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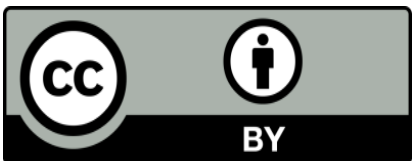

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References

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Al-Kindy College Medical Journal (KCMJ)

Editorial

Lifestyle Medicine: A Promising tool to Restoring Health

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Lifestyle Medicine is the application of evidence-based lifestyle approaches for the prevention, treatment, and even the reversal of lifestyle-related chronic diseases such as diabetes, hypertension, heart disease, obesity, polycystic ovarian diseases, dementia, arthritis, and cancers (1).

Type 1 and type 2 diabetes prevalence is predicted to rise by 54% by 2030. Obesity will affect about 50% of adults, and the prevalence of cardiovascular diseases will grow to 41%.

The goal of lifestyle medicine is to address the health issues brought on by changes in lifestyle during the past three to four decades, rather than to replace traditional clinical therapy

In 2004, the American College of Lifestyle Medicine was established. The lifestyle medicine board exam was offered in 2017, establishing a certification process for physicians and health professionals to validate their competency in this area. Lifestyle medicine should be regarded as a complement to conventional medical strategy and treatment (2).

The six pillars of Lifestyle Medicine are eating a predominantly whole food, a plant-based diet, Regular physical activity, Adequate sleep, Stress management, avoidance of risky substance use, and positive social connections. Lifestyle medicine helps people live healthier by producing personalized treatment strategies targeting these six domains and improving the environments in which people live and work besides its significant effect on chronic diseases, lifestyle medicine is one of the cheapest and most accessible therapy to all populations (3).

A healthy diet is built on eating plant-based foods such as whole grains, fruits, vegetables, nuts, beans, and seeds. Physical activity, such as walking, running, and strength training, should be done on a regular basis for optimal health. Additionally, every element of health can be impacted by stress, which is inescapable. It's crucial to acquire healthy management skills. In addition, reducing the use of addictive substances like alcohol, tobacco, and drugs is crucial for reducing the chance of developing cancer and heart disease. Sleep duration and quality have an impact on the immune system. Other habits and det adjustments can enhance the quality of your sleep. Both mental and physical health are enhanced by having supportive social networks(4,5)

Lifestyle interventions are the first-line approach for treating hypertension and are considered more effective than metformin in preventing the progress of prediabetes to diabetes. Additionally Lifestyle changes can reverse or regress certain cardiovascular diseases

To address the gaps in preparing physicians for the rising burden of chronic diseases,

medical school curriculum reform must include training in lifestyle medicine. A new healthcare system could effectively address non-communicable diseases and lead to wellness as a reality by transforming curriculum and developing new policies to encourage lifestyle medicine teaching in medical education and equipping medical practitioners with the tools they need (6).

Lifestyle-related chronic diseases are likely to increase in the near future. Hence, the prospects for a lifestyle medicine discipline definitely appear healthy. It is now time to use the evidences in lifestyle medicine and promote healthy lifestyles for our patients as well as for ourselves. By incorporating the principles of lifestyle medicine into daily medical practice, there is a significant opportunity to improve patient outcomes and raise the value of medicine as a profession.

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Al-Kindy College Medical Journal (KCMJ)

Review Article

Molecular Microbiology in Clinical Practice: Current and Future Applications

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ABSTRACT

Technological advances have yielded new molecular biology-based methods for the diagnosis of infectious diseases. The newest and most powerful molecular diagnostic tests are available at regional and national reference laboratories, as well as at specialized centers that are certified to conduct metagenomic testing. Metagenomic assays utilize advances in DNA extraction technology, DNA sequence library construction, high throughput DNA sequencing and automated data analysis to identify millions of individual strands of DNA extracted from clinical samples. At present, metagenomic assays are only possible at a small number of special research, academic and commercial laboratories. Continued research in human and pathogen genomic organization and host-pathogen interactions, represent important future goals that will maximize the information obtained from metagenomic assays. To illustrate the power and limitations of metagenomics, we report on a previously healthy 27 year old woman with work related exposure to ill animals, and who developed a rapidly progressive, severe diffuse interstitial pneumonitis that ultimately ended in the need for a double lung transplant. Metagenomic testing on DNA extracted from pleural fluid and nasopharyngeal swabs demonstrated the presence of expected normal bacterial flora along with some unexpected herpesvirus and non-HIV retroviral elements integrated into the patients DNA. Although no specific pathogen was ultimately identified to explain this patient's severe disease, the sample preparation and data analysis methods detailed herein illustrate the powerful benefits and limitations of metagenomic testing.

Introduction

During the past 25 years many extraordinary technological advances have yielded a multitude of molecular microbiology-based methods for the diagnosis and treatment of infectious diseases (1-6). Whereas once the diagnosis of patients with infections always required laborious special culture methods, waiting for pathogen growth in the lab, biochemical testing and in vitro determination of drug resistance, advances in molecular methods now can provide rapid results with highly reliable sensitivity and specificity. When first available, these tests were quite costly, however with time and increased commercial availability from multiple vendors, the cost of such assays is becoming more affordable.

The decision by any institution to invest in an array of molecular diagnostics depends on many important factors: the number and frequency of assay requests, the cost of hardware and software and reagents specific to any one test, the ability of patients or institutions to pay for these tests, the importance of a rapid result, and the availability of so called "reference laboratories" that have higher capacities and thus a wider array of available tests with a reasonably short turnaround time. At our institution, the most commonly

utilized commercially available molecular microbiology diagnostic assays are summarized in Figure 1.

Diagnostic methods for patient care performed at clinical microbiology laboratories in the USA must be certified by CLIA, the Clinical Laboratory Improvement Amendment of the US Federal Centers for Medicare & Medicaid Services (CMS) (7). CLIA requires all labs that perform even one diagnostic test on human samples to meet certain Federal requirements. If an entity performs diagnostic tests, it is considered under CLIA to be a laboratory and must register with the CLIA program. International CLIA laboratory certificates also are available.

A second tier of "reference laboratories" exists for institutions that do not have a high enough volume of samples to make it economical to invest in the required lab infrastructure. Most hospitals have formal contractual relationships with a list of external reference laboratories. The wide range of reference laboratories includes local and state public health laboratories, larger referral center and private institutional clinical microbiology labs (e.g. Mayo Clinic, ARUP, Washington University, University of Washington Seattle, and the Centers for Disease Control, Labcorp Inc., Quest Diagnostics Inc, and the US National Institutes of Health in Bethesda, Maryland, USA. Specific assays are only approved to be performed on specific types of samples, and thus an assay approved for cerebrospinal fluid or a bronchoalveolar lavage, may not be appropriate, sensitive or specific enough to run on a blood or stool sample.

The future or "next generation" of powerful molecular diagnostic assays include various types of metagenomic assays – methods that utilize advances in DNA extraction technology, high throughput DNA sequencing and automated data analysis to identify millions of individual strands of DNA extracted from a clinical samples (8,9). These methods can differentiate viral, bacterial and/or parasitic DNA from background human DNA in complex clinical samples. The extraordinary power of these methods, not only in automated identification of organisms that are difficult or impossible to cultivate in the lab, also provide a glimpse into the human genome of the patient who is being studied. Metagenomic tests are presently done only at specialized centers of commercial laboratories, and the type of sample each lab is authorized to analyze differs greatly. Some labs are only authorized to process specific sample sources (e.g. blood) while others have CLIA certification to process more diverse sample sources such as urine, skin scraping, pleural or cerebrospinal fluid, etc.

Understanding the sensitivity and specificity of new assays is essential for clinicians who choose to use such technology. Sometimes, even with the application of the most advanced forms of molecular diagnostic tests, such as the metagenomic screening described in the case report detailed herein, results are inconclusive. However continued research into the basic science underlying host defense mechanisms, along with better understanding of human and pathogen genomic organization and host-pathogen interactions, represent important future goals that will maximize the value of metagenomic data.

The last three decades saw the emergence of many important but previously unrecognized viral illnesses - viruses such as Ebola, MERS (2012, Middle East Respiratory Syndrome) caused by a

Respiratory samples:

- Respiratory Extended Pathogen Panel Nucleic Acid Amplified test (NAAT) Adenovirus, Coronaviruses (SARS-CoV-2, 229E, HKU1, NL63, and OC43), Human Metapneumovirus (HMPV), Human Rhinovirus/Enterovirus, Influenza A, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, SARS-CoV-2 (2019-nCoV), and Respiratory Syncytial Virus (RSV)) and bacterial pathogens (Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae, and Mycoplasma pneumoniae).
- Atypical Pneumonia Panel Nucleic Acid Amplified Test (NAAT) Legionella pneumophila, Tatlockia (Legionella) micdadei, Mycoplasma pneumoniae, and Chlamydia pneumoniae
- Bordetella pertussis/parapertussis Nucleic Acid Amplified Test (NAAT) Bordetella pertussis/parapertussis

Cerebrospinal fluid

- Meningitis and Encephalitis Panel Nucleic Acid Amplification Test (NAAT): Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae (Group B strep), Streptococcus pneumoniae, Cytomegalovirus (CMV), Enterovirus, Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Human Herpesvirus 6 (HHV-6), Human parechovirus, Varicella zoster virus (VZV), and Cryptococcus neoformans/gattii

Stool

- Clostridium Difficile Nucleic Acid Amplified Test with reflex to Toxin confirmation
- Clostridium difficile PCR positive will reflex to antigen testing

Sexually transmitted infections

- Herpes Simplex Virus Types 1 and 2 Nucleic Acid Amplified Test (NAAT) Herpes Simplex Virus 1 DNA, Herpes Simplex Virus 2 DNA
- Chlamydia trachomatis (CT)/Neisseria gonorrhoeae (NG) Nucleic Acid Amplification Test (NAAT) Chlamydia trachomatis DNA and Neisseria gonorrhoeae DNA
- HIV-1 RNA Quantitative Nucleic Acid Amplified Test (NAAT) HIV-1 RNA Copies/mL, HIV-1 RNA Log10 Copies/mL
- Trichomonas vaginalis Nucleic Acid Amplified Test (NAAT) Trichomonas vaginalis DNA
- Hepatitis C Quantitative Nucleic Acid Amplified Test (NAAT) Hepatitis C RNA IU/mL,
- Hepatitis C RNA Log10 IU/mL

Miscellaneous virus identifications

- BK Virus Nucleic Acid Amplified Test (NAAT) BK Plasma Log IU/mL and BK Virus Plasma Quantitative value in IU/mL
- Epstein Barr Virus Nucleic Acid Amplified Test (NAAT) Quantitative Epstein-Barr DNA Plasma Copies, Epstein-Bar DNA Plasma Log10 Copies, Epstein-Barr DNA Plasma IU, Epstein-Barr DNA Plasma Log IU
- Cytomegalovirus Nucleic Acid Amplified Test (NAAT) Qualitative
- Hepatitis B

Figure 1. Molecular microbiology tests performed in house at our institution, Froedtert and the Medical College of Wisconsin, Regional Medical Center, Milwaukee, Wisconsin, USA.

coronavirus, SARS (2002, Severe Acute Respiratory Syndrome caused by a different coronavirus), the current pandemic COVID19 (yet another coronavirus), Monkeypox and others (10-15). Especially in circumstances where standard methods do not identify a suspected pathogen and the number of possible pathogens is large, application of metagenomic testing is an extremely powerful tool. An example of the power and limitations of metagenomic testing is illustrated herein with a case report of a previously healthy woman who had extensive contact with sick animals and developed a rapidly progressive, nearly lethal pneumonitis and required a double lung transplant.

Case report

A 26 year-old previously healthy female was admitted to the hospital with shortness of breath, fevers and progressive interstitial pneumonitis. She was a student of veterinary medicine and worked with sick animals part time in small animal clinics in the midwestern United States (Wisconsin and Michigan) as well as on the island of Grenada in the Caribbean. She had not travelled outside of the USA for more than one year before she became ill, but had encountered a wide variety of sick small and large domestic animals. She was unaware of any coworkers who had a similar illness. Standard diagnostic assays for bacteria, fungi, parasites and viruses were all negative. Nonetheless there was concern that she had acquired an uncommon zoonotic viral infection that was beyond the capabilities of a standard US hospital based clinical microbiology laboratory. About one month prior to her illness, she was prescribed a short course of the antibiotic, trimethoprim-sulfamethoxazole (Bactrim™) to treat painful cysts, however she stopped that medication after just one day due to the development of a rash. She completed her cyst treatment using a fluoroquinolone antibiotic and recovered completely by the time of her hospital admission for respiratory failure.

Over the course of several weeks, her pulmonary function steadily deteriorated, she developed bilateral diffuse consolidative infiltrates and required intubation and mechanical ventilation with high oxygen concentrations with elevated positive end expiratory pressures to maintain adequate blood oxygenation. After several more weeks, mechanical ventilation failed to maintain adequate oxygenation so she was moved to the cardiac intensive care unit and administered ECMO (extra corporal membrane oxygenation), (16)) via large bore catheters inserted into her venous and arterial systems. These efforts were complicated by both venous thromboembolisms despite anticoagulation and multiple pneumothoraxes. After two months on ECMO, she remained neurologically intact, she was fully alert and oriented and was able to participate in physical therapy and occupational therapy sessions. However her lungs did not recover and she developed densely consolidated lungs bilaterally consistent with rapidly progressive pulmonary fibrosis. The institutional organ transplant committee eventually concluded that her lungs had been irreparably damaged by an unknown cause so therefore she became a candidate for lung transplant. When a donor became available, she was transferred to another medical facility where she received a double lung transplant. At the time of this report, she was recovering well from surgery.

Metagenomic methods

Several nasal pharyngeal swabs and 5ml aliquots of pleural fluid obtained from her chest tubes were frozen at -800 C and used in a metagenomic testing algorithm that has proven valuable for identification of unknown viral, bacterial, fungal and parasitic zoonotic respiratory infections in the USA and globally (17-33). All standard microbiological testing available at our facility did not identify a pathogen. Fortunately, the high cost of metagenomic testing (presently $5,000-$8,000) of this patient's samples was minimized by working with a reference lab that was actively researching the etiology of pneumonitis in patients with animal exposures.

5 milliter aliquots of pleural fluid were centrifuged to process supernatants and cell pellets separately. Sufficient high quality nucleic acid (5-118 nanograms) was extracted from each pleural fluid sample and cell pellets, and nasopharyngeal swabs. DNA extracted from cell pellets, swabs and supernatants were analyzed separately. High quality sequencing libraries were generated from each DNA sample. DNA sequencing yielded the anticipated ~30 million reads per sample along with good quality DNA from positive control samples, and appropriately low background negative controls. The DNA extracted from nasal-pharyngeal swabs was sufficient to yield more than 30 million independent sequences for analysis. A subtraction method was utilized to enrich DNA samples for possible pathogen nucleic acids compared to human DNA. Data was analyzed for the presence of viruses, bacteria, parasites and fungi, as well as any viral elements that were incorporated into the patient's own DNA (20).

Results

Bacteria. No bacteria were detected in any of the pleural fluid samples. However the nasal swab contained a number of bacterial sequences representing normal oral flora: Veillonella parvula, Actinomycetes oris, Gemella haemolysans, Rothia ssp., Corynebacterium ssp., Streptococcus ssp.

Fungi. A polymerase chain reaction (PCR) assay was used that targets the internal transcribed spacer (ITS) region of the fungal ribosomal RNA locus proven to identify basically all fungi. Primers were used for the ITS2 region for screening, which generates an amplification product of 375 nucleotides. No signal was obtained with any of the samples after 35 cycles of PCR. Similarly, no protozoan or metazoan parasite sequences were identified.

Viruses. A single sample revealed Ebstein Barr Virus (human herpesvirus 4) DNA in the pleural fluid supernatants but not the corresponding cell pellets. On the other hand, several different endogenous retrovirus elements were identified in all samples (as expected for any human sample. Figure 2 shows the variety of viral elements found in this patient's samples.

Discussion

Metagenomic testing is an extraordinarily powerful method to explore the human "respiratory virome" when standard methods do not identify a known pathogen. Yet a major drawback at present is the expense and labor involved to yield reproducible data. However when applied judiciously, these methods have proven effective in identifying a wide array of viruses in the lungs of ill patients. The

Vietnam Initiative on Zoonotic Infections (VIZIONS) consortium of researchers has studied persons with severe respiratory distress after animal contacts in an effort to detect new or emerging infectious agents in humans and animals (23,24). The long-term goal of VIZIONS will assess how frequently new pathogens are circulating in human and animal populations and assess how frequently they are exchanged between species. In many of these studies, viruses previously not known to induce human disease have been identified. Whether or not all these new viruses will ever be considered human pathogens versus colonizers, remains to be determined. To date, this pioneering research has found numerous instances of expected human rhinoviruses, enteroviruses, influenza A virus, coronavirus OC43, and respiratory syncytial virus, rotavirus, Torque-teno virus, human papillomavirus, human beta herpesvirus 7, along with novel cyclovirus, vientovirus, gemycircularvirus, and statovirus (17-23). The blood DNA virome in several thousand asymptomatic humans has been reported by researchers in the USA and Singapore (Human Longevity Ltd.) 94 different viruses including DNA viruses, parvovirus and RNA viruses were identified in 42% of patients studied (34). RNA viruses included herpesviruses, anelloviruses, papillomaviruses, polyomaviruses, adenovirus, HIV, HTLV, hepatitis B and C, parvovirus B19 and influenza. Of relevance to transfusion medicine Merkel cell polyomavirus, papillomavirus and parvovirus were also identified. None of these aforementioned viruses were explained by contamination of commercial regents or the environment (35,36). The potential for detection of rare environmental or contaminating DNA sequences emphasizes that the ultrasensitive metagenomic methods now available must be designed and interpreted with great caution.

Despite all our efforts, no single pathogenic microbe was identified in this patient prior to or after double lung transplant. For the purposes of the USA national lung transplant registry database, the diagnosis for this patient was entered as "rapidly progressive interstitial fibrosis, possibly secondary to an adverse immunological reaction to oral (Bactrim™, trimethoprim sulfamethoxazole)". The same month as this patient's hospitalization, the USA Food and Drug Administration added a new labelling warning for Bactrim™ users that in spite of its widely accepted use in tens of millions or persons across the USA for almost 50 years, in the past 10 years a small number of young patients on Bactrim™ were reported to develop progressive pulmonary dysfunction resulting in death, the need for ECMO or lung transplant (37). The absence of an infectious or clear autoimmune etiology for this patient's illness was frustrating for all providers involved, however the results of metagenomic testing did reveal some curious information.

It is intriguing to speculate on the presence of latent herpesviruses such as EBV and endogenous retroviruses found in this patient. Although there is no clear pathogenic role of these organisms in patients with pulmonary fibrosis, there is considerable, sometimes contradictory clinical research ongoing that is exploring roles for latent human herpesvirus 8 (EBV) in the pathogenesis of pulmonary fibrosis (38,39). In our patient there was serological evidence of only two latent viruses - EBV early antigen IgG and CMV IgG, both common in most young healthy adults in the USA.

Lastly, the multiple strains of retroviruses found incorporated into this patient's DNA, are well described in this era of human genome analysis. It has been well documented that the human genome includes repetitive DNA elements, among which human endogenous retroviruses (HERVs) account for about 8% (40). Evolutionary biologists consider HERVs to be DNA sequences of retroviral origin that have been acquired along the last 100 million of years through multiple integrations by now-extinct exogenous retroviruses. Furthermore, a direct immunostimulatory or immunosuppressive role for these elements have been reported in a number of studies. For example, an immunostimulatory role for HERV-W has been reported to play a role in dendritic and monocytic cell activation and the production of pro-inflammatory cytokines (41). In contrast, HERV-P71 and HERV-H have been reported to effect immune-suppression, including a Th1 to Th2 shift in immune tolerance and inhibition of T cell activation (42).

Conclusion

Although metagenomic testing of our patient's pleural fluid and respiratory secretions did not identify a likely pathogen, this work nonetheless illustrates the extraordinary sensitivity and power of such next generation sequencing algorithms (43-45). We remain hopeful that once the high cost is lowered and the complex methods becomes more widely available, metagenomics will lead to many important new insights into the pathogenesis of new and emerging infectious diseases, as well as insights into immunopathological phenomena related to host-pathogen interactions.

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None

Conflicts of interest

The authors declare no conflicts of interest

Family	Genus	Species	---	IPS-567_NPS	IPS-568-PFL-F	IPS-568-PFL-S	IPS-568-PFR-	IPS-568-PFR-S
Herpesviridae	Cytomegalovirus	Stealth virus 1	---	8	0	3	7	4
Herpesviridae	Lymphocryptovirus	Human gammaherpesvirus 4	---	339	0	5	0	2
Papillomaviridae	Betapapillomavirus	Human papillomavirus type 5b	---	3	0	2	5	7
Retroviridae	Lentivirus	Human immunodeficiency virus 1	---	35	4	58	40	47
Retroviridae	Retro_unclassified	Human endogenous retrovirus HCML-ARV	---	3	0	9	8	5
Retroviridae	Retro_unclassified	Human endogenous retrovirus HERV-K(II)	---	7	1	10	3	9
Retroviridae	Retro_unclassified	Human endogenous retrovirus KC4	---	8	1	16	8	7
Retroviridae	Retro_unclassified	Human endogenous retrovirus K	---	75	0	134	117	123
Retroviridae	Retro_unclassified	Human endogenous retrovirus	---	28	1	54	35	60
Retroviridae	Retro_unclassified	Multiple sclerosis associated retrovirus element	---	5	0	9	10	9

Figure 2. Output from metagenomic testing revealed a variety of Herpesviruses, Papilloma viruses and ancient non HIV/HTLV endogenous retroviral elements integrated into this patient's DNA

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Review Article

A Role of Therapy that Targets Immune Checkpoint Proteins for the Treatment of Melanoma Brain Metastasis, Liver, Breast, Pancreatic Cancer and Pancreatic Adenocarcinoma

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ABSTRACT

Checkpoint inhibitors are a type of immune therapy used to treat different types of cancers. These drugs block different checkpoint proteins, for example, CTLA-4, PD-1, and PD-L1 inhibitors.

