are not approved by FDA for treatment of tuberculosis (3). Tuberculosis is a mycobacterial disease, treatable with anti-tubercular therapy. Commonly used drugs are Isoniazid,

Rifampicin, pyrazinamide and Ethambutol. All these drugs are used in combination for a few months (2-6 months).

According to a study, the incidence of drug induced liver injury in India is between 8-36%. Incidence of drug induced hepatotoxicity is higher in Asian countries due to ethnic susceptibility, unusual drug metabolism and due to other risk factors, such as alcoholism, malnutrition and other infections such as hepatitis B (4).

Isoniazid causes peripheral neuropathy and hepatotoxicity (elevated serum transaminases and serum bilirubin), Rifampicin causes immune-allergic reactions and hepatotoxicity (elevated

serum transaminases, alkaline phosphate and serum bilirubin), pyrazinamide causes joint pains (increased serum uric acid) and hepatotoxicity (elevated serum transaminases and serum bilirubin).

These three drugs isoniazid, rifampicin and pyrazinamide have high potential of inducing hepatotoxicity and clinical manifestations of hepatotoxicity include nausea, vomiting, weakness, fatigue and yellowish discoloration of eyes. These side effects can be due to one/two or all of the 3 drugs and some of the patients are not able to tolerate these and as a result stop taking anti-tubercular drugs which leads to decreased effectiveness of the treatment. Hepatotoxicity with ATT drugs increases with following factors such as concomitant usage of other hepatotoxic drugs, age, alcohol abuse and pre-existing liver disease. According to a study, pyrazinamide is 3 times more potent than isoniazid or rifampicin in precipitating serious adverse events (5).

In the present condition, the patient was on a therapy with the ATT drugs like Isoniazid, Rifampicin and Pyrazinamide for two months. After a few days of therapy, she produced signs of loss of appetite, constipation and pale skin. Later, the patient developed signs such as yellowish discoloration of sclera and vomitings.

The pathophysiology of ATT induced hepatotoxicity is explained by four different mechanisms which include direct toxicity, idiosyncratic damage, induction of liver enzymes and allergic reactions.

Direct toxicity is due to production of free radicals which damages the liver tissue and this is dose related. Idiosyncratic damage is due to hypersensitivity reactions and may be a genetic or acquired variations in the metabolic pathway. Induction of liver enzymes may increase the hepatotoxic potential of the drugs. Allergic reaction is caused due to a reactive metabolite.

CONCLUSION

In summary, this is a case of a patient who developed hepatotoxicity following the intake of anti-tubercular drugs which is managed by providing supportive care. It is essential to educate the patients about the possible ADRs associated with the drugs used in the treatment of tuberculosis. Physicians must counsel their patients about signs and symptoms of hepatotoxicity and encourage them to report them as soon as possible.

TAKE HOME MESSAGES/ LEARNING POINTS:

- In pulmonary tuberculosis, a Sputum smear test should be performed once every two or three months to monitor the progression of disease until the end of treatment. If smear test was found positive, the patient must be reevaluated and effectiveness of therapy must be considered.
- Patient should be advised to take full course of treatment although patient might feel better after taking medications for a short span of time.
- Patients advised with DOT therapy is recommended to undergo frequent liver function check-ups, which include serum bilirubin, aminotransferases, alkaline phosphatase as most of the drugs used in DOT therapy has the potential to induce hepatotoxicity.



Authors' contributions

Shravan and Vidya: Collection of the data and preparation of manuscript.

Tabassum and Manashwini: Review of literature and edited the manuscript.

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Case report on pediatric septic arthritis of the hip

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septic arthritis, pain, fever,
staphylococcus aureus and arthrotomy

ABSTRACT

Septic arthritis is a condition from the presence of microbial agents in a joint space.