They block proteins that stop the immune system from attacking the cancer cells. Checkpoints are also described as a type of monoclonal antibody that antagonizes binding between B7 to CTLA-4 and PD-L1 to PD-1.

Immune checkpoint inhibitors are used to treat BARCA mutated triple-negative breast cancer (TNBCS) in patients who do not respond to chemotherapy, and also in the treatment of highly mutated and solid tumors such as brain tumors, liver, and pancreatic cancers.

Immune checkpoint inhibitors exhibit an effect on solid tumors by suppressing CTLA-4, PD-1, and PDL-1. Anti-PD-1 is less toxic than anti-CTLA-4.

For melanoma Brain metastasis immune checkpoint therapy is more effective and Combination therapy has great efficacy and less toxicity which improves overall survival rather than individual therapy.

liver cancer as hepatocellular carcinoma and cholangiocarcinoma used treatment with Genetics based therapy while using alternative immune checkpoint ligands, co-inhibitory (eg. LAG-3) or decreased t-cell infiltration causing therapy failure.

Clinical studies for pancreatic cancer have not been completed yet and treating PDA needs more research as immune checkpoint inhibitors is a new treatment against PDA. A new potent class of nivolumab, pembrolizumab, and ipilimumab have been FDA approved.

For mutated tumors, Combination therapy between checkpoint inhibitors and chemotherapy has great efficacy and improves the city of life and overall survival, rather than individual therapy when using radiation or chemotherapy alone.

Introduction

The integral function of the immune system is its ability to differentiate between self and non-self-cells. For this purpose, the immune system depends on multiple"checkpoints", which are molecules on certain immune cells that need to be activated or inactivated to start an immune reaction. Tumor cells often take advantage of these checkpoints to avoid being detected and attacked by the immune stem. A novel mode of cancer treatment is checkpoint inhibitors. (1,2)

Immune checkpoint inhibitor has been along with the standing goal of cancer research as they activate the immune system to eliminate cancer cell and produce clinically relevant responses, they work by activating anti-tumor immunity. Immune checkpoint inhibitors activate the immune system to eliminate cancer cells and produce clinically relevant responses, they work by activating anti-tumor immunity. To provide immune checkpoint inhibitor, immune therapies enhance the cell-mediated immune response against tumor cells leading to the generation of a long- term memory lymphocyte population patrolling the body to rave growth of any cells, this leads to sustain the therapeutic effect. New clinical results suggest that Combination immunotherapies offer more potent anti-tumor activity. Cancer treatments are categorized into four different classes: one of them is immune therapies which are drugs that target the induction or augmentation of anticancer immune responses (3).

Preclinical data surrounding immune checkpoint proteins, including BTLA, VISTA, CD1 60, LAG3, TIM3, and CD244 exhibit that they are potential targets for inhibition. (4) .

The checkpoint acts as the brakes of the car, they help to prevent The immune system from getting out of control and attacking the body's cells. Checkpoints will suppress the activation of the immune system and activates T-cells into action.

In a normal physiological state, the body uses an immune checkpoint to organize the time and extent of the immune response in tissues to reduce their damage. Research suggested that the immune resistance's mechanism is that tumor cells take control of certain immune checkpoint pathways, ones against T cells that are specific for tumor antigens. Because many immune checkpoints are initiated by ligands- receptor interactions, they can be readily blocked by antibodies or modulated by recombinant forms of ligands. (5).

Immune therapy referred to as the immune checkpoints
exhibit positive outcomes in very advanced and metastatic cancers. These antibodies target specific molecules on immune cells to enhance the immune system to kill cancer cells. Two promising classes of antibodies, anti- CTLA- 4, and anti- PD- 1 are used as monotherapy and as a combination.

Cancer immunotherapies in clinical development can typically be categorized into 3 major types: nonspecific or adjuvant therapies, targeted therapies, and vaccines.

First, nonspecific immunotherapies include drugs that may stimulate an immune response or drive the growth and proliferation of immune cells which target and kill cancer cells. Second, immunotherapies which are called targeted therapies like (monoclonal antibodies, mAbs, and small molecule inhibitors), and third, the vaccines that are antigens made from cancer cells designed to stimulate the immune system to attack the tumor.

ICIs described in this publication are cancer immunotherapies that are categorized under mAbs. (6,7)

T-cells in tumors do not functionally work in all cases so the strategy for the therapeutic target that acts to boost the immune system to modulate either the costimulatory or check into proteins that express is ended on the T-cell surface. (8,9)

Agents that turn on costimulatory proteins could directly activate T cells. In contrast, agents that block checkpoint proteins could enhance the immune system to activate T cells. Anti- CTLA- 4 and anti- PD- 1 are checkpoint proteins that enhance T cell function by preventing inhibitory signals.

They regulate immune responses at different levels (early and late, respectively) and by different mechanisms. (10,11)

The new appearance of immune checkpoint inhibitors eg. T-Lymphocyte antigen4 and PD-1 (programmed cell death) have a role in the treatment of solid tumors, The combination therapy between different immune checkpoint inhibitors therapies has reduced toxicity and improved the response, We make potential synergistic combinations between checkpoint Blockade and newer immune therapies.

The checkpoint Blockade (radiation, chemotherapy, and targeted therapies) and newer immune therapies (cancer vaccine, oncolytic virusea).

There are reliable biomarkers that are necessary to define patients who achieve clinical benefit with minimal toxicity in combination therapy. (12,13)

Immune checkpoints such as CTLA-4 and PD-1 play a role in T cell activation or apoptosis, subsequent Preclinical research showed their important role in the maintenance of peripheral immune tolerance and development of cancer immune therapy.

Mice deficient in immune checkpoints CTLA-4 or PD-1 develop an autoimmune-like disease. Therefore, it was surprising that single-agents-CTLA-4 and anti-PD-1 are so effective anticancer treatments in humans.

These therapies have revolutionized cancer immunotherapy as they showed for the first time in many years of research their effect on metastatic melanoma (14)

Finally, immune inhibitors have had a significant ant role in treatment treating the last two decades the US Food and Drug Administration (FDA) approvals of two different antibodies, (1) against cytotoxic T- lymphocytes antigen-4 (CTLA-4) and (2) against programmed death-1 (PD-1) for treating different types of cancer. (4).

Review

The role of an immune checkpoint inhibitor in the treatment of:

Melanoma Brain Metastasis:

Brain tumors are very rare and most of them are malignant. Glioblastoma is the most common brain tumor and the most common source of metastatic brain tumors that come from lungs, breasts, and skin. (15).

Studies reported that melanoma Brain metastasis is treated with both SRS and anti-CTLA-4 therapy as well as SRS and anti-PD-1 therapy.(16,17,18)

Tumors utilize immune checkpoints as major mechanisms of immunity. The best- characterized immune checkpoints are cytotoxic T- lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (D-1). Ipilimumab(a monoclonal antibody targeting CTLA-4), was the first immune checkpoint approved by the FDA for the treatment of advanced melanoma. (19,20)

Later, nivolumab and pembrolizumab which both are anti-PD-1 (monoclonal antibodies) also was approved by FDA for treating different types of cancer including melanoma.(14,21)

The data suggest that the combination therapy of anti -PD-1and SRS may result in greater and more rapid lesion shrinkage in the initial months after SRS than a combination between anti-CTLA-4 and SRS(22).

Preclinical studies suggest that combination therapy is more effective than individual therapies. Synergism between checkpoint blockade and radiation therapy has been shown in preclinical trials. (23).

The results suggest that immunotherapy with radiotherapy in the treatment of brain metastasis and early lesional has a synergistic effect response that is greater and more rapid. (22).

Liver cancer:

Checkpoint blocking approved its role in the management of the prognosis of liver cancer PD-1, PDL-1, CTLA-4, and B7-1 inhibition seems to be clinically effective in tumor treatment.

PD-1 is found to suppress T cell activation in peripheral tissue in the late stage while CTLA-4 is found to regulate T-cell in lymph nodes at the priming stage. (24).

Genetics-based drugs were used with immune checkpoint blockers as a therapy for liver cancer. sing radiation or chemotherapy in combination with immune checkpoint inhibitors is reported to have a synergistic effect because of their multiple mechanisms. (25)

limitations:

1-Resistance is observed to immune checkpoint therapy.

2-Mutation of cancer immunogenicity is one of the reasons for therapy failure (eg. mutation in transporters B2 microglobulin for example)

3-Using alternative immune checkpoint ligands or alternative co-inhibitory immune checkpoints (eg. LAG-3) can also be a reason for therapy failure.

4-Decreased t-cell infiltration is one of the therapy failure reasons. (18, 26 ,27,28)

Clinical trials of using immune checkpoint inhibitors for HCC and cholangiocarcinoma are under observation. Encouraging clinical outcomes were reported from an ongoing phase I/II trial of the anti-PD-1antibody nivolumab at the 2015 American Society of Clinical Oncology(ASCO)showed that decreasing the tumor size to some extent in all cohorts including uninfected, HBV Infected, and hepatitis C virus-infected HCC patients.

It was proved that nivolumab in the treatment of HCC patients was effective and patients exhibit a response. In other trials, nivolumab showed a manageable safety profile, including acceptable tolerability.

The objective response rate was 20% in patients treated with nivolumab 3 mg/kg. (12,26,29,30,31,32)

Immunotherapy is promising for HCC and cholangiocarcinoma. Despite the ability of the patient's body is responding to only one immune therapeutic drug it is recommended to use combination immunotherapy for being more durable and potent.

In phase l experiment, it was exhibited that using of radiation frequently and cold cryo-ablation extremely for tumors enhancing immune response and anti-CTLA 4 treatment. Using this mixture is safe and results increase in intratumoral CD8+ T cells and activate T cells.

A combination of ablation treatment and immune checkpoint inhibitors was suggested to be used in advanced liver cancer. Using cisplatin as the chemotherapeutic drug can decrease PD-L2 production on tumor cells. Studies show that chemotherapy can enhance anti-tumor immunity so may combine and augment immune checkpoint therapy for the treatment of liver cancer. (29,33,34)

Epigenetic modulators increase immune checkpoints on the cell surface which enhance response to immune checkpoint inhibitors therapy.

A study on the use of histone methyltransferase and histone deacetylase inhibitors (HDACi) has been investigated in affecting immunity and cancer therapy. In vivo experiment showed that in mice with melanoma that HDACi increased expression of PD-1 and PD-2 through up-regulation of histone acetylation, the combination of HDACi and PD-1 blockade led to higher efficiency in tumor progression and improving survival rate than single-agent therapy.

3-Deazaneplanocin A and 5-aza- 2′deoxycytidine, two important DNMTi, enhanced the therapeutic efficacy of PD-L1 blockade in reducing tumor volume, increasing tumor-infiltrating CD8+ T cells, and Th1 -type chemokine expression in ovarian cancer in C57/BL6 mice.

Taking a 5-azacytidine drug makes the cancer cell more sensitive to anti-CTLA 4 and was observed to be more effective than using anti-CTLA 4 or 5-azacytidine alone in a mouse model having melanoma. (9,18, 35,36,37).

Immunotherapy with chemotherapy or radiation therapy when being used as a combination, they have synergistic action which may give hope to patients who have liver cancer with a dismal prognosis.

Breast cancer:

Breast cancer has a poor prognosis and is still facing the problem of tumor recurrence, resistance rate, and relapsing while the treatment has been prolonged. Triple-negative breast cancers' survival rate is worth than estrogen receptor-positive tumors due to a high relapse rate,(TNBC) is more diagnosed in people younger age>50 and people with BRCA mutations particularly(BRCA 1 and BRCA2), So it's Treated by chemotherapy alone but it recurrence again and may cause death, the combination of therapy that achieves an effective and durable treatment response is needed. (38,39).

A breakthrough in cancer immunotherapy was the discovery of an Immune checkpoint inhibitor for the treatment of cancer in combination with chemotherapy-like platinum agents such as cisplatin. (40,41)

Combining chemotherapy with anti-CTLA-4 and anti(PD-1 /PD-L1) causes deficiency in BRCA-1 mutation of breast cancer and improvement of survival. (42)

Due to increasing in activated tumor-infiltrating cytotoxic CD8+ T cells and CD4+ T cells was accompanied by the induction of polyfunctional effector CD4+ T cells, causing a turn up from suppressive to a cytotoxic immune phenotype in TILs.

Toxicity with anti-CTLA4 is higher than with anti(PD-1 /PD-L1) due to the different distribution of ligands due to PD-1 preventing binding between PD-L1 and PD-L2 ligand (, where they found that combination may cause lots of side effects. The combination between Checkpoint inhibitor and chemotherapy shows a great result n TNBC with patient BRCA1 -mutated tumor in the clinical trial, and possibly other hypermutated breast tumors. (43,44)

Results of the animal test suggest that: No increase in toxicity was observed in mice treated with the combination compared to chemotherapy alone,

chemotherapy can act as an immunological adjuvant in the tumor microenvironment by promoting the release of tumor antigens via immunogenic cell death, thus priming DE Novo T cell responses and improving the efficacy of checkpoint blockade. (45)

Mechanism of an immune checkpoint inhibitor for the treatment of breast cancer targeting(CTLA-4)and(PD-1 /PD-L1), ICI used with patients have no response for chemotherapy alone, checkpoint protein such as PD-L1 on tumor cells and PD-1 on T cells help keep immune response in check (when PD-L1 bind with PD-1 send signal off that prevent T-cell to kill tumor cell) by blocking this binding between PD-L1 and PD-1 by using(anti-PD-L1)and(anti-PD-1) send a signal on that enable T cell to kill tumor cell, Immune checkpoint inhibitor blocking the binding of B7 to CTLA-4 by (anti-CTLA-4) activate T cell to kill the tumor.

Pancreatic cancer:

FDA approved the role of immune checkpoint inhibitors in the treatment of solid pancreatic tumors by suppressing CTLA-4, PD-1, and PDL-1 (which were forming protein-expressing tumors and weakened immune response), so by inhibiting them T-cells and immune response increased .(1,46)

Le et al observed the stable disease in five patients (two Arm A, three

Arm B), four according to RECIST (two Arm A, two Arm B) and one according to irRC (Arm B), when treated with 1 0 mg/kg Ipilimumab alone (Arm A) or in combination with GVAX-vaccine (Arm B).

Royal et al, noted delayed progression in one patient when treated with 3 mg/kg Ipilimumab.

Furthermore greater overall survival of 5.7 mo in patients treated with Ipilimumab and GVAX-vaccine, compared to 3.6 mo of Ipilimumab monotherapy was in this study noted.

Aglietta et al demonstrated a partial response in two patients with advanced pancreatic ductal adenocarcinoma, receiving 1 5 mg/kg Tremelimumab.

Among patients with pancreatic ductal adenocarcinoma, immune checkpoint therapies also appear to be effective.(5,47,48,49)

These studies are limited, these results of treatment with immune checkpoint inhibitors in PDA indicatthatat further research is needed. pancreatic adenocarcinoma (PDAC) is an exocrine pancreatic cancer that begins in the cells that line the ducts of the pancreas.PDAC is the most common type of pancreatic cancer, accounting for more than 90% of pancreatic cancer diagnoses. It has been reported that up to 10% of PDAC have a hereditary component. (50).

The extent of mutational changes in pancreatic tumors generates gene instability that appears to play an essential role in PDAC tumor growth and resistance to treatments. PDAC is the fourth most common cause of cancer-related deaths in the United States and a major health issue.

(PDAC) is a highly aggressive cancer with poor patient survival due to a lack of understanding of the etiology and tumor biology, early diagnostic markers, diverse genetics, rapid metastasis, screening, treatment of PDAC and delayed detection which is due to symptoms do not appear until the disease has progressed and metastasized to distinct sites.

Multiple studies have demonstrated that The poor prognosis and the difficulty in establishing efficacious therapeutic strategies for PDA come from the PDAC micro-environment. (51,52).

The Tumor micro-environment (TME) is characterized by dense desmoplasia and extensive immunosuppression which results in decreased stromal vascularization, altered immune cell infiltration, and hypoxia, inducing tumor growth and hindering drug activity. (53,54,55)

The surgical resection with chemotherapy provides the best treatment option for PDAC and is beneficial in patients whose cancer cells have not spread to critical abdominal vessels and adjacent organs.

Traditional treatments for PDAC are limited and ineffective such as radiation therapy or chemotherapy which despite recent advancements in systemic chemotherapeutic regimens, the median survival time of advanced pancreatic cancer patients remains 4-11 months.

During the last decades, Immunotherapy like immune checkpoint inhibitors offered encouraging results in preclinical models and several clinical trials have explored its therapeutic application in PDAC but in combination with other therapies which need more clinical research. (56,57).

clinical studies on treating PDA need more research as immune checkpoint therapy is a new treatment against PDA, A new potent class in treating different types of tumors like ipilimumab has been approved by FDA for different types of cancers and further information is needed about PDA to personalize combination treatment.

So in this research, we will study the effect of a combination between immune checkpoint inhibitors and stromal targeted therapy.

The clinical trials that are running now, describe that immune checkpoint therapy is a new and novel treatment perspective in the fight against PDA. A couple of trials are combining two of the checkpoints inhibitors, Durvalumab and Tremelimumab,

Other trials are testing checkpoint inhibitors in combination with cytostatics, in combination with a vaccine, or as monotherapy. Currently, no results from these trials are present.

Further knowledge is needed regarding the tumor of pancreatic ductal adenocarcinoma, to determine the activated immune suppressors within an individual patient's tumor to develop a personalized combination treatment. (58,59)

LAG-3(checkpoint molecule) is blocked by IMP321 (immune checkpoint blocker). There are other checkpoints TIM-3 and BTLA that can treat cancer. The combination therapy of ipilimumab and GVAX-vaccine improve survival more than monotherapy.

Cytostatic and radiation therapy leads to tumor cell death and antigen release that leads to t-cell activation in the tumor(60,61,62)

Conclusion

Immunotherapy by checkpoint inhibitor makes a breakthrough in In recent years that use immune checkpoint inhibitors to eliminate cancer cells of various types of solid tumors for example breast, pancreatic, liver, and brain tumors.

Nivolumab has been proved its efficacy and safety in hepatocellular carcinoma, on the other hand, Ipilimumab, Tremelimumab, and Durvalumab appear to be effective with pancreatic ductal adenocarcinoma. Also, Ipilimumab is approved by FDA to treat advanced melanoma.

FDA permitted the use of ipilimumab, pembrolizumab, and nivolumab which are immune checkpoint drugs for the treatment of non-small cell lung cancer and also for bladder cancer, and renal carcinoma, and lymphoma.

Combinations using immune-based therapy like vaccines, radiosurgery, chemotherapy or radiotherapy, and others with immune checkpoint inhibitors targeting CTLA-4, PD L1, and PD- 1 or a combination of multiple immune points inhibitors may be more effective in producing an anti-tumor immune response (Kyi and Postow,201 6), (Vaddepally RK, et al., 2020), like, checkpoint inhibition and SRS may be safe to administer, produce favorable survival outcomes and not increase the toxicity of therapy in the treatment of melanoma brain metastases .

Also in breast cancer, anti-PD1/PD L1 and anti-CTLA-4 can be used in different types of breast cancer, particularly in TNBC and it will increase the efficiency of treatment of breast cancer or advanced pancreatic cancer when a combination occurs between checkpoint inhibitor and chemotherapy or radiotherapy .

Finally, treatment with an immune checkpoint inhibitor shows that it needs more studies on a patient level to prove its true efficacy and it will be success.

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Research Article

Changing the Trends in Surgery during the COVID-19 Times: An Experience from the Eastern Uttar Pradesh State, India

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ABSTRACT

Background: The COVID-19 pandemic has an immense effect not only on the social and economic lives of people but also on the surgical lives of surgeons, residents, nursing staff, and patients as well as ground level staff. Amidst this COVID pandemic, emergency surgeries were being done but at a decreased rate, whereas elective cases depended on the will of hospitals, surgeons, and patients. Study aims to promulgate a "Neo–Surgical Check Box" by amalgamating the WHO surgical checklist and the results obtained from the questionnaires.
Subjects and Methods: After receiving ethical clearance from the Institute Ethical Committee, an online questionnaire with 50 questions divided into three sections was distributed to 235 surgeons in Uttar Pradesh.
Results:Two hundred and eight surgeons had responded, out of which 154 were male and 54 were female. Only 41.4% (86) were actually conducting the surgery. The emergency surgery rate was 60.3%, whereas 18.6% of elective surgeries were done, 11.8% of surgeries were avoided, and the rate of minimal access surgery was 9.3%.
Conclusion: The "Neo-Surgical Check Box" will not only increase the number of surgeries per day but also provide protection to the healthcare workers while handling not only COVID-positive patients but also any airborne communicable diseases.

Introduction

A global influenza pandemic in 1918 and 2009, H1N1, i.e., swine flu, wreaked havoc on humanity. (1, 2) Lots of people died and suffered. It has been observed that surgical emergencies are complicated by respiratory infections. (3) As a result, surgical preparedness is critical in order to reduce the unwanted infection rate, mortality, and morbidity of not only patients but also healthcare workers. The coronavirus attack, which had its epicentre in Wuhan, China, illustrates a similar situation when the whole world was unprepared for the worse outcome. (4) The recent public health emergency declared by WHO had an immense effect on the surgeons and patients who required surgical care. (5).

As India was facing a major challenge not only in terms of scarcity of resources but also in mobilizing human resources at the cost of surgical branches, (6) The infectiousness, widespread fear, and loss of life among front-line workers had wreaked havoc, resulting in a drop in surgical rates. According to Orhan et al., out of 321 consulted emergency cases, 129 were admitted, out of which 49 patients (38%) were operated on. (7) A reduction in emergency intervention as compared to previous years has been noticed. (8)
The WHO Surgical Safety Checklist has been proposed to reduce the rate of surgical site infection, which as a result will reduce morbidity and mortality. (9). Similarly, the combination of the WHO SSC and new normal norms will almost certainly benefit patients, frontline

workers, and surgeons. The Neo-Surgical Checkbox (10) will provide a comprehensive description of the preoperative assessment, intraoperative requirements, and post-operative outcome, benefiting surgical care seekers while protecting healthcare workers. Therefore, it was vital to preserve manpower by minimizing infections among surgeons. However, they were not at the forefront, but the chances of getting infected in the operating room were quite high. (11) Therefore, operating room protocol needs to be modified starting from the entry till the discharge of the patient. (12) To overcome this crisis type of situation in the future, to handle the droplet infectious cases without any hesitation and to provide them with appropriate surgical care on time, we promulgate a "Neo–Surgical Check Box."

The "Neo-Surgical Check Box" has been developed by taking into account the standards developed by World Health Organization Surgical Safety Checklist (WHO-SSC) and results obtained from experienced surgeons.

The "Neo-Surgical Check Box" will not only increase the number of surgeries per day but also provide protection to healthcare workers while handling not only COVID-positive patients but also any airborne communicable diseases.

Subjects and Methods

Study type: Questionnaire-based prospective study Material used: After obtaining ethical clearance from the Institutional Ethical Committee. The questionnaire has been circulated online among the surgical branch doctors of Eastern Uttar Pradesh working in tertiary centres. The survey has been conducted for months (March 2021-Nov 2021). This questionnaire has been sent to 235 doctors. Those who have responded within the time period are included in this study.

Questionnaire Validation: The questionnaire has 3 segments divided into pre, intra, and post-operative sections containing 19, 14, and 17 questions, respectively.

Statistical analysis: Analysis of data is done by using excel sheets, pie charts, percentages, and bar graphs.

Results:

Two hundred and eight surgeons had responded, out of which 154 (74%) were male and 54 (26%) were female. Only 41.4% (86) were actually conducting the surgery, where the male-to-female ratio is 3:1.

Pre-Operatively-Team briefing on surgical plans for 86.3%, anaesthesia plans for 50.8%, and both for 9.6%. As an emergency (48.5%), followed by elective (33.8%), and minor surgeries (17.7%). Before shifting the patient to the operating room, 84.3% used proper donning of personal protective equipment, whereas 11.7% still used only gloves and N95mask. (Figure1).

Intra-operatively- 83.1% agreed that the viral filter is present at the patient end of the breathing circuit. An aerosol box is used for intubation only by 27.7%. Maximum and minimum aerosol generation have been seen in general and in local anaesthesia, respectively. (Figure 3).

Surgeons are predisposed to infection when dealing with exploratory laparotomy, i.e., an emergency as compared to elective and minor surgery. (Figure 4).

25.1% agreed that part of the patient's preparation takes place in the operating room. For sanitization, sodium hypochlorite is mostly used. (Figure 2). Other yields of pre-operative assessment have been briefed under Table 1.

Post-operatively all the operative room (OR) team members remain in quarantine for 4–14 days, whereas 15.3% agreed that they should receive quarantine only in symptomatic or in suspicion cases. RTPCR testing on the OR team was done immediately on 29.2% of the doctors, whereas the maximum were tested after 7 days irrespective of symptoms or in asymptomatic conditions. (Table 3) Of 80 surgeons who had operated, 28 had gotten COVID test positive within 7 days of operating. Table 2 provides the rest of the questionnaire statistics

Table 1: Preoperative outcome

Pre – operative Questionnaire (n=208)	Agreed (%)	Denied (%)
Separate pre-operative room	87.5	12.5
Status of covid patient briefed	95.5	4.5
Covid notification sign on door	90.5	9.5
patient wearing surgical mask	95.5	4.5
RTPCR of OR team	81.8	17.7
Recovery plan and location confirmed	97	3
Stretcher cleaned after patient transferred to OR	93.4	6.6
WHO Sign IN done	96.4	3.6
All OR staff wearing Personal Protective Equipment (PPE) kit is confirmed	88.8	10.2
Identification of OR staff and names are listed in register	94.9	5.1
OR is under negative pressure	82.4	16.6
OR runner present outside OR	82.4	16.6

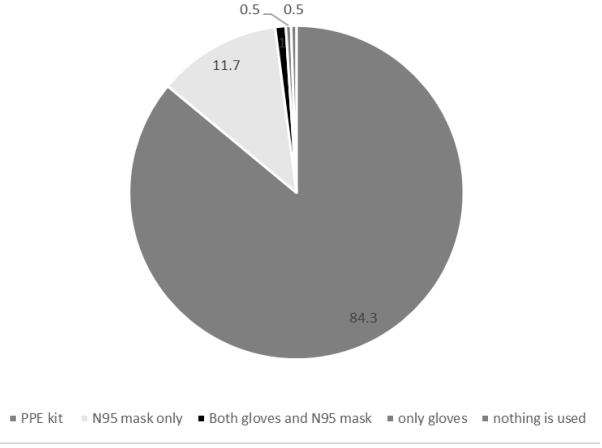

Figure 1: Use of personal safety materials

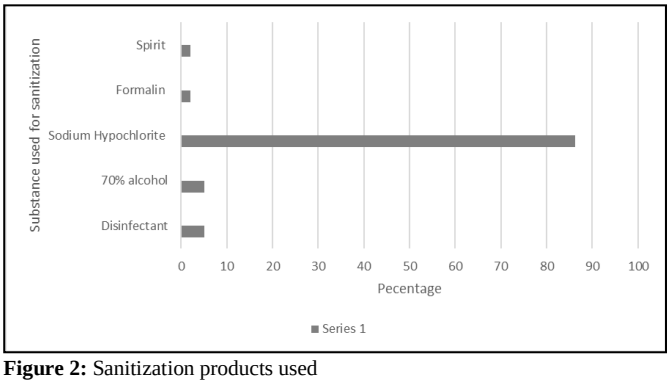

Figure 2: Sanitization products used

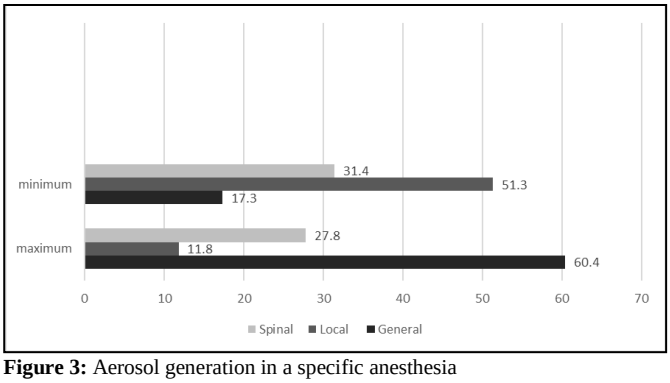

Figure 3: Aerosol generation in a specific anesthesia

Table 2: Intraoperative outcome

Intraoperative requirements (n=208)	Agreed (%)	Denied (%)
Non-essential staff not required in OR	68.4	31.6
No.of Staff should be		
<6	93.3	6.7
>10	5.6	94.4
Surgery is aerosol generating	51.6	48.4
WHO Time Out preferred	92.7	7.3
Extubation process is aerosol generation	88	12

Table 3: Post-operative outcome

Post -operative outcome (n=208)	Agreed (%)	Denied (%)
Separate post-operative room available for COVID positive	95.9	4.1
Same gloves are used during surgery and for the shifting of patient	68.2	31.8
Separate way to shift the patient	92.3	7.7
COVID Notification sign on post-operative door	92.3	7.7
Oxygen flow available in post-operative room	96.9	3.1
While shifting patient PPE kit is used	86.7	13.1
Cleaning of OR is done		
Immediately	84.3	15.7
Within 6hrs	6.8	93.2
Within 24hrs	2.6	97.4
Before next case	6.3	93.7
Stretcher santitizer after shifting the patient	90.2	9.8
Biomedical waste disposal followed	94.9	5.1
COVID task force constituted	60.4	39.6

Table 4: Neo – Surgical Check Box (NSCB)

Pre- operative assessment	Put "√" for the checked ones	Intra-operative requirement	Put "√" for the checked ones	Post-operative observation	Put "√" for the checked ones
COVID notification sign on the door of the patient room		Patient wearing N95 mask		Separate post-operative room available	
Status of the patient on COVID briefed to OR team		Staff limited to ≤ 6		Change of gloves while shifting the patient	
Patient wearing N95 mask		Viral filter present at the patient's end		Separate way to shift the patient	
Briefing on surgery and anaesthesia plan to the OR team		Aerosol box for intubation		COVID notification sign on post-operative doors	
Recovery plan and location confirmed		Type of surgery performed		Oxygen flow availability checked	
Part preparation of the patient		Cautery fumes continuously sucked out		Patient wearing N95	
RTPCR status of the OR team		Slow Extubation		PPE kit wore by shifting team	
PPE kit worn by the shifting team		Biomedical waste disposal rules followed		COVID task force vigilance on OR team	
Separate way for the transportation of the patient		WHO "Time Out"		Quarantine period minimum of 7 days provided	
Stretcher cleaned after patient transfer		Immediate cleaning of the operating room		RTPCR of OR team sent on Day 5	
10%sodium hypochlorite used for the sanitization		Immediate Disposal of unused or left over medicines			
OR team members identified and names listed in the register		Disposal of waste according to BMW guidelines			
Full PPE Kit worn by the OR team with proper donning		Disposal of drain fluid according to BMW guidelines			
1 OR runner present outside OR					
OR under negative pressure					
WHO "Sign In" done					

Discussion

The global death toll stands at around 1.3 million (13) and, as per the Indian Medical Association COVID registry, the maximum number of doctor deaths is reported in Delhi, followed by Bihar and Uttar Pradesh. This survey had a broad perspective on the surgical cases faced by the eastern part of Uttar Pradesh. Whether it's a natural calamity or a man-made disaster, the health issues of the people will remain, casualties will come, and deliveries are going to happen. Hence, it's important to look forward to managing the situation, keeping in mind patient safety and security as well as keeping surgeons healthy. Banerjee et al. are correct in recommending that surgical techniques (5) be adjusted. Orhan has reported a dramatic reduction in emergency as well as in elective cases in pandemic time as compared to 2018 and 2019 in the same month. Here, in this survey, the number of elective surgeries being performed was less as compared to emergency surgeries.

Refusal of surgeries by the hospital and doctors, as well as patient reluctance to report to the hospital due to fear, always had a negative impact on the number of cases performed, which in turn had a negative impact on the health care system. A study was conducted in Ontario between March 15 and June 13, 2020, which revealed a backlog of 148364 surgeries. (14) According to Uimonen et al., the mean waiting time for the surgery was 92.6 days, which was an 8% increase as compared to the usual time. Therefore, delays in surgery increase complications and mortality rates. (15)

To keep a check on the backlog and perform usual surgeries even at the time of a pandemic, we are proposing a "Neo-Surgical Check Box" keeping in mind the WHO surgical checklist (Table 4).

Conclusion:

Due to lack of knowledge of the pandemic situation has moved the surgical resident towards the theoretical aspect of surgery as compared to the practical one .Mobilizing the surgical resident to handle the medical part of the COVID patient would hamper or delay their progression of surgical career .Hence, this checkbox will bring the residency life towards normalcy where they can handle all sorts of carefree surgeries

It's good to be ready with all the possible outcomes. This check box not only will be beneficial for the surgeons and covid positive patients but it will be a boon in any sort of respiratory ailments for the near future.

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Conflict of Interest

No conflict of interest.

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Al-Kindy College Medical Journal (KCMJ)

Research Article

Comparative study on Various Tube Voltages and Contrast Media Doses in CT Pulmonary Angiography to detect Pulmonary Thromboembolism

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Keywords: pulmonary thromboembolism, computed tomography for pulmonary angiography, contrast media.

ABSTRACT

Background: Lowering the amount of iodinated contrast material and tube voltage may increase pulmonary artery opacification and thrombus identification without compromising picture quality.

Objectives: To explore the efficiency of using lower tube voltage and a lower contrast medium dose for conducting computed tomography for pulmonary angiography (CTPA) aiming to increase its accuracy in detecting pulmonary thromboembolism (PTE).

Subjects and Methods:100 patients scheduled for CTPA with a preoperative diagnosis of PTE were grouped into two: group A, (50 patients) got 1 mL/kg at 120 kV and group B, (50 patients) received 0.5 mL/kg at 80 kV.The technique of bolus tracking was implemented.Values of midpoint of multiple pulmonary artery divisions were used to assess attenuation.Significant values are those that exceed 300 Hounsfield units (HU). The dosage of radiation received by the patient, defined as the effective dose, was compared between groups.

Results: Total mean attenuationdata for the pulmonary arteries have shown to be substantially greater in group-B compared to group-A (39110.4HU vs. 335.97.9HU, P=0.001). Moreover, total dose length product (DLP) values were substantially lower in group-B (934.9 mGy.cm vs. 384.443 mGy.cm, P=0.001). While effective dose values have shown to be significantly lower in group-B (2.90.3 mSv vs. 13.21.8 mSv, P 0.001).

Conclusion: Low-kilovoltage technique and low quantity of iodinated contrast are useful in conducting (CTPA) with high accuracy in detection of pulmonary thromboembolism.

Introduction

Pulmonary thrombo-embolism (PTE) is a frequent consequence of deep venous thrombosis and is regarded as third most common acute cardiovascular illness after stroke and myocardial infarction(1). CTPA is often used to test individuals clinically assumed of having pulmonary embolism (PE) in order to confirm or rule-out this diagnosis (2).

Narrow slices and high number of cross-sections in multi-detector computed tomography (MDCT), implies higher radiation dosage;however, by reducing the kV value, the radiation dose may be lowered without compromising the diagnostic accuracy of PTE(3).

Apart from radiation risk, anotherpoint of concern is injectable intravenous contrast media needed for CTPA. Because of potential

contrast medium-induced nephropathy, its usage is troublesome in individuals suffering compromised renal function(4).

Unfortunately, the majority of patients liable to develop pulmonary embolism are old with several coexisting diseases that raise the risk of contrast medium-induced nephropathy (5).

Thus, lowering the iodine load in such patients may effectively avert contrast-induced nephropathy. Additionally, lower CM volume is beneficial in individuals having compromised right ventricular function, since even low osmolar CM will draw fluid from interstitium during its venous return, resulting in right ventricle volume overload(4)

Low-voltage protocols for pulmonary CT angiography may result in significant dose reductions. The mean reduction would reach 40% as setting is reduced from 120 down to 80kVp. It is most likely due to the enhanced vascular enhancement caused by the greater weakening of iodinated contrast material at low tube voltage. This would increase the pictures contrast/noise ratio. Second, reducing tube voltage, would improve vascular enhancement, since at low tube voltage, the attenuation of iodinated contrast medium rises (6).

Reduced tube voltage, on the other hand, increases noise while boosting picture contrast. As the relative increase in noise is greater than the equivalent rise in picture contrast, the signal-to-noise ratio (SNR) would decrease(7).

When low kV is utilized, image noise might significantly rise. This is especially critical in parts of the body with a large surface area, for instance abdomen. Low kilovoltage applications in lungs, on the other hand, do not pose a problem due to the large density difference between interstitium of alveoli and the vasculature. The attenuation and absorption of x-ray in alveoli are quite low. This improves visibility of adjacent vascular and parenchymal structures(8).

Aim of Study

Investigate whether MDCT may be utilized to examine pulmonary arteries (pas) in individuals suspected of having PTE by utilizing low dose CM and low tube voltage.

Subjects and Methods

CT pulmonary Angiography Image Acquisition: This cross-sectional study was carried out in Medical City at Baghdad Teaching Hospital, Department of Radiology.

The study extended for 6 months from September 2018 to February 2019. CT pulmonary angiography examination was carried out using 64–slice CT scan (Brilliance 64: Philips medical system corporation, best, the Netherland), the tube voltage was 120 KV for group-A, and 80 KV for group-B.

The other parameters kept constant as the following:

- Tube current: 200 mAs
- Collimation: 0.6 mm
- Slice increment: 0.5 mm
- Pitch: 0.891
- Rotation time: 0.5 sec
- FOV: 350 mm
- Scan time: 5 seconds
- Bolus tracking threshold: 150
- Post threshold delay: 6 sec
- Window level of CTPA: 80
- Window width of CTPA: 700
- Window level of parenchyma: 600
- Window width of lung parenchyma: 1200For CTPA scans, iopromide (Ultravist; Bayer Schering Pharma AG, Germany) wasutilized at a dosage of 370/100 mg/ml administered at a rate of 5 ml/s.

The CTPA examination was initiated automatically utilizing thebolus tracking approach; this method initiates image acquisition when the augmentation in the major pulmonary artery reaches a predefined threshold.

Patients Population: CT pulmonary angiography requested for 115 patients with clinical suspicion of pulmonary embolism to confirm their provisional diagnosis. All patients signed a written informed consent, (age range 15-85 years, mean age = 49.1 year), 15 patients are precluded from the study 5 patients had a known allergy to contrast media, 10 patients had impaired renal function (serum creatinine more than 1.5 mg/dL), the other exclusion criteria were pregnancy and respiratory distress.

The study group subdivided into Group-A (50 patients) received 120 kV value and 1 ml/kg contrast media, and group-B (50 patients) received 80 kV value and 0.5 ml/kg contrast media, the examination was carried out at the end inspiration in supine position, in cranio-caudal orientation extending from thoracic inlet down to adrenals.

The density of several pulmonary arterial segments was determined from their centers. More than 300 HU values were considered significant. All pulmonary arterial segments had appropriate attenuation values for diagnosis.

Objective Image Quality Assessment: The attenuation values from main and lobar pulmonary segments in HU were computed by centering a circular region of interest (ROI) on the center of these vessels and adjusting the size of the circular area to vessel diameter, up to 2 cm. To determine the HU of the peripheral arteries, measurements have been taken in several segmental and sub-segmental branches in the basal and apical branches. Seven arteries were separately analyzed, (main pulmonary artery, Rt and Lt pulmonary arteries. The apical section was chosen between the upper and lower aortic arch boundaries. The basal segment was defined as the area situated in-between the inferior pulmonary veins and the diaphragm. The highest attenuation value estimated from the peripheral vessels takenwas done solely in axial plane due to their small caliper.

After the sections were obtained, the PA attenuation value, sufficiency of image quality, and radiation dose received by the patients have been estimated. The CT scan results were read by two consultant radiologists who check the exam separately. The examining radiologists documented radiological findings from the CTPA examination, including thrombus location, consolidation, pleural effusion, the existence of pulmonary embolism, and assessments of PAs density. As PTE is an emergency case, CTPAs are often made in an emergency situation. As a result, our research excluded patients' weight, height, and body mass index.

Subjective Image Quality: All studied cases exams are assessed by two specialist radiologists separately.They evaluated the image quality of each exam subjectively and recorded their observations on evaluation sheets which is prepared for this purpose. They scored the image quality in three main scores:

1. Very high to outstanding quality: That enables accurate identification of even tiny structures.
2. Adequate quality: The enhancement was less than score 1, but still sufficient for diagnosis.

3.Inadequate quality: Where there is sub-optimal opacification that could not be used for diagnosis.

Radiation Exposure: The dosage-length product was automatically determined by the CT equipment, and the effective dose for CTPA was estimated using the quality standards for CT established by the European research group (18).

The effective dose was estimated using this approach by multiplying dose length product (dose divided by length of anatomical region inspected; dose length product (DLP), mGy.cm) by the anatomical chest area conversion factor (k = 0.017mSv.mGy-1.cm-1).

Ethical consideration and official approvals: verbal permission was taken from each patient preceding data collection, and the details were kept anonymous, administrative approvals were conceded from: The council of Arab Board of Health Specialization, Baghdad teaching hospital in Medical City.

Statistical Analysis: The collected data were introduced into Excel sheet and loaded into IBM-SPSS V24 software to be used in statistical analysis.Descriptive statistics were presented through tables and graphs.Two samples independent T-test, Chi-square test were used to find out significance of differences between continuous numerical variables and associations between categorical variables. P-value less than 0.05 was considered for discrimination between levels of significance.

Results

In this cross-sectional study, 100 patients with provisional diagnosis of pulmonary embolism enrolled the study, the range of age (15-85 years old), mean age of studied cases was 49.14±16 years. Group-A; mean age 48.3±14.5 years, group B; mean age 49.9±17.4 years, there is no significant difference between both groups in age according to independent 2 sample T-test, p-value 0.637.

Males constituted 38% of studied cases, females constituted 62%. Group-A: males=17, females =33, Group-B: males=21, females=29, there is no significant association between gender and the group of study, P- value=0.410

Pulmonary arterial thrombosis was revealed in seventeen patients (17%) in both Groups, 7 patients had central thrombosis (7%), and 10 had segmental and sub segmental thrombosis (10%).The pleural effusion: Found in 25 patients, 25%. Consolidation: Found in 25 patients, 25%. Dilatation of the main pulmonary artery and increased in the aorto-pulmonary ratio present in 4 patients, 25%

Objective Image Quality:

The attenuation values of pulmonary arterial tree were taken from each case in both groups:

1. The main PA
2. The right main PA
3. The left main PA
4. The right-left apical PA
5. The right-left basal PA Segments.

The attenuation value of pulmonary arterial segments was significantly higher in group-B where low contrast and low kv given to the patient in contrast to group-A where the ordinary dose given.The attenuation value of main pulmonary artery in group-B = 411 ±78 HU, while in group-A, was 347±60 HU, the percentage of difference is 15.6%, which is significant P-value is less than 0.001.The density of Rt and Lt pulmonary arteries in group-A were 342±60 HU and 339±53 HU respectively, while in group-B the readings were 396±89 HU and 389±87 HU respectively, percentage of difference is 13.7% in right pulmonary artery and 12.7% in Lt pulmonary artery, which is statistically significant. P-value of all cases was less than 0.001. The peripheral pulmonary arteries density values also found to be significantly higher in group-B.

The attenuation values and percentage of difference between both groups are shown in table (1) and figure (1& 2).