A 9 year old boy presented with pain in right hip joint which was tender on palpation, associated with fever (38.3°C). Blood tests showed elevated levels of C reactive protein and erythrocyte sedimentation rate. No pathological findings were found in X-ray of the hip and ultrasound of right hip joint showed mild increase in joint space. Pus culture and sensitivity yielded plenty pus cells in pairs of chains and Methicillin sensitive Staphylococcus aureus was isolated. Treatment with antibiotics was started and right hip arthrotomy and debridement was done followed with skin traction. Follow up was done and the boy had regained his full range of motion with no sign of complications.

INTRODUCTION

Septic arthritis (SA) of the hip is a true orthopedic emergency; delay in diagnosis or treatment may result in irreversible damage to the joint.¹ Staphylococcus aureus is the usual offending microorganism, present in 50% of cases.^{2,3,4} Early diagnosis and accurate treatment is key in avoiding complications such as joint destruction, ankylosis, growth arrest or spread of infection leading to osteomyelitis or nerve lesions.^{5,6} SA is a challenging clinical problem because (1) signs and symptoms may be subtle and overlap with those found in other conditions, (2) screening laboratory studies and synovial fluid cultures are relatively insensitive, and (3) optimal management, including duration of antibiotics and surgical approach, is not evidence based.¹