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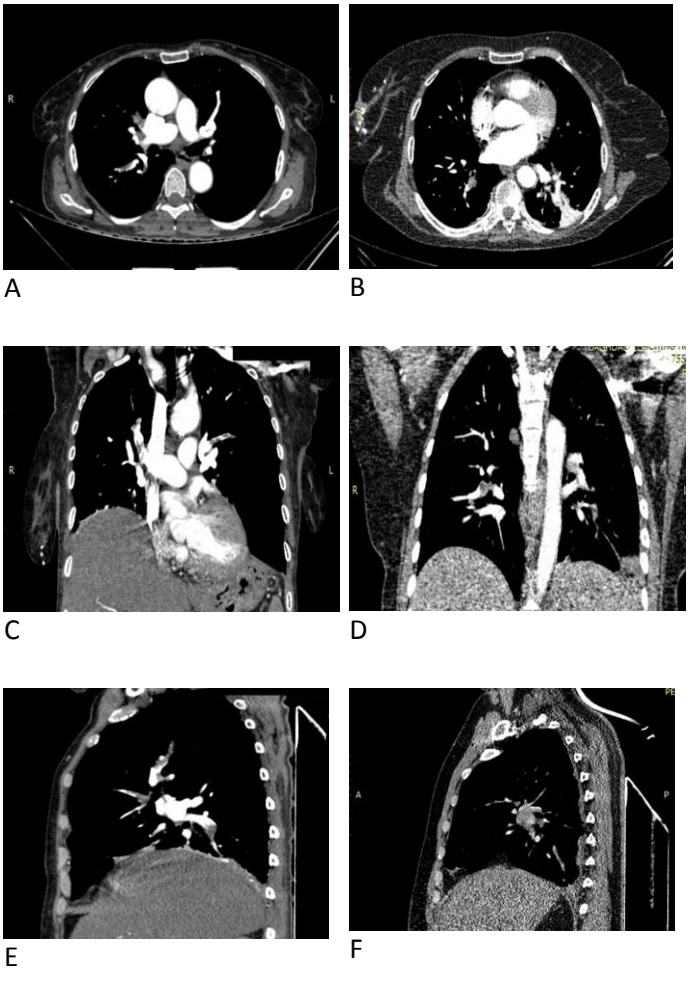

Figure 1: Pulmonary CTA performed using the conventional protocol (a,b,c) and the evaluated low-voltage, low contrast medium protocol (d,e,f).Axial images (a,d), reformatted coronal images (b, e), and reformatted sagittal images (c, f). The study performed with the conventional pulmonary CTA technique. A similar example is shown in the study obtained with the 80 kV protocol demonstrated segmental PE.

Subjective Image Quality

There was inter observer agreement between two radiologists regarding the image quality who evaluate the images separately. The vast majority of exams where low voltage protocol used (80 KV) and low dose of contrast media (0.5 ml/kg) given was scored as 1 – very good to excellent image quality, few cases scored as 2 where the enhancement was inferior to score 1 but still suitable to sufficient diagnosis.

Onlyone case shows inadequate opacification and poor arterial enhancement so scored as 3

Table 1: Two sample t-tests showed differences between means of attenuation values of different pulmonary arterial segments according to the tube voltage exposure of patient

Pulmonary arteries	KV value	Mean	% Of difference	Std. Deviation	P value
MPA	120kv	347.3700	15.6%	60.5	0.001
	80kv	411.9200		78.2	
RMPA	120kv	342.5000	13.7%	60.7	0.001
	80kv	396.9400		89.2	
LMPA	120kv	339.9200	12.7%	53.7	0.001
	80kv	389.6300		87.16	
RAPA	120kv	327.2300	15.1%	57.6	0.001
	80kv	385.6000		79.8	
LAPA	120kv	326.9600	15.4%	55.7	0.001
	80kv	386.9100		67.7	
RBPA	120kv	330.7300	13.6%	65.2	0.001
	80kv	382.8600		82.1	
LBPA	120kv	336.7000	12.1%	70.5	0.004
	80kv	382.9400		85.9	
Average of all data	120kv	335.9	14.1%	7.9	0.001
	80kv	391		10.42	
DLP	120kv	384.44	-313.4%	43.4	0.001
	80kv	93		4.9	
Effective dose	120kv	13.2	-355.2%	1.8	0.001
	80kv	2.9		0.3	

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The Patients Radiation Dose:

By statistical analysis: patients in group-B received lower radiation dose than group-A.The mean value of total dose length product of group-A was 384 mgray.cm-1, while in group-B was 93 ml gray.cm-1, which is significantly lower in group-B with P value less than 0.001.

The effective dose also was significantly lower in group-B 2.9 than group-A which is 13.2 with p- value less than 0.001.

Discussion

In our study: the most fundamental feedback is that the use of new protocol of CTPA including low kv value and smaller amount of contrast media results in better enhancement of pulmonary arterial segments including central and peripheral branches. The new protocol that we used provided diagnostic dependability that was not substantially different from our standard protocol and yet significantly decreasedDLP and effective dose between two groups with P value 0.001, implying that the effective dose decreased by 300% in group-B as compared to group-A.

Because both CTPA methods have produced comparable rates of pulmonary embolism and adequate subjective image quality, the slightly inferior objective picture quality owing to noise appears to be irrelevant in practice.Our study were in line with many previous studies of the same purpose, such as the study done by Mustafa Kara et al who examined by 80 and 120 kv protocol. They found significantly higher attenuation of the different pulmonary arterial segments with changing tube voltage from 120Kv to 80Kv (as in our study) and also decreased the amount of contrast media given to the patient by 60%, thus,lessening the risk of contrast media nephrotoxicity, and associated allergic reactions, in addition to less effective dose (3).

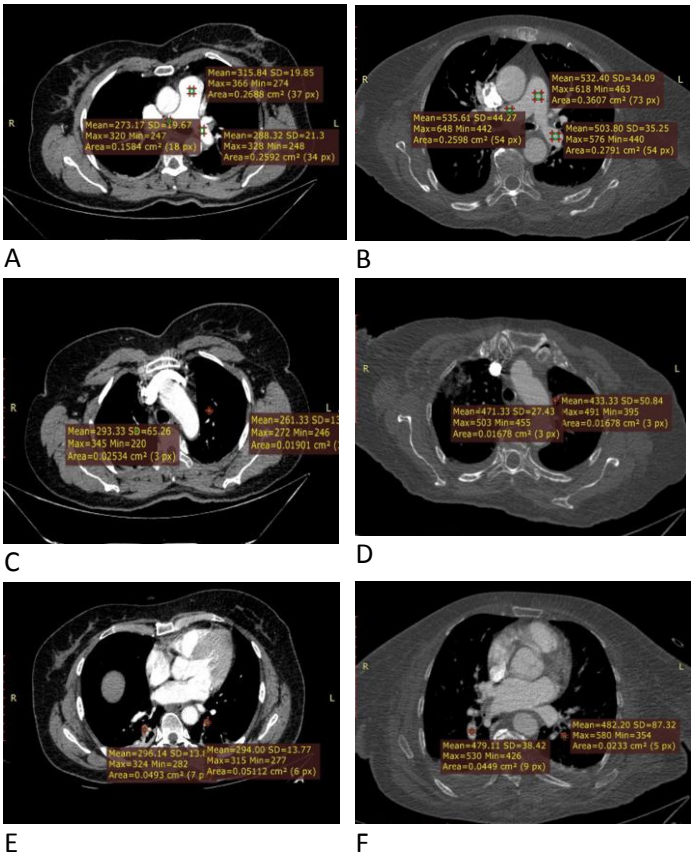

Figure 2: A, Mean attenuation values of group A; main pulmonary artery 315 HU, right pulmonary artery 273 HU, left pulmonary artery 288 HU. B, Mean attenuation. Values of group B; main pulmonary artery 532 HU, right pulmonary artery 535 HU, left pulmonary artery 503 HU. C, Mean attenuation values of group A; right upper zone pulmonary artery branch 293 HU, left upper zone pulmonary artery branch 261 HU. D, Mean attenuation values of group B; right upper zone pulmonary artery branch 471 HU, left upper zone pulmonary artery branch 433 HU. E, Mean attenuation values of group A; right lower zone pulmonary artery branch 296 HU, left lower zone pulmonary artery branch 294HU. F, Mean attenuation values of group B; right lower zone pulmonary artery branch 479 HU, left lower zone pulmonary artery branch 482 HU.

Note/ group-A: where conventional protocol used and group-B: where the studied low Kv and low contrast used

Viteri-Ramirez et al. conducted another research in which they compared the two aforementioned CTPA methods. The author evaluated 70 individuals with a preliminary diagnosis of PTE in this study. Patients were placed into two groups: A (80 kV/60 mL) and B (100 kV/80 mL). The mean attenuation values were 362.4-100.2HU for group-A and 262.4 -134.3HU for group-B, with a P-value less than 0.001 in both central and peripheral arteries. Additionally, the total DLP and effective dosage values were decreased, and the reductions were statistically significant (P=0.001) (9).These findings are compatible with our study.

Viteri-Ramírez G the author have changed the tube voltage from 120 kv down to 80 kv(9) (as our study), and the amount of contrast from 80ml to 60 ml, in our study we give the patients halved the standard

dose 0.5ml/kg instead of 1ml/kg), and in spite of this decrement, the attenuation value of central and peripheral pulmonary segments has been increased.

Other studies, such as Leithner Det al., reduced only the tube voltage and observed the effect on contrast attenuation in the vessels and exposure dose. They discovered significantly greater attenuation values at 90 kV compared to 120 kV in central and peripheral Pas (10).

Conclusion:

Pulmonary CTA protocol which performed using 80 KVP and 0.5 ml/kg iodinated contrast media produces high attenuation of central and peripheral pulmonary arteries and thus produces the same diagnostic accuracy as the traditional method in confirming or excluding the diagnosis of pulmonary embolism.

Study Limitations

Lack of patient information's about body mass index, since the CTPA is an emergency condition and most of patients are tired. Additionally, we didn't compare the two separate kV values and two contrast media protocols intra individually because it will be unethically to expose the same patient to extra dose of radiation.

Funding

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Conflict of Interest

No conflict of interest.

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Research Article

Assessment of Serum Level of Protein Carbonyl as a Marker of Protein Oxidation in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

***Background*:** Diabetes mellitus is a chronic disease with an increasing prevalence worldwide and characterized by an increase in oxidative stress and inflammation. The most important factor that is responsible for oxidative stress and production of reactive oxygen species (ROS) is hyperglycemia. The major targets of ROS are proteins. The most common and widely used biomarker of severe oxidative protein damage is protein carbonyl content.

The study was designed to assess the serum level of protein carbonyl as a marker of protein oxidation in patients with type 2 diabetes mellitus and to evaluate the effect of age, body weight, waist circumference, diabetic control and disease duration on the level of protein carbonyl.

***Subjects and Methods*:** This is a case-control study that included 91 patients with type 2 diabetes mellitus Eighty-five non-diabetic apparently healthy subjects matched for both age and sex with cases were enrolled as controls. Fasting blood samples were collected after an overnight fasting to measure protein carbonyl, fasting blood sugar, lipid profile, and glycated hemoglobin.

***Results*:** The level of serum protein carbonyl was significantly higher in diabetic patients than in controls and positively correlated with glycated hemoglobin, age of participant and disease duration as well as with body mass index and waist circumference.

***Conclusion*:** Diabetes mellitus is associated with an increase in protein oxidation in term of increase in the level of serum protein carbonyl with significant association in those who had poor glycemic control, obesity, higher age, and prolonged disease duration suggest that the carbonyl content of protein may be useful in evaluating the disease progression. Significant positive correlation of protein carbonyl together with waist circumference suggest that individual with central obesity are more susceptible to protein oxidation.

Introduction

Diabetes Mellitus (DM) refers to a group of diverse metabolic disorders characterized by elevated blood glucose levels caused by insufficient insulin release, resistance to action of insulin, or both (1). The prevalence of DM in Iraq had significantly raised from 19.58⁄1000 in the year 2000 to 42.27⁄1000 in 2016 (2). In Basrah, the age-adjusted prevalence of diabetes in individuals aged 19-94 years is 19.7 percent (3). The high prevalence of DM in Basrah, Iraq,

which affect one in every five adults, will put a strain on the financial resources of health care systems (3), therefore several studies have been conducted in Basrah to study diabetes from various aspects (4,5). Protein oxidation is a major class of post-translational modifications that affects proteins, caused by reactions between protein amino acid residues and reactive oxygen species (ROS) or reactive nitrogen species (RNS) (6). Protein oxidative changes are classified into two types: irreversible oxidation and reversible oxidation, both of which can be produced selectively by ROS and RNS (6). According to several studies, DM has been linked to an increase in the formation of ROS or RNS as well as a decrease in anti-oxidant potential (7). Oxidative stress plays a crucial role in the development of diabetes complications, both microvascular and macrovascular (8). Generally, reactive species can generate damage to all cellular components, including proteins, carbohydrates, lipids, and DNA (9). Proteins are the most common targets for oxidation reactions due to their high abundance in cells, extracellular tissues, and body fluids as well as their rapid reaction rates with oxidants. Additionally, oxidative stress is able to degrade lipids and carbohydrates to highly reactive intermediates, which eventually attack proteins at various functional sites (9). It has been estimated that proteins can scavenge a majority (50%–75%) of generated reactive species (10). The variety of reaction sites leads to a wide range of post-translational protein modifications which change protein composition and folding as well as their net charge and hydrophobicity/hydrophilicity. This has an impact on their functions as receptors, enzymes, carrier and structural proteins (11). The attack of ROS modifies amino acid residues resulting in generation of protein carbonyl (PC) groups, while chloraminated oxidants, primarily hypochlorous acid and chloramines, which are produced in activated neutrophils by myeloperoxidase, form dityrosine-containing cross-linked protein products called advanced oxidation protein products (AOPPs). Both have been identified as early markers for oxidative stress and are used as a measure of protein oxidative damage (12).

There are four major pathways for the production of PC: (i) backbone fragmentation via the α-amidation pathway and β-scission , (ii) binding of non-protein carbonyl compounds derived from lipid peroxidation by Michael addition of 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) to amino acid side chains of protein including histidine imidazole, cysteine sulfhydryl, and lysine amino groups, (iii) direct oxidation of amino acid side chains including arginine, lysine, proline, and threonine, (iv) addition of reactive carbonyl derivatives (ketoaldehydes, ketoamines, and deoxyosones) generated by reaction of reducing sugars and their oxidation products with lysine (13).

As PC groups occur on multiple amino acid residues on selected protein targets, its magnitude is much greater than any other modifications that occur only on a specific amino acid residue, and thus is more readily detectable(6). Because of their relative early formation and relative stability, the usage of PC as a biomarker of oxidative protein damage has some advantages in comparisons with the measurements of other oxidation products (14). The aim of this study is to assess the serum level of PC in patients with type 2 diabetes mellitus (T2DM) and to evaluate the effect of age, body mass index (BMI), waist circumference (WC), diabetic control and disease duration on the level of PC.

Subjects and methods

Study population:

This is a case-control study done in Basrah governorate, southern Iraq, from December 2020 to December 2021, on 176 participants categorized into two main categories; 91patients with T2DM as cases, and 85 apparently healthy individuals matched for both age and sex. Each participant in this study signed an informed written permission form. A detailed questionnaire contains demographic data include (Age, Gender, Residency, Duration of disease, Type of treatment whether dietary, oral, insulin or mixed therapy, and Family history of diabetes) were obtained from each participant. Blood pressure, body weight, height and WC were measured for each participant, and body mass index (BMI) was calculated as (kg/m2) (13).

Blood collection:

After an overnight fast for at least 8 h, five ml venous blood samples were obtained from each participant by venipuncture and divided into two parts: 2ml was dispended in a tube contains 1.5mg/ml Ethylenediaminetetraacetic acid (K3EDTA) for the determination of Glycated hemoglobin (HbA1c%). The rest of blood was placed in a serum separator tube (SST) that contains gel and clot activator without anticoagulants and was left at room temperature (20-25) °C for 30 minutes, and centrifuged at 3000 rpm for 5 minutes to collect serum. Then a part of the serum was used to estimate the routine biochemical tests promptly. The other part of serum was frozen in tightly closed eppendrof tubes and stored at -20°C for subsequent analysis of PC.

Laboratory investigation:

Fasting blood sugar (FBS), serum creatinine, total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C), were measured by automated colimetric methods using kits provided by Roche diagnostics Gmbh, Germany. Glycated hemoglobin was measured by ion exchange high performance liquid chromatography (HPLC) using VARIANT II TURBO HbA1c Kit-2.0 provided by Bio-Rad, USA. The estimation of PC was done by sandwich enzyme-linked immunosorbent assay (ELISA) kit, according to instruction of manufacturer (My Biosource, USA, REF MBS161516). Absorbance was measured at 450 nm and standard curve constructed from the known dilution of PC. Results were compared with standard curve and the lower detection limit was 2ng/ml. The inter-assay precision was <10%, while intra-assay precisions was <8%.

Statistical analysis:

The data of this study were analyzed by a computer program Statistical Package for Social Science (SPSS) version 23 and the results were expressed as Mean±Standard Deviation (SD) and percentage. For analysis of continuous data Independent t-test was used, while categorical data; were analyzed using Chi-square test (χ^2

test). Bivariate Pearson correlation was used to find out the correlation coefficient (r-value) between the parameters. P-value<0.05 was considered the lowest limit for significance.

Results

The demographic, clinical and biochemical data of the patients and controls were shown in (Table 1). There were no significant differences between the patients and the controls regarding the age, gender, WC, TC, LDL and creatinine (p>0.05). More than half of the individuals were females (54.9% for patients and 55.3% for controls respectively). The mean of BMI was significantly higher in diabetes than the controls (p<0.05). Hypertension was significantly more frequent in diabetic patients than controls (p<0.05), in addition to that the majority of diabetes (76.9%) with family history of DM. There were statistically significant differences between the diabetic patients and the controls in FBS, HbA1c, TG, VLDLC (P<0.001) and HDL-C (P<0.05). Regarding the marker of protein oxidation, the mean value of serum PC was significantly higher in diabetic patients than in controls (3.93± 0.18 vs. 3.36± 0.13 ng⁄ml; P <0.05).

To investigate the effect of age on the serum level of PC, participants were categorized in to five age groups (Table 2). The mean value of PC levels increased with the age of participants with significant difference only in control group (P<0.05). Furthermore, the study showed that the mean value of PC levels in the diabetes patients was higher in comparison with apparently healthy controls with respect to their similar age groups, the difference was significant between the age of 36-65 years (P <0.05).

Comparison of PC among the studied groups according to BMI categories revealed that the mean of PC levels was higher but not significant in overweight and obese participants when compared to those with normal weight (P>0.05), in addition to that, the mean of PC was higher in diabetic patients than in control group, the difference was significant in those with overweight and obesity (P<0.05). Regarding WC, the study found that the mean of PC was higher in individuals with central obesity than those with normal WC with significant differences in control groups (P<0.05). Moreover, the mean of PC was higher in diabetic patients than controls across the studied groups with significant differences in females (P <0.05) (Table 3).

With respect to disease duration, the mean of PC levels was higher but not significant in patients with disease duration more than 5 years than those with a duration ≤5 years (p>0.05). Comparison of PC among the studied groups according to glycemic control found that the level of PC was higher in patients with poor glycemic control than those with good glycemic control and fair control (P<0.001) (Table 4). There was significant positive correlation of PC level with HbA1c and duration of disease in patients with known disease as well as with age, BMI and WC in all studied population (Table 5):

Table 1: Demographic, clinical and biochemical data of the participants.

Variables		Cases (n=91)	Controls (n=85)	P value*
Age (years)		50.29±10.32	49.59±10.18	NS‡
Gender	Male	41(45.1%)	38(44.7%)	NS♣
	Female	50(54.9%)	47(55.3%)	
BMI (kg/m^2)		31.81±5.57	30.19±4.89	<0.05‡
Waist circumference (cm)		103.59±13.30	99.92±11.64	NS‡
Hypertension (n, %)		49(53.8%)	25(29.4%)	<0.05♣
Family medical history of DM (n, %)		70 (76.9%)	47 (55.3%)	<0.05♣
Duration of disease (years)		----	6.80±4.93	-----
Fasting blood sugar (mg/dL)		210.51±76.13	89.45±11.20	<0.001‡
HbA1c (%)		9.07±2.21	5.13±0.46	<0.001‡
Serum total cholesterol (mg/dL)		175.46±40.67	173.31±38.17	NS‡
Triglyceride (mg/dL)		168.80±90.93	119.71±44.50	<0.001‡
HDL-C (mg/dL)		44.20±9.80	49.02±9.43	<0.05‡
LDL-C (mg/dL)		120.70±36.37	118.02±35.70	NS‡
VLDL-C (mg/dL)		33.75±18.09	24.06±8.90	<0.001‡
Creatinine (mg/dL)		0.73±0.19	0.77±0.19	NS‡
PC (ng/ml)		3.48±0.27	3.36±0.13	<0.05‡

Data were represented as mean ± SD or percent
* P Level of significance between cases and controls
‡ Student t-test
♣ Chi-square test

Table 2: Distribution of PC of the study population according to age groups

Age (Years)	PC (Mean±SD) (ng⁄ml)		P value*
	Cases n=91	Controls n=85	
26-35 year	3.82±0.25#	3.76±0.15##	NS
36-45 year	3.89±0.15#	3.77±0.12##	<0.05
46-55 year	3.94±0.17#	3.86±0.11##	<0.05
56-65 year	3.97±0.17#	3.87±0.11##	<0.05
≥66 year	4.04±0.24#	3.93±0.16##	NS

Data were represented as mean ± SD
* Level of significance between cases and controls
# Level of significance between age categories
# P> 0.05
P< 0.05
Student t-test

Table 3: Distribution of PC of the study population according to BMI and WC

BMI (Kg/m2)	PC (Mean±SD)(ng⁄ml) Cases n=91	Controls n=85	P value*
Normal weight 18.5-24.9	3.85±0.12 #	3.79±0.17 #	NS
Over weight 25-29.9	3.93±0.18 #	3.80±0.14 #	<0.05
Obese ≥30	3.95±0.19 #	3.88±0.09 #	<0.05

Waist circumference (cm)		PC (Mean±SD)(ng⁄ml) Cases n=91	Controls n=85	P value*
Male	≤102	3.87±0.18£	3.79±0.14££	NS
	>102	3.90±0.14£	3.88±0.11££	NS
Female	≤88	3.96±0.18£	3.73±0.16££	<0.05
	>88	4.01±0.20£	3.86±0.11££	<0.05

Data were represented as mean ± SD
* Level of significance between cases and controls
# Level of significance between normal weight, overweight and obese
# P> 0.05
£ Level of significance between normal waist and central obesity
£ P> 0.05
££P<0.05
Student t-test

Table 4: Distribution of PC according to duration of DM and glycaemic control

Parameter		PC (Mean±SD) (ng⁄ml)	P value*
Duration of DM	≤5 years (n=46)	3.41±0.28	<0.05
	> 5 years (n=45)	3.55±0.24	
HbA1c%	Good control <7% (n=14)	3.30±0.19	<0.001
	Fair control 7-8% (n=24)	3.32±0.26	
	Poor control >8% (n=53)	3.58±0.23	

Data were represented as mean ± SD
* Level of significance
P<0.05 statistically significant
P<0.001 highly significant
Student t-test

Table 5: Correlation of the study variables with PC levels in the study population

Variables		PC (ng⁄ml)
Age (years)	Correlation Coefficient	0.283**
	P-value	0.001
BMI (Kg/m2)	Correlation Coefficient	0.196**
	P-value	0.009
Waist Circumference(cm)	Correlation Coefficient	0.150*
	P-value	0.046
Duration of DM	Correlation Coefficient	0.213*
	P-value	0.043
HbA1c%	Correlation Coefficient	0.465 **
	P-value	0.001

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Discussion

Diabetes mellitus type 2 is the most common type of diabetes, which is a multifactorial chronic metabolic disorder with a rising global prevalence (14). It has been stated that, PC groups are the most common and reliable biomarker of oxidative/nitrosative stress (15). The role of protein oxidation in diabetes has received a lot of attention over the last years (16). The results of present study showed that the mean value of PC levels was significantly higher in diabetic patients as compared with controls (P <0.05). Similar finding results were reported by several other studies (17, 18, 19), while Odetti p et al. (20) reported no significant difference in plasma PC levels between diabetic and non-diabetic individuals. Increased oxidative stress is the most likely cause of protein oxidation in diabetic patients (21). Several studies which support the presence of accelerated oxidative stress in DM reported that there was an increased level of lipoperoxidation markers as well as an excess of antioxidant consumption (22). Furthermore, glycation, a nonenzymatic reaction, that is highly activated by chronic hyperglycaemia, catalyzes the release of free radicals during the formation of early and late glycation products, which contributes to the enhancement of oxidative stress (23). Increased ROS levels can interact with proteins, resulting in oxidative protein modifications (24). The current study revealed that the mean value of PC levels increased with age of participants with significant difference in control group. In addition to that, there was significant positive association between the level of PC and age of the study population. These finding were in agreement with the results of several other studies (17, 25, 26, 27). However, Odetti p et al. (20) found no correlation between age and PC in both diabetic patients and controls. Aging causes an oxidative and nitrosative redox imbalance in plasma, and the reactive products of oxidative and nitrosative damage tend to accumulate during the aging process (28). Disruption of redox regulation is likely to contribute to the significant rise in oxidized protein levels with age and the development of disease (29). In this study a significant positive correlation was reported between the level of serum PC and BMI (r=0.196, p=0.009). These findings were in agreement with the results of Bollineni RC et al (30). Furthermore, there was significant positive correlation between PC level and WC of the study population. These findings were consistent with the results of Caimi G et al (31). The elevation of PC could be due to several causes; the first of which is that, despite the fact that the mitochondrion is the primary source of ROS and the major regulatory node for ROS synthesis, adipocytes contain a variety of enzyme systems that produce ROS (32). Secondly: individuals with overweight and obesity with metabolic syndrome have lower activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) (33). Goyal R et al have reported that there was suppression of GPX activity in diabetic patients compared to non-diabetic control subjects, with the magnitude of the suppression being greater in the presence of obesity (34). Thirdly: presence of high percentage of unsaturated fatty acid in adipose tissue which are a significant target of oxidation by hydroxyl radical (.OH-)(35). Peroxidation of poly unsaturated fatty acid (PUFAs) result in release of lipid aldehydes (36) which are highly diffusible electrophilic and prone to nucleophilic attack by the side chain of amino acids: histidine, cysteine and lysine residue of protein resulting in protein carbonylation (37). The present study showed that there was significant positive correlation between the level of PC and the

duration of DM (r=0.213, p=0.043). These findings were in accordance with the results of other studies (17, 38). oxidative stress may be increased with chronicity of DM and provide a strong evidence of the involvement of it in progression of the disease and development of diabetic complication (17). In the present study a positive significant correlation was found between the level of PC and glycaemic control of the patients with DM. These findings are going with the results of several other studies (17, 20, 27, 39). Patients with poor glycaemic controls have a higher level of lipids, which are a largely peroxidizable substrate, and more activation of glycation cascade, which produces an excess of free radicals, resulting in an increase formation of carbonyl groups (20).

Conclusion

The main finding of the current study was that the serum concentration PC was significantly higher in patients with T2DM than in apparently healthy controls. The results demonstrate that there was an increase in the oxidation of protein in diabetic patients in terms of an elevation in content of PC which might play an active role in progression of disease. The significant positive correlation of PC levels observed in the present study together with age, glycaemic control and the duration of DM may provide an evidence of the involvement of oxidative stress and protein oxidation in the development of DM and its complication as well as, suggest that the carbonyl content of protein may be useful in evaluating the disease progression and illustrating the mechanism of disease pathogenesis. There was a significant positive association of PC levels with WC suggest that individual with central obesity are more susceptible to protein oxidation even if BMI was normal.

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Conflict of Interest

No conflict of interest

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Research Article

Pulmonary CT findings in Patients Recovered from COVID-19 Pneumonia

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ABSTRACT

***Background*:** The COVID-19 infection is a more recent pandemic disease all over the world and studying the pulmonary findings on survivors of this disease has lately commenced.
Objective: We aimed to estimate the cumulative percentage of whole radiological resolution after 3 months from recovery and to define the residual chest CT findings and exploring the relevant affecting factors.
***Subjects and Methods*:** Patients who had been previously diagnosed with COVID-19 pneumonia confirmed by RT-PCR test and had radiological evidence of pulmonary involvement by Chest CT during the acute illness were included in the present study. The radiological data of chest CT scan of all patients were collected and analyzed after recovery (confirmed by negative RT- PCR) three months after their initial diagnosis of having COVID 19 pneumonia.
***Results*:** A total of 40 patients who had a second CT scans were assessed; there were 27 (67.5%) male and 13 (32.5%) female, with a mean age of 40.3 years old. The collective percentage of whole radiological resolution was 65% (26 patients). Patients >40 years old showed a significantly lower cumulative percentage of complete radiological resolution than patients ≤40 years old at the 3 months follow-up. The predominant patterns of abnormalities observed at discharge were ground-glass opacity (GGO), fibrous stripe and reticular opacities.
***Conclusion*:** Lung findings in COVID-19 pneumonia patients can be resolved completely during medium-term follow up with no sequelae. The older age and co-morbidities are the main significant risk factors for residual radiological findings of COVID-19 disease.

Introduction

The COVID-19 infection is a more recent pandemic disease all over the world and studying or monitoring the data on survivors of this disease has recently begun (1). The CT findings usually detected in patients with COVID-19 pneumonia are representer of acute interstitial lung injury and subsequent parenchymal changes caused by the cytokine storm activated by the incorporation of the virus into the pneumocytes (2,3). While reverse transcription-polymerase chain reaction (RT-PCR) is the essential laboratory test to confirm the diagnosis of COVID-19, non-contrast chest CT may represent a valuable tool in assessing this patient population (4). The histological changes in the lungs of COVID-19 patients as evaluated by post-mortem studies revealed the occurrence of pulmonary edema, alveolar cellular exudates and hyaline membranes (5). These

changes are probable base for the common recorded CT findings, such as ground-glass opacities (GGO) and focal consolidation (6,7). The parenchymal lesions were commonly distributed as bilateral, multilobar, and peripheral, with common involvement of the posterior areas of the lungs (8,9). With a lower prevalence, other chest CT findings have been reported such as interlobular septal thickening, bronchiectasis, "crazy paving," and halo sign (10,11). Mediastinal lymphadenopathy, pleural effusions, and pulmonary nodules have been rarely observed (12).

The initial data imply that lung changes do not resolve in all COVID-19 recovered patients and may progress into lung fibrosis (13). In the last two years, some published studies have evaluated the changes in lung findings in a short-term follow-up, usually after four weeks from recovery (14-17).

To identify patients at risk of long-term COVID-19-induced lung compromise, comprehending the intermediate-term lung changes on CT may provide a starting point for picking patients for prospect trials concerning antifibrotic treatment.

This study aimed to analyze the CT imaging findings in patients recovered from COVID 19 pneumonia and to estimate the cumulative percentage of complete radiological resolution.

Subjects and Methods

This descriptive observational case series study was conducted at the radiology department in Al-Yarmook Teaching Hospital, Baghdad, Iraq from August 2020 to April 2021. This study was conducted under the Declaration of Helsinki and was approved by our hospital's ethics and scientific research committees. We gained informed consent from all the patients involved in the study, and we safeguarded their personal health information .

Initially, a total of 70 patients previously diagnosed with COVID-19 were selected. Of these, 27 patients refuse to have a second CT examination, one patient with previous TB and pulmonary fibrosis and two patients with heart failure were excluded from the study. Then, 40 patients underwent a second chest CT study, (27) man and (13) women were included in this study with age ranging from (20) to (63) years. The basic information included gender, age, co-morbidities and history of smoking or occupational exposure were obtained

Any patient who had been previously diagnosed with COVID-19 pneumonia confirmed by RT-PCR test and had radiological evidence of pulmonary involvement by Chest CT during the acute illness were included by this study. The radiological characteristics of all patients were collected and analyzed after recovery (confirmed by negative RT- PCR) by chest CT scan three months after their initial diagnosis of having COVID 19 pneumonia .

Patients with preexisting interstitial lung disease or heart failure were excluded from the study to avoid the overlapping of the radiological pictures. Additionally, those with normal chest CT during the acute stage of disease were not involved by our study.

Thin-section CT examination was achieved on a multi-detector CT machine (Philips Ingenuity Core128, Philips Medical Systems, Best, the Netherlands; SOMATOM Definition AS, Siemens Healthineers,Germany) with a single inspiratory phase. The patient lay supine with head in advance and hands up. CT images were then acquired during a single breath-hold. The tube voltage was set as 120 kVp with automatic tube current modulation.with WL (-600) and WW (1600). CT images were reconstructed from the raw data with a sizable matrix of 512×512 as transverse images (1.5 mm thickness and 1.5 mm increment) with pulmonary B70F kernel and a mediastinal B30f kernel (Siemens Healthineers, Germany) or hybrid iterative reconstruction (iDose level 5, Philips Medical Systems, the Netherlands). No contrast media was administered. All images were transmitted to the post processing workstation to be reconstructed by high-resolution algorithms and conventional algorithms.

The CT images were evaluated by two experts' radiologists (five years of experience for each). The major CT findings were described by using internationally standard nomenclature defined by the Fleischner Society glossary (18) using terms including ground-glass opacity (GGO), sub pleural fibrous band, thickening of adjacent pleura or reticular shadows. Ground glass opacity is defined as is hazy increased attenuation of the lung, with preservation of bronchial and vascular margins. Sub pleural band is defined as a linear opacity up to 5 cm long with 1–3 mm thickness that typically spreads to the visceral pleura. This band reflects pleuroparenchymal fibrotic change and is frequently linked with alteration of the lung architecture. A reticular pattern is a gathering of immeasurable minor rectilinear opacities that yield an appearance similar to a net (synonym: reticulation). This finding usually represents interstitial lung disease. Intralobular lines, Interlobular septal thickening, or the cyst walls of honeycombing are the elements of a reticular pattern at thin- section CT. The distribution of lung abnormal findings was reported as chiefly subpleural (involving the peripheral one third of the lung), random (without predilection for central or subpleural regions), or diffuse (generalized involvement without respect to lung segments).

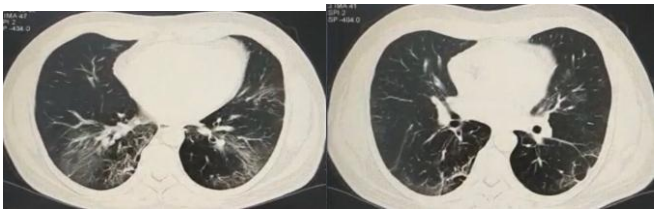
Statistical analysis:

Collected data were introduced into an excel sheet (Microsoft excel sheet 16) and loaded into Statistical Package for Social Sciences (SPSS), SPSS® for Windows, Version 24.0 (IBM Corp, Armonk, NY). Results of analysis were arranged in categorical variables and in scales variables (means & standard deviation). Fishers' exact test was used for categorical variables. To compare between two means, an independent sample t-test was used. The P value of 0.05 or less was regarded as significant.

Results

This study included 40 patients who recovered from COVID-19 pneumonia with a mean age of 40.3±10.2 years; 55.5% were less than 40 years of age, and 44.5% of them were 40 years of age and more. Male patients were more than females with a male to female ratio of 1.35:1.

The smoking was present among 20% of COVID-19 patients, while 80% of them were not smokers. The co-morbidities were absent in 70% of COVID-19 patients, while the present co-morbidities were hypertension (50%), hypertension and diabetes mellitus (25%), diabetes mellitus (16.7%) and ischemic heart disease (8.3%). The CT scan revealed that 26 patient (65%) of them had complete radiological resolution, while 14 (35%) of them had residual radiological findings as in Figure 1.



A B

Figure 1 (A & B): Axial chest CT images of adult patient 3 months after recovery from initial infection with Covid-19, showing fibrous stripes with reticular parenchyma opacification and ground glass appearance seen in the lower lobes of both lungs. Both images have the same window level of − 600 and window width of 1600

The residual radiological findings, patterns, and distribution were summarized in Table 1.

Table 1: Residual pulmonary CT findings of patients included in this study

Variable	n (%)
Residual CT findings	
GGO	13/14 (92.9%)
Fibrous stripes	13/14 (92.9%)
Parenchyma reticular opacification	10/14 (71.4%)
Pleural thickening	8/14 (57.1%)
Pleural effusion	1/14 (7.1%)
Traction bronchiectasis	2/14 (14.3%)
Distribution of findings	
Unilateral	3/14 (21.4%)
Bilateral	11/14 (78.6%)
Patterns of findings	
Predominantly subplueral	8/14 (57.2%)
Random	3/14 (21.4%)
Diffuse	3/14 (21.4%)

GGO; ground glass opacity

A significant association was observed between the increased age of COVID-19 patients and residual radiological findings (p=0.03). The mean age of patients with residual radiological findings was significantly higher than patients with complete resolution (p=0.03). We observed no significant differences between COVID-19 patients with full radiological resolution and COVID-19 patients with residual radiological findings regarding their gender (p=0.4), as in Table 2

Table 3 shows no significant differences between residual CT scan findings of COVID-19 patients between unilateral and bilateral distribution (p=0.3).

We observed no significant differences between residual CT scan findings of COVID-19 patients between different patterns distribution (p=0.4) as in Table 4.

Discussion

The COVID-19 infection is a more recent pandemic disease all over the world and studying or monitoring the data on survivors of this disease has newly started. However, most of data available at present emphases on the short-term sequelae of this disease and data on medium-term radiological findings are uncommon (19).

Table 2: Distribution of patients' demographic characteristics according to radiological findings

Variable	Complete resolution		Residual findings		P value
	No.	%	No.	%	
Age					0.03* Sig.
<40 years	18	69.2	5	35.7	
≥40 years	8	30.8	9	64.3	
Mean±SD (years)	36.2±10.8		45±11.3		0.03** Sig.
Gender					0.4* non sig.
Male	19	73.1	8	57.1	
Female	7	26.9	6	42.9	

*Fishers exact test, **Independent sample t-test. SD; standard deviation

Table 3: Distribution of residual CT scan findings; Unilateral versus bilateral

CT findings	Unilateral	Bilateral	
GGO	2	11	
Fibrous stripes	3	10	
Parenchyma reticular opacification	0	10	
Pleural thickening	2	6	0.3*
Pleural effusion	0	1	
Traction bronchiectasis	1	1	

*Fishers exact test. GGO; ground glass opacity

Table 4: Distribution of residual CT scan findings according to their patterns

CT finding	Subpleural	Random	Diffuse	P value
GGO	7	5	1	
Fibrous stripes	9	1	3	
Parenchyma reticular opacification	5	3	2	0.4*

*Fishers exact test, GGO; ground glass opacity

Current study showed that 65% of COVID-19 patients had complete radiological resolution, while 35% of them had residual radiological findings. These findings are close to results of Tabatabae et al (20) study in Iran on 52 patients with COVID-19 pneumonia with at least two chest CT scans and interval duration of 3 months which revealed that 57.5% of patients had complete radiological resolution and 42.3% of patients had residual radiological findings. However, our study findings are inconsistent with results of Han et al (21) prospective study on 114 patients with severe COVID-19 pneumonia which found that after six months of recovery, the CT scan showed that 35% of patients showed fibrotic like changes, while 65% of them showed either complete radiological resolution (38%) or residual ground-glass opacification or interstitial thickening (27%). This inconsistency might be attributed to many factors such as virulence variances of COVID-19 infection between different populations, differences in healing periods and discrepancies in

radiological technologies and interpretation of CT scan findings in addition to difference in study durations. Liu et al (22) stated that the lung abnormalities of COVID-19 disease could disappear completely through short time duration leaving no residuals and optimum time required for identification of early complete radiological resolution was mainly two weeks after patient's discharge.

Present study showed that residual radiological findings of COVID-19 patients detected by CT scan were GGO, fibrous stripes, parenchyma reticular opacification, pleural thickening, traction bronchiectasis and pleural effusion. These findings are consistent with results of Brogna et al (23) study in Italy which found that the main residual radiological findings by CT scan were ground glass obacities (GGO), fibrous stripes and parenchyma reticular opacification.

The radiological characteristics of chest as detected by CT scan are usually differ according to time of CT scan, the stage of COVID-19 disease at time of follow-up, the age of patients, immune status, co-morbidities and type of management provided for patients. Ding et al (24) study found significant changes in radiological findings with time and the prevalence of GGOs, consolidations, crazy-paving patterns and linear opacities decreased with time. Pan et al (25) study found that in high cases of COVID-19 pneumonia, the residual CT scan findings like GGOs and consolidations might be absorbed within period of 26 days after discharge. However, another Chinese study conducted by Li et al (26) found that after two weeks follow up of patients with COVID-19 pneumonia, 27% of the patients had residual CT scan findings commonly GGOs parenchyma reticular opacification and pleural thickening.

The current study showed that 78.6% of residual findings were bilateral, while 21.4% of them were unilateral in distribution. Our study also found that CT scan residual finding patterns were predominantly subplueral (57.2%), random (21.4%) and diffuse (21.4%). These findings are similar to results of Wang et al (27) study in China which documented that the common residual CT scan findings detected among COVID-19 patients were bilateral and subplueral in pattern. The present study found that mean age of patients with residual radiological findings was significantly higher than mean age of patients with complete resolution (p=0.02). This finding is consistent with results of Parry et al (17) study in India on 81 COVID-19 patients revealed that increased age, obesity, higher co-morbidities, low oxygenation, increase duration of hospital stay, admission to intensive care unit, increased WBC count, increased CT severity score and low steroid intake were the main risk factors for residual radiological findings detected by CT scan for COVID-19 patients. Santesmasses et al (28) study in USA reported that the COVID-19 disease is an emergent disease of aging and the age or the age-related disorders are the main risk factors for this disease. However, Ho et al (29) population-based cohort study in UK found that higher death rates of COVID-19 disease among older age population is related to other older age diseases and healthier older age population at low risk of COVID-19 disease mortality.

In current study, there was a significant association between positive co-morbidities of COVID-19 patients and residual radiological findings (p=0.02). This finding is similar to reports of George et al (30) study in UK which stated that co-morbidities accompanying COVID-19 infection such hypertension and diabetes mellitus are the main significant risk factors of residual radiological findings detected by CT scan after follow up.

Our study has few limitations. Firstly, this study involved small number of patients, as many patients refuse to undergo a second CT examination, and the short duration of the study. Secondly, we could not estimate the effects of different treatment regimens to the patients as well as the disease severity during the acute illness and its consequent effects.

Conclusion

The rate of residual radiological findings of COVID-19 disease detected by computerized tomography is within acceptable range. The common residual radiological characteristics of COVID-19 disease detected by computerized tomography are ground glass opacities, fibrous stripes, parenchyma reticular opacification and pleural thickening. The bilateral distribution is the common anatomical residual distribution and the subpleural pattern is more prevalent residual pattern. The age and co-morbidities are the main significant risk factors for residual radiological findings of COVID-19 disease.

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Conflict of Interest

No conflict of interest

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Research Article

Evaluation of Streptocin SH3, a Bacteriocin produced by Streptococcus sanguinis isolated from Human Dental Plaque

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ABSTRACT

***Background*:** Bacteriocin is a peptidic toxin has many advantages to bacteria in their ecological niche and has strong antibacterial activity**.** Objective: The aim of this study was to evaluation of bacteriocin using *Streptococcus sanguinis* isolated from human dental caries**.**
***Subjects and Methods*:** Thirty five streptococcus isolates were diagnosed and tested for their production of bacteriocin, and then the optimal conditions for production of bacteriocin were determined. After that, the purification of bacteriocin was made partially by ammonium sulfate at 95% saturation levels, followed by and gel filtration chromatography using Sephadex G-50 column. Finally, physical characteristics were also studied and determined the bacteriocin stability.
Results: Among all streptococcal isolates, the *S. sanguinis* SH3 isolate with batter antimicrobial activity was selected, and used in further experiments. The optimum conditions for bacteriocin production were in Todd Hewitt broth (THB) supplemented with 1% glucose at 37°C for 24h under anaerobic conditions and inoculum size of (1.2×10^9CFU/ml) and pH 7, where the inhibition zone diameter reached to 29 mm against *Enterococcus faecalis*. The purified bacteriocin had high inhibitory activity against *S. pneumoniae*, *S. pyogenes*, *E. faecalis*, *E. faecium* and *Leuconostoc mesenteroides*. Streptocin SH3 keeps its effectiveness within the pH range 3-10, and temperature until 80ºC for 20 min.
Conclusion: Bacteriocin produced from *S. sanguinis* has high activity against many pathogens and has high stability over wide ranges of temperature and pH, which makes it a good alternative in the medical and food preservation fields.