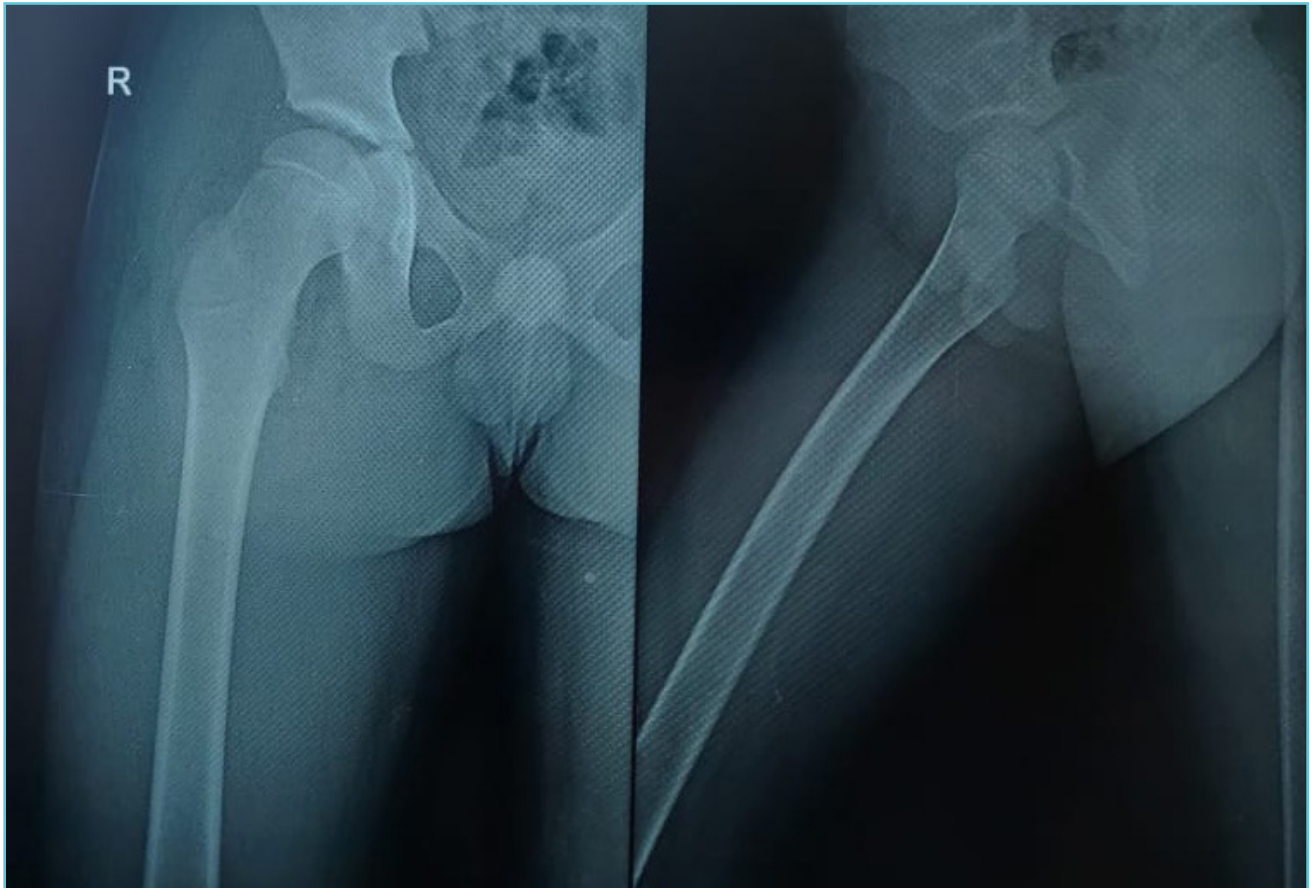
CASE PRESENTATION

A 9 year old boy weighing 30 kg presented with fever (38.3°C) and pain in his upper right thigh, having begun one day prior; and which had increased on the day of his presentation. Parents also reported the pain was sudden in onset and there was restriction in movement. Birth history was normal and uneventful. There was no history of trauma.

Physical examination revealed right anterior superior iliac spine (ASIS) at lower level; flexion abduction altitude; anterior joint line tenderness over right thigh; painful extension of hip and flexion with painful external rotation and internal rotation.

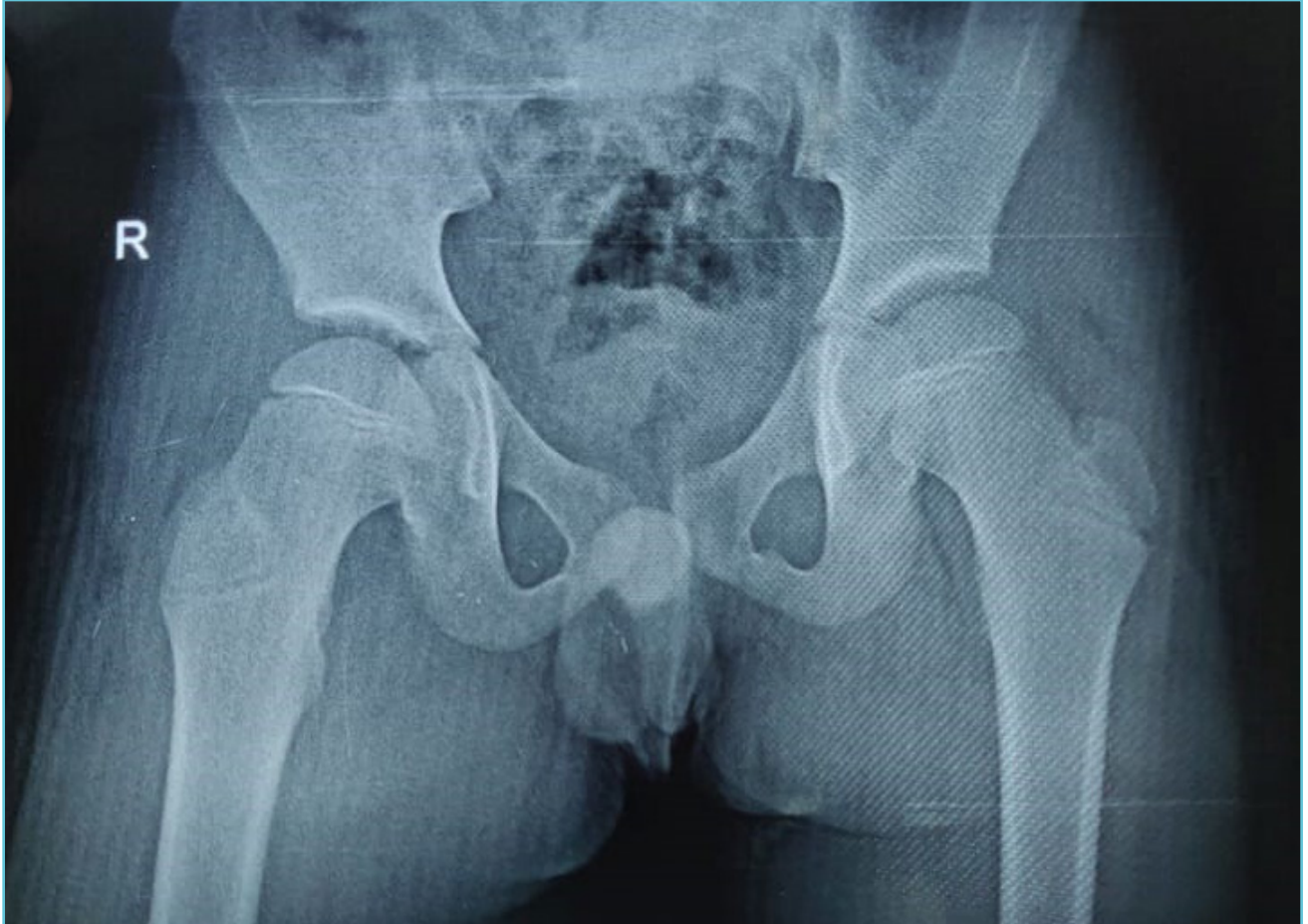
No distal neurovascular deficit was reported.