Introduction

Bacteriocin is an antimicrobial peptidic toxin produced by Gram-positive, Gram-negative bacteria, and members of the archaea (1,2). Bacterial species produce these peptides as an ecological feature in their niches for self-protection and competitive advantage. Bacteriocin may has function as a killing peptide to eliminate or inhibit the competing species (3), and facilitate the dominance of a producer strain into the niche (4). Alternatively, bacterial strains may produce bacteriocins as a signal peptides for cell to cell communication within microbial communities (5). Bacteriocin exhibits significant potency against antibiotic resistant pathogenic bacteria, a major problem in the world due to excessive use of

antibiotics in humans and animals(6,7). As a result of the increase in antibiotic resistant strains, resistance to cephalosporins, a broad-spectrum antibiotic used in the management of infections caused by Pseudomonas areuginosa. Also, vancomycin resistance in enterococci, and methicillin resistance in Staphylococcus aureus that caused problems in hospitals. So, there is an urgent need to develop new antibiotics to eliminate multi-drug resistant pathogens (6). Like that, to control the misuse of antibiotics in food, bacteriocins are considered as a safe alternative due to their high therapeutic and nontoxic features. In food preservatives, bacteriocin has received exciting interest as a preservative and for control of spoilage and pathogenic bacteria (8). Moreover, bacteriocin has potential activities on human health, such as anticancer and antiviral agents, in recent years (9,10). Streptococcus sanguinis (S. sanguinis) plays an important role in promoting oral health, but may come to be pathogenic, including dental plague and endocarditis (11). It is lactic acid, gram-positive bacteria, nonspore-forming, nonmotile, catalase and oxidase negative, facultative anaerobic arrangement in chains or pairs of cocci (12). So a bacterium can produce bacteriocin, it requires suitable growth medium. Similarly, different factors affecting the production of bacteriocin are the type of bacterial species, fermentation conditions, and enough presence of nutrition (13). The production of bacteriocin is growth associated due to the fact that production takes place for the duration of the mid-exponential phase and will increase to attain a maximal level at the end of the exponential phase or the start of the early-stationary phase (14, 15). Specifically, many lactic acid bacteria (LAB) bacteriocins have been tested with high safety and tolerance to acids, heat, and bases (16). This study at pursuits optimization, purification, and characterization of bacteriocin produced with the aid of S. sanguinis and observes its effectiveness in opposition to pathogenic bacteria.

Subjects and Methods

Isolation of producer isolate and screening for bateriocin production

The producer isolates *S. sanguinis* SH3 was isolated from a patient with dental plaque and identified by morphological and biochemical tests, then confirmed by vitek 2 system. It was selected from among 35 isolate of streptococci (*S. mutans, S. parasanguinis, S. mitis, S. sobrinus*) isolated in this study for having the highest activity against indicator isolates.

The Agar Well Diffusion (AWD) method was used to evaluate the activity of the isolate according to (14) as follows: Tubes contained 10 ml of THB were inoculated with 1.2×10^9 CFU/ml of an overnight culture of the producer isolate. Then, tubes were incubated for 24 h and 37°C under CO_2 (5%) using candle jar . After incubation, the cultured broth was centrifuged at 6000 rpm for 15 min and the cell-free supernatant (CFS) was collected. Then, 0.1 ml of an overnight growth (24 h) culture of the indicator bacterium (1.5×10^8 CFU/ml)) was spread on the surface of MHA. In the plate, circular well 5mm in diameter was cut by using a cork borer after that 100 µl of CFS were put in wells, then plate was incubated for 18 h. After that, the activity was measured in mm.

Determination of the optimal conditions for bacteriocin production

To determine the medium and culture conditions that support the maximal production of bacteriocin, several optimization experiments were performed. Different culture media such as Todd Hewitt Broth (THB), Tryptic Soy Broth (TSB), Brain Heart Infusion Broth (BHIB), Nutrient Broth (NB), supplemented with 1% Glucose were inoculated with the isolate ($1.2 * 10^9$cells/ml) inoculum size. They were further incubated at 10, 15, 25,25, 30, 37, and 40°C for 24 h and to check the effect of culture medium as well as temperature on bacteriocin production. Also, to determine the effect of initial pH on bacteriocin production, the culture medium adjusted at different pH ranges from 4–10 and incubated at 37ºC for 24 h. Subsequently, the effect of the incubation period on the production of bacteriocin was also determined, where the isolate was incubated for 18 , 24, 48, 72 h at 37◦C. The effect of initial inoculum size on bacteriocin production also measured at different sizes (3.0×10^8, 6×10^8, 9×10^8, and 1.2×10^9). Finally, the effect of aeration on production of bacteriocin was determined and the producer isolate was cultured under aerobic, anaerobic (gas pack) and co_2 (5%) conditions. The activity was measured in mm in terms of inhibition zone diameter .

Production and purification of bacteriocin

According to (17), the producer isolate was grown in 750 ml THB supplemented with 1% Glucose and incubated at 37°C under anaerobic conditions, pH 7, and inoculum size $1.2 * 10^9$ CU/ ml. After 24h of incubation, it was centrifuged at 10,000 rpm for 15 min at 4◦C. After that, the supernatant was collected and the pH was adjusted to 6.5 with 1 M sodium hydroxide (NaOH), then, the antimicrobial activity was determined by agar well diffusion method. To precipitated bacteriocin, ammonium sulfate was added at 50% and 95% saturation levels to crude supernatant and held overnight at 4◦C then, centrifuged at 10,000 rpm at 4° C for 30 min. The precipitate was dissolved in phosphate buffer saline (0.1 M; pH 7). The remaining solution was assayed next level 95% saturation and also the precipitate was collected and dissolved in 5 ml of buffer and bacteriocin activity was measured at each saturation level against *E. faecalis* by AWD method.

After that, the precipitates were dialyzed overnight against the same buffer by using dialysis membrane (SIGMA) of molecular weight cut-off 1000 DA . The precipitate was further purified using Sephadex G-50 column (2x46 cm). Column was loaded with 15 ml of dialyzed partially purified samples. After the complete entry of the sample into the column, the peptides were eluted by using potassium buffer pH 7 with a flow rate of 1 ml/ min. 5ml for each fraction was collected, the absorbance of each fraction was read at 280 nm by spectrophotometer, and the plot was drawn between fraction number and its absorbance . All the collected fractions were examined for bacteriocin activity against *E. faecalis*. Active fractions were pooled, assayed for a specific activity, and protein concentration.

Measuring protein concentration

Protein concentrations of crude, precipitated and purified bacteriocin were determined according to Lowry (18).

Determined the activity of purified bacteriocin and activity unit

Activity of bacteriocin was measured by AWD **method** on MHA against *E. faecalis, S. pyogenes, S. enterica, S. pneumoniae, E. faecium, Staphylococcus aureus, and L. mesenteroides* as indicator strains. The activity was determined by measuring of inhibition zone diameter around the wells and activity unit was expressed as AU/ml according to (19) with the following equation:

$$AU = \frac{Inhibition\ area\ (mm^2) - Well\ area\ (mm^2)}{Volume\ sample\ (ml)}$$

Temperature and pH stability of streptocin SH3

To determine the effect of pH, and temperature, Streptocin SH3 was incubated at 40,60, 80, 100◦C for 20 min and at 121˚C using autoclave for 15 min, and then cooled to room temperature. To test pH stability, Streptocin SH3 was treated with either 1N HCI or 1N NaOH to obtain the desired pH between (2- 12) then, incubated for 30 min. After that, the aliquots were neutralized to pH 6.5 and the residual activity was determined by the AWD method against indicator isolates (20) .

Statistics analysis

Data analysis was carried out using SPSS-V.16 software. The mean, standard error, and significant differences between values were determined by ANOVA and Duncan test.

Results

Screening and optimal conditions for bacteriocin production

The cell-free supernatant of producer isolate was obtained from Todd Hewitt Broth (THB) inoculated with 1.2 * 10^9 cells/ml and incubated at 37˚C for 24h under 5% co_2 conditions give 18 mm inhibition zone diameter by agar well diffusion method against *E. faecalis* isolated from urine as in figure (1). The results showed that the production of bacteriocin is affected by the culture conditions. The activity of the bacteriocin disappeared under aerobic conditions and at a temperature between 10˚-25˚ C, also when using tryptone soya broth medium and pH of 8.5-10. At temperature 30, pH 5.5-7, incubation time 18-48h, inoculum size from 3×10^8 - 9×10^8, and using nutrient broth, and brain heart infusion broth, the activity ranged between 7.5 ± 0.5- 21 ± 0.52 mm but, the maximum activity was 29 mm obtained from culture under anaerobic condition at 37 ˚C for 24 h and pH 7 in THB supplemented with 1% glucose against *E. faecalis*.

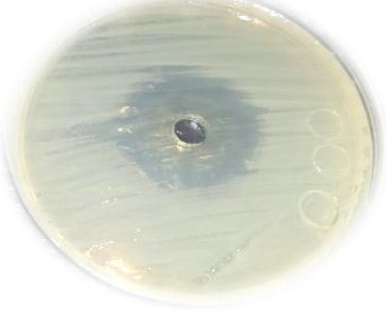


Figure 1: Activity of Cell- Free Supernatant of *S. sanguinis* SH3 against *Enterococcus faecalis*

Purification of streptocin SH3

The curve of purified bacteriocin was plotted between the absorbance and fraction numbers of protein eluted by gel as shown in figure (2). Each eluted 5ml fraction read at 280nm. When measuring the effectiveness of bacteriocin in each fraction , highest activity obtained from fraction 13. During the purification procedures, each step resulted in a considerable loss of protein concentration while the activity increased.

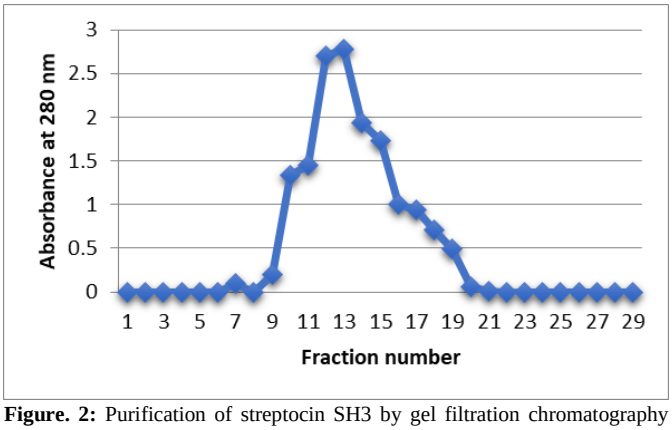


Figure. 2: Purification of streptocin SH3 by gel filtration chromatography using Sephadex G-50 column with dimensions (2x46) cm that equilibrated and eluted by 0.02 M of sodium buffer saline (pH7) and flow rate was 1ml/min with 5ml for each fraction.

Estimation of protein concentration

The protein concentration of crude, dialysis, and purified streptocin SH3was determined by Lowry method. There was gradually decrease in the protein concentration. In crude bacteriocin, the protein concentration was 10.461 mg/ml after dialysis, the concentration decreased to 6.201 mg/ml while after purification, the concentration reached 3.21 mg/ml. The activity after purification increased for streptocin SH3, the concentrations of streptocin SH3 shown in figure (3).

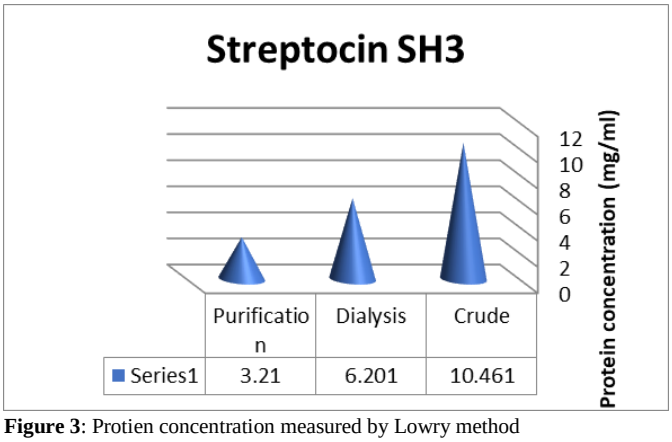


Figure 3: Protien concentration measured by Lowry method

Spectrum activity of streptocin SH3

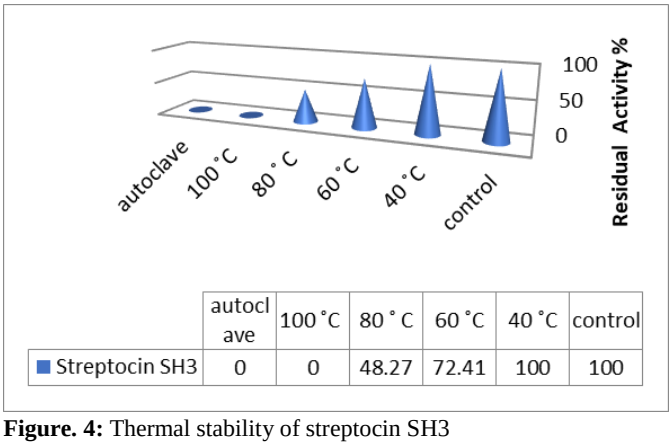
Streptocin SH3 was active only against some pathogenic closely related gram positive bacteria as in table (1).

Table 1: Spectrum activity of streptocin SH3 against some pathogenic bacteria.

Bacterial isolates	Inhibition zone (mm)	Activity Unit/ ml
S. aureus	0 ± 0.0	-
S.pyogenes	19 ± 0.53	3610
S. pneumoniae	15 ± 0.27	2250
E. faecalis	35 ± 0.17	12250
Salmonella enterica	0 ± 0.0	-
pseudomonas aeruginosa	0 ± 0.0	-
E. faecium	39± 0.12	15210
Leuconostoc mesenteroides	9 ± 0.07	810

Thermal stability of streptocin SH3

The streptocin SH3 was remained active until 80˚C for 20 minutes , but the activity lost after exposed to 100˚ C and autoclaved as in figure (4).

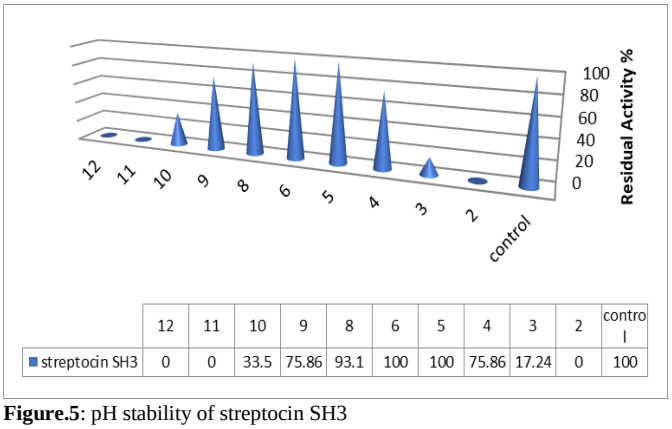


	autoclave	100 ˚C	80 ˚C	60 ˚C	40 ˚C	control
Streptocin SH3	0	0	48.27	72.41	100	100

Figure. 4: Thermal stability of streptocin SH3

3.7 pH stability

The bacteriocin activity of the crude streptocin SH3 at different pH levels is shown in figure (5). streptocin SH3 was active in a wide pH range (4-8) , at pH 3, 9, and 10 the bacteriocin activity decreased, and did not inhibit the indicator bacteria and completely lost at pH 2, 11, and 12.



	12	11	10	9	8	6	5	4	3	2	control
streptocin SH3	0	0	33.5	75.86	93.1	100	100	75.86	17.24	0	100

Figure.5: pH stability of streptocin SH3

Discussion

The use of bacteriocin to eliminate of disease-associated bacteria as an alternative to antibiotics is gain more attention. In this study, *S. sanguinis* SH3 produced bacteriocin with high activity against gram positive bacteria. Bacteriocin was produced in large quantities under anaerobic conditions more than aerobic and this result may due to increase of the bacterial growth rate as observed. As mentioned by (21) that the anaerobic growth condition is favored in *S. intermedius* with a 24 % faster growth rate than aerobic, and associated with the up-regulation of the central carbon metabolism also, its adaptation to anaerobic conditions and accelerated growth allows *S. intermedius* to multiply in complex polymicrobial environments , both as a pathogen and a commensal. The current results also agree with obtained by other researchs (22,23) who found that always anaerobic conditions favor bacteriocin production by *streptococcus* spp. include Mutacin by *S. mutans* and Bovicin HC5*S by S. bovis* HC5 in simple media, respectively. Also the present results showed that the neutral medium was the best for bacteriocin production and this agree with the results of other studies (24, 25) on optimize of bacteriocin of *E. faecalis*. In general, if the density of bacteria is bigger in the lag and exponential phases of growth, the bacteriocin concentration will be higher, and in this point is agree with (26, 27). The present results show an increase in bacteriocin production when added glucose to the culture medium and this agree with (28) who found the biomass amounts increased the initial glucose concentration was between (0.5–2%) during fermentation of *E. faecium* DPC1146. For optimization of bacteriocin by *S. sanguinis* in this study using culture media TSB with 2% yeast extract, which appear the best compared with THB medium and this results coincide with (29) who used TSB with 2% yeast extract for bacteriocin production by *S. salivarius*. The bacteriocin production increased with increasing bacterial growth rate to reach the highest production at incubation time 24h. After that, the activity decreased. These results agree with (30) who found that the high activity of bacteriocin from by *E. faecalis* 478 was obtained after 24h. This suggested that bacteriocin production depending on the cell density. Similar results have been obtained in some bacteriocins of Lactic acid bacteria, for example, bacteriocin FGC-12 (31). At the end of stationary phase, due to decrease in the number of viable cells and pH, the bacteriocin activity decreased (7). Also decreasing of activity after prolonged incubation of the producer strain has been reported to occur as a result of extracellular proteases, protein aggregation or readsorption to the producer cell surface (32).

The reduction in the bacteriocin production at high and low temperatures referred to slow growth of producer bacteria. This result agrees with (33) who study the effect of temperature on bacteriocin production by *E. faecium* and show that the bacteriocin production reduced at (4, 10 , 15, and 30) ˚C compared to the control incubated at 37˚C. Some of bacteriocins produced by streptococci and enterococci purified from culture incubated at 37˚C such as enterococcin produced by *E. faecalis* CG-9 (34) and streptocin STH_1 produced by *S. sanguis* (35).

Some of bacteriocin from *Streptococcus* precipitate by ammonium sulfate such as sanguicin produced from *S. sanguinis* isolated from dental caries and the highest activity of sanguicin was at a saturation level 70% of ammonium sulfate (36).

The streptocin SH3 active against gram positive bacteria and this agree with the results of many researchers on the spectrum activity of some lactic acid bacteria such as the bacteriocin produced from *E. faecium* (37, 38), and benterocin Gr17 produced by *E. faecalis* (39).

The Streptocin SH3 was remained active until exposed to 80˚ C. There were some different results obtained by (36) where the activity of sanguicin produced by *S. sanguinis* was lost after heating at 60˚C for 10 min and by Skilton (40) , whose showed that the streptoccin san-K11 produced by *S. sanguinis* was heat stable until at 100˚C for 10 minutes, but in current study, streptocin SH3 was active at 60 ˚ C and lost its activity after 100 ˚ C , this differences may due to the difference in the period of exposure of the bacteriocin to heat, as in the previous studies it was 10 min, but in the current study 20 min.

The effect of pH on bacteriocin production was studied, some of bacteriocins lost their activity at pH 2, 11, and 12 such as bacteriocin produced from *E. faecium* KY11240 lost their activity at pH 2 and 10 but the activity in pH range 5 to 7 was stable and decreased at pH 3, 4, and 9 after 2h of treated (37), and bacteriocin produced from *S. sanguis* (41) where the bacteriocin was stable at pH range (3-10).

Conclusion

The mouth is a reservoir for many bacteriocin-producing bacteria; including Streptococcus spp. *S. sanguinis* SH3 produces streptocin SH3 in greater quantities under anaerobic conditions, which raises a broad topic for physiological study about the relationship between growth conditions and bacteriocin production. The produced bacteriocin is highly stable within wide temperature and pH ranges , which can be used to eliminate bacterial resistance to antibiotics and in the field of food preservation.

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Conflict of Interest

No conflict of interest

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Research Article

The Association between CRP Levels with Comorbidities, Species, and Complications of Severe Malaria

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ABSTRACT

Background: Malaria remains a leading cause of mortality in sub-Saharan Africa (including Sudan). C-reactive protein (CRP) is useful as a marker of severity in malaria. African studies have shown that serum CRP levels correlate with parasite burden and complications in malaria, especially falciparum. However, there are no data on CRP levels in Sudanese malaria patients.

This study aims to evaluate the association between CRP levels with comorbidities, species, and complications of severe malaria

Subjects and Methods: A cross-sectional study enrolled 65 severe malaria patients at Khartoum state hospitals during the period from April to June2021. Manifestations of severe malaria were defined according to WHO criteria. Data regarding demographics, presenting symptoms & signs, laboratory investigations, complications, length of hospital stay and outcomes were collected. CRP was classified as elevated when the measured level was >10 mg/l.

Results: Among 65 patients, 33(50.8%) were females and 32(49.2%) were males, and mean age was 48±21 years. The main manifestation of severe malaria diagnosis criteria was anemia in 26(40%) patients. Most of the patients had density 1+ (n=53; 81.5%) and were infected by P. falciparum (n=61; 93.8%). The overall case fatality rate for malaria was 8% (n=15 patients). The mean of CRP was 72±57 mg/L and 84% (n=55) of patients had elevated levels above 10 mg/L (ranged from 10-100 mg/L in 52%, and above 100 mg/L in 32%). The elevated CRP levels were significantly DM (P= 0.048), high malaria parasite density in blood film (P= 0.001), P. falciparum (P= 0.33), presence of complications (P= 0.001) and death (P= 0.003)

Conclusion: Elevated CRP levels were found in a considerable proportion of severe malaria patients. CRP is an effective biomarker in assessing malaria severity and poor prognosis in term of complications development and mortality.

Introduction

Anopheles mosquito bites from females carry the intracellular parasite that causes malaria. P. falciparum produces the bulk of infections and fatalities among the five Plasmodium species known to infect humans (P. vivax, P. ovale, P. malariae, and P. knowlesi). (1). In Sudan, malaria is a significant public health issue. Malaria poses a risk to over 75% of the population (2). The reported malaria cases represent 8.7% and 12.2% of total outpatient attendance and of hospital admissions respectively (2). Sudan is on track to achieve a 20–40% drop in incidence by 2020, according to the WHO's 2018 malaria report, despite a rise over the previous three years (3). There can be a wide range of symptoms associated with malaria parasite infection, from none at all or very minor to severe illness and even death. There are two types of malaria disease: mild (uncomplicated) and severe (complicated) (4). Clinical characteristics that are linked to a bad poorgnosis, such as prostration, decreased consciousness, convulsions, respiratory distress, anemia, jaundice, and shock, are used to define severe malaria (SM). This criteria identifies individuals who need hospitalization and parenteral treatment and captures the majority of patients who are at risk of dying; but, in situations with severe resource constraints, a definition with higher specificity may be required (5). Acute phase reactants include CRP. In response to pro-inflammatory reactions brought on by infection, stress, and tissue injury, the liver produces it. However, it is also thought to play a pathogenic function in malaria. According to some reports, CRP binds to diseased erythrocytes and aids in their removal. Numerous harmful indications are also brought on by this immunological activity in response to contaminated RBCs. Additionally, CRP triggers the complement pathway and platelet activation, which has a number of negative effects. Therefore, CRP testing can be helpful in figuring out how serious malaria develops (6). (CRP) as a biomarker in malaria patients is being researched. It is particularly crucial in areas with a high parasite burden, where patients may not exhibit typical illness symptoms like fever. CRP levels were found to substantially correspond with parasite density in patients' blood in a Tanzanian investigation, regardless of whether those patients experienced clinical symptoms or not (7). Additionally, a different study from Gambia discovered that CRP levels were useful for analyzing malaria in a community (8). CRP levels were employed in this investigation as a stand-in measure for malaria infection and sequelae. In a Tanzanian study, Hurt et al. discovered a correlation between CRP levels and morbidity in children with malaria, particularly in cases where falciparum infection was present (9). The goal of the current investigation was to evaluate the association between CRP levels with comorbidities, species, and complications of severe malaria.

Subjects and Methods

This is a descriptive cross-sectional hospital-based study conducted in Khartoum state hospitals (governmental and private) in the period from April to June 2021. All patients admitted at Khartoum state hospitals with severe malaria diagnosed based on malaria tests (positive BFFM thick and thin blood film or positive ICT) with clinical or laboratory criteria or both of them. Total coverage of all severe malaria patients those fulfill the inclusion criteria, enrolled 65 patients during the study period.

Inclusion and exclusion criteria: Adult patients and both sexes were included. Adult patients admitted with severe malaria with sepsis either (bacterial or viral) on same presentation like (viral or bacterial chest infections, urosepsis, meningitis, etc.) or obvious focus of sepsis like (Infected bed sores or infected DSF) because sepsis causes high CRP. All patients with severe malaria with active inflammatory bowel diseases (ulcerative colitis and Crohn's disease) or active rheumatoid arthritis were excluded.

Data collection tools: Data collection carried out by the principal investigator. Data was collected through structured questionnaires used to collect data consisting of: demographics, presenting symptoms & signs, laboratory investigations, complications, length of hospital stay and outcomes. Manifestations of severe malaria were defined according to WHO criteria.

Data analysis: Data were analyzed by using Statistical Package for Social Studies Program (SPSS, V. 21.0. IBM; Chicago). The analyzed data presented in tables and figures designed by Excel Microsoft 2010. ANOVA test was used as significance test for continuous variables and Chi-Square for categorical variable. P. value is significant at level 0.05.

Ethical considerations: An ethical approval was obtained from Sudan medical specialization board (SMSB). Approval of acceptance to the hospital authority was given. Data used anonymously by using identity numbers instead of names is order to protect patient identity and kept securely and in a separated file. No reference to any individual participant made in study reports. Subject identities were being known only by study staff

Results:

The main manifestations of severe malaria diagnosis criteria were; anemia in 26(40%) patients, AKI in 25(38.5%): and cerebral malaria in 19(29.2%) patients .Concerning to the malaria microscopic features, most of the patients had density 1+ (n=53; 81.5%) and infected by P. falciparum (n=61; 93.8%) .15(32.1%) patients developed complications (with mean duration 4±1 days) as gram –ve sepsis in 10(15.4%) patients, anemic heart failure in 3(4.6%), ARDS in one (1.5%) and bleeding tendency in one (1.5%) patients. In outcomes, 60(92%) patients were normally discharged and 15(8%) were dead. Revealed that, CRP levels were not significantly affected by the age (P= 0.766) and gender (P= 0.111) of the patients. The association between CRP levels and comorbidities. Diabetes mellitus was significantly correlated with elevated CRP levels (P= 0.048). However, hypertension (P= 0.336), history of CVD (P= 0.564), HIV (P= 0.645), liver diseases (P= 0.771), CKD (P= 0.300) and other comorbidities (P= 0.151) were not significantly associated with CRP levels .In the association between CRP levels and malaria density, elevated CRP >100 mg/L was found in all patients (100%) with density 3+ (CRP average= 155), 75% of patients with density 2+ (CRP average= 119) and in 20.8% of those with density 1+ (CRP average= 58), the difference was statistically significant (p = 0.001) .In the association between CRP levels and malaria species, elevated CRP was frequent among patients infected by P. falciparum (CRP average= 75) more than those infected by P. falciparum and P. vivax (CRP average= 13), and the difference was statistically significant (P= 0.033) . The CRP levels were not significantly correlated with length of hospital stay (P= 0.612).CRP levels above 100 mg/L were presented in all non-survived patients

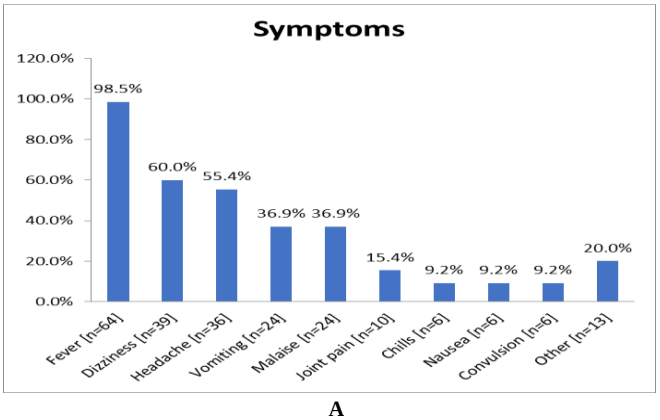
(100%; CRP average= 155) comparing to 26.7% (CRP average= 65) of survived patients (P= 0.003). In total, this study enrolled 65 severe malaria patents, 33(50.8%) were females and 32(49.2%) were males, their mean age was 48±21 year, and most of them 25(38.5%) belonged to the age group from 40-59 year and also, the majority of the patients were workers (n=31; 47.7%) and resided in Khartoum state (n=52; 80%) (Table 1). More than one-half (n=36; 55.4%) of the patients had comorbidities and mainly as diabetes mellitus (n=17; 26.2%) (Table 2).Regarding to symptoms, almost all the cases had fever (n=64; 98.5%); in addition, dizziness (n=39; 60%) and headache (n=36; 55.4%) were the major symptoms in more than 50% of the patients. Tachycardia (n=43; 66.2%) was the predominant sign among our study group (Table 2).In hematological parameters, the mean of ESR was 38±19 mm/hr, hemoglobin 9.5±3 g/dl, MCV 85±9, leucocyte count 8±3 x 10^3 cell/Cumm, PMN differential 55±16%, lymphocyte differential 45±16% and platelets count was 220±140 x 10^3 cell/Cumm. In biochemical parameters, the mean of blood glucose was 140±106 mg/dl, creatinine 2.3±2 mg/dl, urea 74±70 mg/dl, sodium 136±7 mmol/L and potassium was 3.7±0.6 mmol/L (Table 3). Regarding to symptoms, almost all the cases had fever (n=64; 98.5%) and more than 50% had headache (n=36; 55.4%), dizziness (39; 60%), and Tachycardia (n= 43; 66.2%). (Figure 1) .The mean of CRP was 72±57 mg/L, also 10(16%) patients had CRP levels below 10 mg/L, 34(52%) from 10-100 mg/L and 21(32%) patients above 100 mg/L (Figure 2).The Pearson's correlation illustrated that, CRP levels showed significant direct correlation with ESR levels (r= 0.231; P= 0.04) and blood glucose levels (r= 0.262; P= 0.039) (Table 8). The presence of complications was significantly associated with elevated CRP levels above 100 mg/L comparing to those without complications (73.3% vs 20%; P= 0.001; complication CRP average= 130.4 and non-complication CRP average= 54). Also, patients those developed anemic heart failure (66.7%) were more tended to had CRP levels ranged from 10-100 mg/L, while patients those developed ARDS (100%), bleeding tendency (100%) and gram –ve sepsis (80%) were more tended to had CRP above 100 mg/L (P= 0.015) (Table 4).

Table 1: The demographic characteristics of severe malaria patients

	N	%
Age (yrs.)	**48±21**	
<20	6	9.2
20-39	16	24.6
40-59	25	38.5
60+	18	27.7
Gender		
Female	33	50.8
Male	32	49.2
Residence		
Khartoum	52	80.0
Central	9	13.8
West	4	6.2
Occupation		
Worker	31	47.7
Housewife	24	36.9
Student	7	10.8
Employee	1	1.5
None	2	3.1

Table 2: The co morbidities among severe malaria patients

	N	%
Comorbidities (Yes)	36	55.4
DM	17	26.2
Hypertension	9	13.8
History of CVD	9	13.8
HIV	3	4.6
Liver disease	2	3.1
CKD	2	3.1
Other	10	15.4



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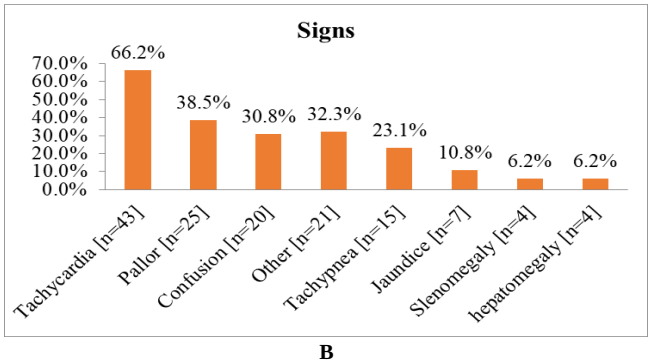

B

Figure 1: The distribution of symptoms and signs among severe malaria patients

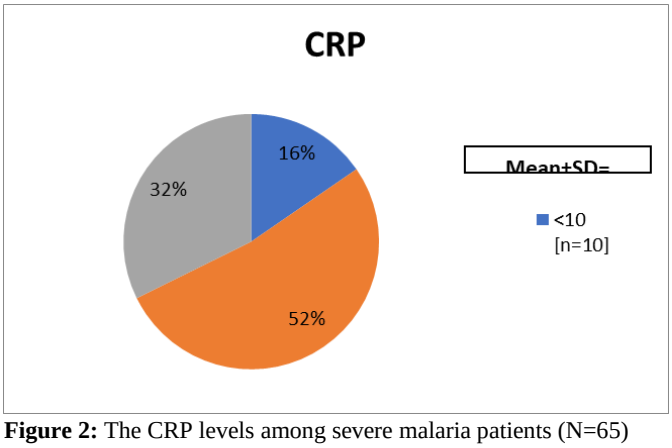

Figure 2: The CRP levels among severe malaria patients (N=65)

Table 3: The laboratory investigations of severe malaria patients (N=65)

	Mean	SD
Hematology		
ESR (mm/hr)	38	19
Hemoglobin (g/dl)	9.5	3
MCV	85	9
TWBC (*10^3 cell/Cumm)	8	3
PMN diff (%)	55	16
Lymph diff (%)	45	16
Platelets (*10^3 cell/Cumm)	220	140
Biochemistry		
RBG (mg/dl)	140	106
Creatinine (mg/dl)	2.3	2
Urea (mg/dl)	74	70
Na+ (mmol/L)	136	7
K+ (mmol/L)	3.7	0.6

Table 4: The Pearson's correlation between CRP levels and laboratory investigations

	Correlation coefficient (r)	P
Hematology		
ESR (mm/hr)	0.231	0.040*
Hemoglobin (g/dl)	-0.183	0.879
MCV	-0.055	0.687
TWBC (*103 cell/Cumm)	0.139	0.272
PMN diff (%)	0.015	0.912
Lymph diff (%)	-0.014	0.914
Platelets (*103 cell/Cumm)	0.009	0.942
Biochemistry		
RBG (mg/dl)	0.262	0.039*
Creatinine (mg/dl)	-0.158	0.210
Urea (mg/dl)	0.044	0.731
Na+ (mmol/L)	0.028	0.825
K+ (mmol/L)	0.139	0.269

Discussion

In the present study, we aimed to determine the significance of CRP levels among 65 severe malaria patients in Khartoum state . Severe malaria was approximately affected males (49.2%) and females (50.2%) equally, and was common among middle-aged patients aged from 40-59 year (38.5%) a mean age 48±21 years. This is in accordance with Sudanese studies (10, 11) those reported severe malaria affected male and female equally. Also, another study in India reported severe malaria affected male and female equally with mean age 42 years (12). Fever was the common symptom in all cases, in addition to dizziness, and headache. Also, in the study of Hasan et al in Khartoum state, fever was presented in all severe malaria patients (81 patients) and vomiting combined with diarrhea . Our study similar to another study reported that fever and vomiting were the main symptoms (14). P. falciparum was the causative agent of severe malaria in 93.8% of the patients, and this confirmed that P. falciparum is the commonest malaria species in Sudan as reported by other studies (2, 13 and 10). Our results showed the frequency of P. vivax infection was 6.2% and this rate was comparable to the study in Eastern Sudan (6). This may be due to an influx of people from Ethiopia where : P. vivax infection is increasing (15). Severe malaria anemia (SMA) was reported in patients with severe disease, which is a result from intravascular hemolysis caused by malaria species. These findings were higher than the studies in Sudan (13) and in Ethiopia (16) those reporting severe malaria anemia (SMA) were presenting in 14.2% and 15.9% of severe malaria patients, respectively. And lower than that reported by Zeidan A et al in Sudan (44), Achidi EA et al in Cameroon (17) and Oduro et al in Ghana (18) those reported severe malaria anemia in 62%, 87.9%, and 81%, respectively. This variation may be due to the multifactorial etiology of SMA that could also be influenced by the nutritional status and severity of malaria as well as late presentation. The current study demonstrated that, cerebral malaria (CM) was present in 19(29.2%) of patients. These findings were similar to the studies in Sudan (13) and in Cameroon (17): In the present study, we recorded acute kidney injury in 25(38.5%) patients with severe disease. And this disagree with the result the results of several studies in Sudan (13,19,20) those reported acute renal failure in 17.4%, 15.4% and 18.3% of patients, respectively. The current study illustrated that, the overall case fatality rate of malaria was 8% (n=15 patients). Other studies (24,25,26), had different results where the percentages of mortality were (17.2, 23, 24, 24)%, respectively. These disparities in fatality rates might be attributed to differences in geographical areas, causative malaria species and management protocols and guidelines used. This study showed that, 15(32.1%) patients developed complications (with mean duration 4±1 days) and gram –ve sepsis was the major complication in 10(15.4%) patients. Recent studies in sub-Saharan Africa showed that 4–23% of patients with severe malaria had concomitant sepsis (27, 28). Bacterial sepsis is less common but still considerable, with a reported incidence of 13% in Asians population (29). C-reactive protein (CRP), an acute phase reactant, whose plasma concentration increases during inflammatory disorders, has gained considerable attention as a biomarker in malaria. Our findings were comparable with tothe study that found 92% o SM patients had elevated CRP levels above 10 mg/L (30). Also, our finding is consistent with results from studies conducted in The Gambia, Mozambique and Malawi (8, 9,31) where malaria was also found to be associated with elevated CRP levels. In contrast, our CRP mean was higher than that reported (31.29 ± 20.4 mg/L) (6). The association between CRP levels and co morbidities showed that, DM was significantly correlated with elevated CRP levels (P= 0.048). Additionally, CRP levels showed significant direct correlation with blood glucose levels. These findings were confirmed by the study of Tabassum R et al who found that the mean CRP level was significantly higher in diabetic patients -diabetics . Also, they concluded and supported a possible role of inflammatory markers in diabetogenesis (32). Similar study showed a significant association between C-reactive protein levels and hyperglycemia concluding that hyperglycemia is a related factor to the increase of serum CRP levels (33). Our study demonstrated that, elevated CRP levels were significantly increased with malaria parasite density in blood film .These observations are indicating that, the levels of CRP are directly correlated with the severity of malaria. Correspondingly, researchers in Nigeria reported the predictors of the C-reactive protein response in malaria (CRP ≥ 10mg/l) were malaria parasite count (34). Also, study in Ghana reported that the median CRP level was significantly higher in high malaria parasitaemia compared to moderate and low malaria parasitaemia(35). In a study from Tanzania, CRP levels were found to correlate strongly with parasite density in the blood of patients, whether they had clinical features or not (7). Other researcher found

that, patients with increased CRP levels had more than an eight-fold likelihood for parasitemia (30). In this study we noticed that elevated CRP was frequent among patients infected by P. falciparum (CRP average= 75) more than those infected by P. falciparum and P. vivax (CRP average= 13); the difference was statistically significant (P= 0.033). However, in India, reported that average CRP levels did not significantly affected by malaria species either P.f or P.v (6). Considerably, our study illustrated that the levels of CRP were significantly greater in patients with complicationsthan those without complications (P< 0.05). Consistently, several studies (6,9,36) mentioned that patients with complications had significantly elevated CRP levels compared to those without complication (P< 0.05). Although, found CRP levels at presentation showed positive correlation with duration of hospital stay (r = 0.59; P < 0.05) (6), we showed that CRP levels were not significantly correlated with length of hospital stay . Interestingly, the present study revealed that, elevated CRP levels were significantly associated with mortality among our study participants . These findings should emerge the use the CRP s monitor and prognostic marker for all patients with malaria. Our results were confirmed where patients who died had higher CRP levels compared to survivors (6). We recommended that use of CRP levels as a surrogate marker for malaria infection and complications. CRP should be used as tool in assessment of prognosis in malaria. Further prospective studies with larger sample size are needed.

Conclusion:

The present study concluded that, elevated CRP levels were found in a considerable proportion of severe malaria patients. Also, elevated CRP levels were significantly correlated with DM in comorbidities, high malaria parasite density in blood film, P. falciparum infection, complications, and mortality.

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Conflict of Interest

The authors declared no conflict of interests

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Research Article

Thyroidectomy in elderly; is it safe?

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ABSTRACT

Background: The prevalence of thyroid nodules rise with age and different data available about the risks of thyroid surgery in old age people. In general, old age could be a predictor of perioperative mortality and morbidity. The aim of this study is to prove if there is increased risk accompanying thyroidectomy in elderly patients.

Subjects and Methods: Prospective study of one surgeon of study sample undergoing thyroid surgery at Al-Kindy teaching hospital and Saint Raphael hospital .This study was including two groups; group (A) involved patients 65 years and older, group (B) involved patients below 65 years old who were subjected to thyroidectomy. Taking in consideration histopathology results, indications of surgery (compressive symptoms, suspicious or confirmed malignancy, toxic goiter and recurrent goiter) and complications (including rates of temporary and permanent hypocalcaemia, temporary and permanent RLN paralysis, postoperative hematoma, wound infection and seroma), in addition to the risk of perioperative mortality.

Results: There were 574 patients below 65 years and 127 elderly patients (>=65 years) who underwent thyroidectomy between January 2015 and December of 2018. There were no deaths in either group; no one had bilateral RLN paralysis. Old age patients had a lower frequency of complications in comparison to the younger counterparts, including transient hypocalcaemia (3.1% vs 14.8%, respectively) and temporary RLN injury (0% vs 0.69%, respectively), in addition to permanent RLN injury (0% vs 0.34%, respectively).

Conclusions: Thyroidectomy in elderly is safe as compared to younger patients regarding perioperative complications.

Introduction

Who had defined elderly patients in developed world as people older than 65 years [1]. Thyroid diseases including benign or malignant occur commonly in the old people and this may be partly related to that the incidence of thyroid nodules increase with age [2]. The prevalence of thyroid nodules and thyroid malignancy increases with age [3-5]. Thyroid gland dysfunction is common among older patients, and can lead to significant morbidity when left untreated [3].