Figure 1A Plain radiograph of the right hip, showing no pathological findings



To exclude bony lesions X-rays in anterioposterior and lateral view were performed.

Figure 1B Plain radiograph of pelvis with both hip joints, showing no pathological findings



LABORATORY INVESTIGATIONS

Blood tests revealed an elevated level of C reactive protein of 4.8 mg/dL. Erythrocytes sedimentation rate was 80 mm/1 hr. Blood cell count showed 16.8×10^9 leucocytes/L with a differential of 87% neutrophils and 18% lymphocytes. Haemoglobin was 99 g/L and mean cell volume was 72 fL, revealing a microcytic anaemia with neutrophilia.

X-rays of the pelvis with both hips and of right hip with thigh revealed no abnormality. The ultrasound of the right hip joint showed mild increase in joint space of right hip joint as compared to the left hip joint, which indicated signs of inflammation.

Plain radiograph of the right hip showing no pathological findings. To exclude bony lesions X-rays in anteroposterior and lateral view were performed (Fig. 1A, 1B).

TREATMENT

Due to functional limitation, pain, and elevated CRP and leucocytes the decision was taken for arthrotomy & debridement of the right hip. The hip was aspirated with 16 G needle; pus was confirmed. An incision of 10cm posterior and lateral tip of greater trochanter and about 5cm upto proximal femur was done. The pus welled out into the wound which was sucked and drained. The capsule is retracted and thorough irrigation

Table 1 Laboratory parameters

Parameter	Result	Reference
C Reactive Protein	4.8mg/dL	<0.6mg/dL
Erythrocyte Sedimentation Rate	80mm/1hr	0-15mm/1hr
Haemoglobin	99g/L	138 -172g/L
Mean Cell Volume	72fL	80-96fL
Leucocytes	16.8x10 ⁹ cells/L	4.3-10.8x10 ⁹ cells/L
• Neutrophils	87%	40-60%
• Lymphocytes	8%	20-38%
• Eosinophils	1%	2-7%
• Monocytes	5%	4-7%
• Basophils	0 %	<1-2%

with copious amount of saline was done. A piece of synovium was taken for biopsy. Post operated limb was given skin traction to prevent flexion and internal rotation. Abduction pillow was also used.

Pus for gram stain revealed plenty of pus cells gram positive cocci in pairs and chains (Acid Fast Bacillus negative). Methicillin sensitive staphylococcus aureus was isolated from pus culture and sensitivity. Post operative treatment included intravenous Ceftriaxone, Amikacin, Diclofenac sodium, Pantoprazole. Oral medications include tablet Chymoral forte and Limcee.

The patient's condition remained stable after surgery. The CRP and white cell count (WCC) dropped on sequential postoperative testing. After 10 days the boy started to mobilise his leg spontaneously. No further surgical intervention was required and intravenous antibiotic treatment was continued for 14 days. At 2 weeks the

patient was discharged following normal clinical and laboratory findings. The parents were instructed to continue the child on oral antibiotic treatment for 4 weeks. Follow-up examination of 2 years revealed a healthy boy with a full range of motion and no sign of complications.

DISCUSSION

Septic arthritis in children is one of the few true orthopaedic emergencies. It is very important to make the diagnosis as early as possible to avoid sequelae such as cartilage destruction, osteomyelitis, ankylosis, growth aberration due to physical damage, joint instability and restriction of movement.^{7,9}

Early diagnosis in young children can be particularly challenging. In addition, clinical symptoms in these young patients can range from subtle signs, such as restlessness and poor appetite without fever, to the more common diagnostic

criteria of inflammation such as swelling, erythema, warmth, functional limitation and location specific pain.^{3,7} The medical history is, as always, crucial to diagnosis, in particular other foci of infection such as the respiratory tract.⁸

In contrast to children with septic arthritis (SA), children with transient synovitis appear well and are usually afebrile with just a mild limp.⁹ Differentiating septic arthritis from transient synovitis of the hip is particularly important given the need for urgent surgical intervention for the former condition.

The radiological investigations in this case did not reveal much important findings. X-ray findings are often unremarkable, as was the situation here, except in cases that have proceeded to chronic infection.¹⁰ Ultrasonography has a greater sensitivity than plain radiography and is becoming the modality of choice to reveal hip effusions.¹¹ When septic arthritis (SA) is suspected, synovial fluid should be obtained for a complete blood count (CBC), glucose, Gram stain, and culture. The C-reactive protein (CRP) is a more sensitive marker for septic arthritis than is the peripheral WBC count.¹¹ In one study, a CRP of more than 2 mg/dL was found to be a strong independent risk factor for SA of the hip among children presenting with hip pain.¹²

Septic arthritis of the hip requires emergent irrigation and drainage to minimize risk of aseptic necrosis of the femoral head. The optimal duration of antibiotic therapy is not defined, and recommendations vary from 1-6 weeks.

CONCLUSION

We presented a case where immediate surgical intervention and follow up helped in recovery of the patient. Our case uniquely demonstrates that a minimally invasive arthroscopic irrigation of the hip was adequate, and resulted in an excellent clinical outcome.

TAKE HOME MESSAGES/ LEARNING POINTS

- Time to diagnosis is the most important prognosticating factor in septic arthritis. Early institution of therapy helps to prevent degenerative arthritis.
- If lower extremity joints are involved, parents often report that children cannot bear weight and that they resist all efforts to move the involved joint.
- The presence or absence of fever may be helpful in distinguishing septic arthritis from transient synovitis of the hip.
- The C-reactive protein (CRP) is a more sensitive marker for septic arthritis than is the peripheral WBC count.
- Hospitalize all children presumed to have septic arthritis (SA) for empiric intravenous antibiotic therapy.
- Post operative skin traction is beneficial to prevent flexion.



Authors' contributions

Shireen conceived the idea and wrote and edited the manuscript.

Dr. Tulasi was the paediatrician managing the patient and contributed to the manuscript.



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Retraction of Publication

Editor of the eJIFCC

Retracted: Pathophysiology of Metabolic Syndrome

The Editor of the electronic Journal of the International Federation of Clinical Chemistry and Laboratory Medicine (eJIFCC) has retracted the article “*Pathophysiology of Metabolic Syndrome*” by Aganović and Dušek, which was published in eJIFCC. 2007 Feb 26;18(1):3-6 [1]. Upon notice from a reader, based on similarity index analysis, it was found that more than half the text is a copy of a previously published paper by Eckel *et al* [2].

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