Some other studies say that about 90% of women after the age of 60 years demonstrate the thyroid nodules and about 60% of men after the age of 80 [6]. The percentage of the elderly people to the younger people had increased by ninety percent over the last thirty

years, and some authors predict that by the year 2020, the ratio of the people over sixty-five years will increase from 12.4 % to 20% [7].
Other authors estimated that if in 2000 in the world there were 600 million people in age of 65 years or more, in 2050 there will be 2 billion [6]. According to the German Federal Statistical Office, 28% of the population in 2030 and 33% in 2060 will be older than 65 years [8]. Therefore, more elective surgery is performed on aged patients than in the past decades [9].
Approximately fifty percent of all surgical operations are involving patients over sixty-five years [10]. In general surgery, one of the main predictive factors for perioperative morbidity is age [11]. Although age is not a contraindication for major surgery [12-14], few elderly patients receive programmed thyroid surgery due to the major risk of morbidity [13-15].
Common surgical indications in the elderly include secondary thyrotoxicosis resistant to non-surgical treatment, compressive symptoms, suspicious thyroid nodule requiring histologic diagnosis or confirmed thyroid malignancy [16, 17].
In general, some authors suggest that old people have a much stronger or emergent indications for surgery like trachea infiltration or severe compressive symptoms due to thyroid carcinoma [18]. Some authors suggest that thyroid surgery presents various risks for older people [19]. Nevertheless, the decision to perform surgery is not often straightforward as reoperation for persistent or recurrent thyroid cancer is generally associated with increased surgical morbidity [20].
Thyroid surgery remains the treatment of choice for benign and malignant thyroid diseases. Because of improved methods of disease detection and treatment, there will be an increased demand for thyroid surgical procedures in the elderly patients' population [21].
A recent study from Germany also reported that thyroid surgery could be performed safely and without impairment of quality of life [22].
It had been proposed that thyroid surgery in elderly should be limited to those whose lesions are at high risk of malignancy, whereas low risk lesions may be followed [23]. Since thyroid surgery remains one of the most commonly performed operations worldwide, thyroid surgery in the elderly has become an important topic in endocrine surgery [24, 25].
Furthermore, over the last 10 years, major advances have occurred in general and endocrine surgery, anesthesia, perioperative care, instrumentation, and technology and they tend to reduce the overall operative risk, allowing greater proportion of elderly patients to undergo elective surgery [26].
The aim of this study is to prove if there are increased risks associated with thyroidectomy in the elderly patients.

Subjects and Methods

This prospective study was conducted in Al-Kindy teaching hospital (surgical unit) and Saint Raphael hospital during a period from January 2015 to December 2018. Patients were divided into two groups: Group (A) patients were 65 years and older. Group (B) patients were those below 65 years of age.

Perioperative measures:

Preoperative assessment for all patients were done including thorough history and clinical examination and thyroid function tests, preoperative vocal cords assessment and serum calcium level were done in addition to necessary laboratory investigations (complete blood count , blood urea , serum creatinine , random blood sugar, liver function test , viral screen , chest x-ray , ECG , ultrasound of neck. CT scan of the chest in special circumstances including retrosternal extension and echo study in the elderly and as needed) were done.
All patients with comorbidities were sent for medical consultation for control of comorbidity preoperatively.
All patients were subjected to total thyroidectomy, near total thyroidectomy or hemi thyroidectomy depending on patients' condition using Ligasure scalpel for hemostasis and one surgeon did all of these surgeries.
All patients were given intravenous one shot of broad-spectrum Antibiotic at the time of induction of anesthesia. All patients were sent for serum calcium level on first postoperative day and patients with symptomatic hypocalcaemia were given I.V infusion calcium and put on calcium tablets and one alpha capsules, Redivac drains were removed at 24-48 hours after surgery.
All patients put on levothyroxine tablet at morning before breakfast starting with 100 micrograms and adjusting dose according to follow up results.

Inclusion criteria

The indications of surgery were Compressive symptoms (dysphagia, dyspnea), Suspicious/confirmed malignancy, toxic goiter and recurrent goiter.

Exclusion criteria

Patients with inoperable anaplastic carcinoma (hard, fixed), those who refuse to participate in the study, and those who had no enough information or lost follow up were excluded

Ethical considerations

The research proposal of the study was fully discussed by the scientific and ethical committee of the Iraqi board of general surgery.
The agreement of health authority in Al-Kindy teaching hospital was approved before starting of data collection.
A written consent was taken from each included patient after a full explanation about the type of surgery and the possible postoperative complications and the aim of the study and insurance of included patients that the collected data will be used for scientific purposes only and will be anonymous.

Statistical analysis

The collected data were introduced into Microsoft excel sheet and loaded into IBM-SPSSV24 statistical package for statistical analysis. Descriptive statistics were presented using tables (frequency and percentages). Inferential statistics were presented through chi – squares test and fisher's exact test to find out associations between related studied variables. P-value less than 0.05 were considered as discrimination point of significant association.

Results:

Group (A) patients were 65 years and older (127 patients) (oldest was 82 years). Group (B) patients were those below 65 years of age (574 patients) (youngest was 12 years).

Ninety-three patients in the group (A) were female (n = 93; 73.2%), and 465 in the group (B) were female (n = 465; 81.0%). While male patients were 34 patients in group (A) (n = 34; 26.8%) and 109 patients in group (B) were females (n =109; 19%) as shown in table 1.

Ninety eight patients in group (A) had 24 hours hospital stay, (n = 98; 77.2%) and 527 patients in group (B) stay for 24 hours (n = 527; 91.8%), while 29 patients in group (A) had stay for 48 hours (n =29; 22.8%) and 47 patients in group (B) had stay for 48 hours (n =47; 8.2%) as shown in table 1.

While regarding histopathological results, 118 patients in group (A) had a benign pathology (n = 118; 92.9%), and 511 patients in group (B) had a benign pathology (n = 511; 89.02%). Regarding malignant pathology, only 9 patients had malignancy in group (A) (n =9 ; 7.1%) and 63 patients in group (B) (n =63 ; 10.98%) as shown in table 1.

Table 1: Distribution of patients' socio-demographic and clinical characteristics of the study sample

List	Age group	Group (A)		Group (B)		P-value
		Frequency	Percent	Frequency	Percent	
	Gender	**Group (A)**		**Group (B)**		**P-value**
1.	Male	34	26.8	109	19.0	0.800 (NS)
	Female	**93**	**73.2**	**465**	**81.0**	
	Total	127	100.0	574	100.0	
	Hospital stay	**Group (A)**		**Group (B)**		**P-value**
2.	24 hours	**98**	**77.2**	**527**	**91.8**	0.000 (HS)
	48 hours	29	22.8	47	8.2	
	Total	127	100.0	574	100.0	
	Histopathology results	**Group (A)**		**Group (B)**		**P-value**
3.	Benign	**118**	**92.9**	**511**	**89.02**	0.090 (NS)
	Malignant	9	7.1	63	10.98	
	Total	127	100.0	574	100.0	

F= frequency; %= percentage; NS= Not Significant;
HS = highly significant

Both groups experienced Compressive symptoms as a main indication of the surgery; group (A) (n = 71; 55.9%), group (B) (n = 393; 68.5%), followed by suspicion or confirmed malignancy (n = 25; 19.7%) in group (A) and (n = 104; 18.1%) in group (B) as shown in table 2.

Table 2: Table 2. Distribution of indications of surgery

Indication of surgery	Group (A)		Group (B)		P-value
	Frequency	Percent	Frequency	Percent	
Compressive symptoms	71	55.9	393	68.5	0.331 (NS)
Suspicious/confirmed malignancy	25	19.7	104	18.1	
Toxic goiter	24	18.9	64	11.1	
Recurrent goiter	7	5.5	13	2.3	
Total	127	100.0	574	100.0	

F= frequency; %= percentage; NS= Not Significant

There were no recorded patients in group (A) who had temporary Unilateral RLN injury as a complication after surgery (n = 0; 0%) in comparison to 11 patients in group (B)(n = 11; 0.69%) as shown in table3

With regard to Permanent Unilateral RLN injury, there is no patients in group (A) (n = 0; 0%), while there were 2 patients who had permanent unilateral RLN injury from the patients in group (B) (n = 2; 0.34%).

There was no bilateral RLN injury recorded in this study.

No tension hematoma was recorded in group (A) (n = 127; 100%), while in group (B), only one patient was developed hematoma and it was evacuated in the theatre at night of surgery and the bleeder was ligated (n = 1; 0.2%) as shown in table 3

Table 3: Distribution of post-operative complications (RLN injury and tension hematoma) of the study sample:

	Temporary Unilateral RLN injury	Group (A)		Group (B)		P-value
		Frequency	Percent	Frequency	Percent	
1.	Yes	0	0	11	0.69	
	No	**127**	100	**563**	99.31	
	Total	127	100.0	574	100.0	
	Permanent Unilateral RLN injury	**Group (A)**		**Group (B)**		**P-value**
		Frequency	**Percent**	**Frequency**	**Percent**	
2.	Yes	0	0	2	0.34	
	No	**127**	**100.0**	**572**	**99.66**	
	Total	127	100.0	574	100.0	
	Hematoma	**Group (A)**		**Group (B)**		**P-value**
3.	Yes	0	0	1	2	
	No	**127**	100	**573**	99.8	
	Total	127	100.0	574	100.0	

F= frequency; %= percentage; NS= Not Significant

Table 4: Table 4. Distribution of post-operative complications (hypocalcaemia and seroma) of the study sample

	Temporary Hypocalcaemia	Group (A)		Group (B)		P-value
		Frequency	Percent	Frequency	Percent	
2	Yes	4	3.1	85	14.8	0.938 (NS)
	No	123	96.9	489	85.2	
	Total	127	100.0	574	100.0	
	Permanent Hypocalcaemia	**Group (A)**		**Group (B)**		**P-value**
3	Yes	0	0	7	1.2	
	No	127	100.0	567	98.8	
	Total	127	100.0	574	100.0	
	Seroma	**Group (A)**		**Group (B)**		**P-value**
4	Yes	4	3.1	12	2.09	0.122 (NS
	No	123	96.9	562	97.91	
	Total	127	100.0	574	100.0	

F= frequency; %= percentage; NS= Not Significant

Only 4 Patients in group (A) (n = 4; 3.1%) had temporary hypocalcaemia, while in group (B) there were 85 patients (n = 85; 14.8%) with temporary hypocalcaemia as shown in table 4.

There were no patients in group (A) who had Permanent Hypocalcaemia (n = 0; 0%), we found 7 patients in group (B) (n = 7; 0.34%) as shown in table 4.

Moreover, concerning Seroma, 4 patients in group (A) had Seroma (n = 4; 3.1%), and 12 patients for those in group (B) had Seroma after surgery (n = 12; 2.09%) as shown in table 4.

Concerning post-operative wound infection, 2 patients had signs of infection in group (A) (n = 2; 1.6%), while in group (B), 16 patients had infected wound (n = 16; 2.8%) as shown in table 5.

Table 5: Distribution of post-operative complications (wound infection) of the study sample

	Wound infection	Group (A)		Group (B)		P-value
		Frequency	Percent	Frequency	Percent	
1.	Yes	2	1.6	16	2.8	0.791 (NS)
	No	**125**	98.4	**558**	97.2	
	Total	127	100.0	574	100.0	

F= frequency; %= percentage; NS= Not Significant

There was no perioperative mortality recorded in this study

Discussion

The management of thyroid disorders has become increasingly specialized. The techniques of safe anesthesia and operative skills have led to an increase in the proportion of these patients being managed in specialized endocrine centers [17]. Recent studies stated that increased mortality in elderly was influenced by biological age than chronological age [14] and on the number of concomitant comorbidities [27, 28]. Passler et al., [15] indicated surgery only when absolutely indicated.

The difference between the indications of surgery in the two groups in this study:

1- Compressive symptoms 55.9% in group (A) , 68.5% in group (B)
2- Suspicion Confirmed malignancy 19.7 % in group (A) , 18.1 % in group (B)
3- Toxic goiter 18.9% in group (A) , 11.1% in group (B)
4- Recurrent goiter 5.5% in group (A) , 2.3% in group (B)

In this study, the main indication of surgery was compression symptoms next suspicion or confirmed malignancy then toxic goiter. In a study performed by Rios et al., They revealed that elderly patients more often than younger ones presented compressive symptoms and then suspicion for malignancy, recent goiter, or patient request [5].

In another study done by Raffaelli et al., they analyzed the indications for thyroid surgery in those patients whose age is 70 years and more, they noticed that the most common indication was bilateral multi-nodular goiter, next suspicion or confirmed malignant process, and toxic goiter [16].

In similar observations revealed by Lang and Lo [26], they confirmed that in patients aged 70 years and more the most common indication for thyroid surgery was retrosternal goiter, but they added that in this group of patients the volume of goiter was significantly higher.

K. Kaliszewski et al., showed that the main indication for surgery in the elderly patients group was compression symptom. The second was verified malignant tumor or suspicion of malignancy. The number of retrosternal goiters in elderly patients was significantly higher than in younger ones [29].

The next important issue of indications and thyroid surgery in the elderly patients is recurrent goiter; some authors said that the number of recurrent thyroid operations is significantly higher in elderly patients than in younger group [15, 17].

These observations were confirmed in this study, which encourage for total or near total thyroidectomy in case of multi-nodular goiter in general for all patients in endemic countries as in Iraq to prevent recurrence.

The difference between the rates of complications in the two groups regarding:

* Tension hematoma 0 % in group (A), 2% in group (B).

F. Tartaglia et al., said that in older group it was 3.34% vs 1.49% in younger [30]. N.Tabriz show that it was 1% [31].

The low incidence in our study might be related to secured hemostasis especially by using Ligasure scalpel and proper timing of drain removal.

*Temporary hypocalcaemia 3.1% in group (A), 14.8% in group (B)

*Permanent hypocalcaemia 0% in group (A), 1.2% in group (B)

C. Passler et al., said that the risk of hypo-parathyroidism in older group it was 13.6% vs 14.1% in younger [15]. Patients who were ≥70 years had a lower (but non-significant) postoperative and definitive hypocalcaemia rate than patients < 70 years: 14.85% vs 20.44% regarding temporary hypocalcaemia and 0% vs 2.15% regarding permanent hypocalcaemia [32]. N. Tabriz et al., showed that temporary postoperative symptomatic hypocalcaemia is (20%) [31].

*Temporary unilateral RLN injury 0% in group (A), 0.69% in group (B)

*Permanent unilateral RLN injury 0% in group (A), 0.34% in group (B)

*Bilateral RLN injury 0% in group (A), 0% in group (B)

F. Tartaglia et al., said that in older group it was 4.01% vs 3.92% in younger as nerve palsy in general [30]. Abnormal vocal cord motility rate was 12.00% in patients ≥70 years vs 9.75% in patients <70 years regarding temporary RLN injury, and 2.06% in patients ≥70 years vs 0.86% regarding permanent RLN injury [29]. N. Tabriz et al., showed that temporary vocal cord paralysis is (9%) [31].

This low percentage is strongly related to the mandatory identification of RLN in all of our patients, and presence of RLN injury may be due to thermal injury of Ligasure scalpel.

* Wound infection 1.6% in group (A), 2.8% in group (B)

C. Passler et al., stated that the rate in older group is 2% [15].

N. Tabriz show that infection is (5%) [31].

* Seroma 3.1% in group (A), 2.09% in group (B)

F. Tartaglia et al., said that in older group it was 3.12% vs 2.85% in younger [30].

Regarding histopathological results, in group (A) 92.9% benign and 7.1% malignant, in group (B) 89.02% benign and 10.9% malignant .F. Tartaglia et al., found higher incidence of malignant pathology in younger (21.25% vs 18.37%) [30].

Regarding hospital stay, in group (A) was 99.2% only 24 hours and 0.8% required 48 hours (mean =1.23) and in group (B) was 99.8% for 24 hours and 0.2% for 48 hours (mean = 1.08). Grogan et al., had

found that elderly (1.4 days) had significant increases in the hospital stay compared with the young (1.1 days) [33].
N. Tabriz showed that younger patients (<70 years, median = 3 days) had significantly shorter postoperative hospital stays than older patients (≥70 years, median = 4 days) [31]. This highly significant difference may be because elderly were kept more hours for close monitoring of comorbid illnesses.
The actual proportion of elderly patients undergoing elective thyroid surgery ranged between 2.5% to 21.2%, depending on patient selection and type of referral [15, 17, 26, 33-36]. In this study, group (A) was 22.1% and group (B) 81.8% from total number of thyroidectomies.
Regarding perioperative mortality which was 0% in this study, mortality in other studies is low; Passler [15] reported no perioperative mortality; Har- El [37] reported 1.9 % in old age patient with CA thyroid. This was related to proper preparation of old age patients for surgery especially those with comorbidities.

Conclusion:

Thyroidectomy in elderly is a safe surgical operation showing a better morbidity and mortality rate in relation to younger age group.
The chronological age by itself is not a contraindication to surgery.
The most common indication of surgery was benign thyroid lesions.
Postoperative complications rate in elderly does not differ from those noticed in younger patients.
In well selected patient group done by experienced surgeon, there was no significant increase in morbidity and mortality could be documented. Elderly with medical comorbidities need a complete and thorough preoperative work up before undergoing any thyroid intervention.

Recommendations

For old patients who have clear indication of surgery, we recommend a careful preoperative evaluation and risk stratification.
We further suggest improving pre and postoperative care with better monitoring. In the future, more data are required for analyses concerning indications for thyroid surgery in elderly in relation to overall benefits, including long-term survival, life quality, and recurrence of goiter.
Larger population-based studies will most likely provide optimal understanding and better approach to the elderly people in thyroid surgery

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Conflict of Interest

No conflict of interest

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Research Article

The Efficiency of Corn Solution as a Cytological Fixative in Buccal Smear

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Keywords: Corn syrup, cytological fixative, maltose, oligosaccharide.

ABSTRACT

Background: Corn Syrup is food syrup higher of carbohydrate, depending on grade. The study aimed to assess efficiency of Corn syrup as cytological fixative.
Subjects and methods: This was laboratory based study, it has been conducted at Elrazi University included apparently 30 healthy students have been involved in this study.
Results: Out of 30 smears fixed with 95% alcohol, 76.7% (n=23) shows excellent nuclear stain, 23.3% (n= 7) shows good nuclear stain. 70% (n=21) show excellent cytoplasmic stain, 26.7% (n=8) shows good cytoplasmic stain, 3.3% (n=1) shows poor cytoplasmic stain.
Out of 30 smears fixed with corn solution, 60% (n=18) shows excellent nuclear stain, 40% (n=12) good nuclear stain, 3.3 % (n=1) shows excellent cytoplasmic stain, 83.3% (n=25) shows good cytoplasmic stain, 13.3% (n=4) shows bad cytoplasmic stain.
Conclusion: Study concluded that Corn syrup can be used as cytological fixative alternatively to 95% ethyl alcohol.

Introduction

Corn Syrup is primary ingredient in most brands of commercial "pancake syrup "as a less expensive substitute for maple syrup (1; 2; 3).

Corn syrup is used in baked goods and other many food products, and dark corn syrup is also used (4).

Study has shown that sugar preserve cell morphology and causes no difficulties in routine processing and staining. These substances are harmless, eco-friendly, well suited for laboratory processing and staining.

Exofliative cytology is the microscopic study of cells shed or obtained from body especially for diagnostic purposes (as in determining the presence or absence of cancerous condition) (5).

Corn syrup can also be used as a good cytological fixative alternatively to common 95% ethanol alcohol.

Fixation is an important step in cytological diagnosis and the basis or foundation of cytological technique. Ethanol is traditionally a popular and widely used fixative for cytological diagnosis. Commercially ethanol is expensive and not freely available in some institution, flammable, beside its toxicity, it has a

pungent, irritating odor. Corn syrup is less costly, harmless, eco-friendly, well suited for laboratory processing and staining (5).
The study aimed to assess efficiency of Corn syrup as cytological fixative.

Subjects and Methods

This was laboratory-based study, it has been conducted at Elrazi University included apparently 30 healthy students have been involved in this study.

Ethical approval and consent to participate

Ethical approval was obtained from Ministry of Health Ethical Research Committee in accordance with the Declaration of Helsinki Principles, and the agreement was taken from all hospital administration before sample and data collection. The patient's information were highly secured and not used for other purposes than scientific inquiry.

Each participant was asked to sign a written ethical consent form during the interview, before the specimen was taken.
Ethical clearance code number: MH-RES/8-021-09
Date: 3/5/2021

Statistical analysis:

Data was analyzed using statistical package for social science software version (20) SPSSV20 (IBM Corp, Armonk, NY, USA).

Method of specimen collection:

A commercially available tongue depressor was used for the collection of the samples. The scraps were smeared onto the center of the glass slides and spread over a large area, preventing the clumping of cells. The smears were immediately fixed with 95% alcohol (control) and corn syrup (test) (6).

Corn syrup preparation:

30 g of corn were boiled with 300 ml of water and then filtered to remove any excess deposits. Then we added 45 g of sugar and 3.5 ml of lemon juice to the filtered syrup and boiled it again till it gains its viscosity. The syrup has diluted first before fixation, 1:2 with DW (5).

Staining technique:

The smear were fixed by corn syrup for 15-30 min, then it was treated with 70% alcohol 2 min , rinse in Distilled water 3 min, stain with Harris's for 3 min, wash by water, differentiated by 1% acid alcohol for seconds, rinse in water then dehydrate in 70% alcohol for 2 min , stain with Orange G6 for 2min,then rinse in 95% alcohol (2) for 2 min , then 95% alcohol (1) 2 min, stain Eosin Azure for 3min , rinse in 95% alcohol (2) 2 min , then 95% alcohol (1) for 2 min , mount cover slip with DPX (Distyrene, plasticizer and xylene) , to be examined by light microscopically (6).

Staining assessment:

- Nuclei appear blue/black.
- Cytoplasm blue/green.
- Keratinizing cell pink-orange

Assessment of cytological smears for staining quality

The smears were assessed and evaluated by an experienced cytotopathologist. For comparative analysis of both techniques, parameters such as thickness, cellular distribution were evaluated, adopting criteria reported elsewhere (7; 8). Also, given that a good staining method must show the shapes and sizes of the cell, provide crisp delineation of nuclear chromatin, and demonstrate the cytoplasm, each slide was evaluated as follows: (i) excellent;(ii) good; (iii) poor. All parameters were compared to standard parameters illustrated elsewhere, (9) and the degrees were given (10).

Results

From each case involved in this study two samples have been collected, one sample was fixed with 95% ethanol alcohol as control, while the other was fixed with corn syrup as test.

Out of 30 smears fixed with 95% alcohol, 76.7% (n=23) shows excellent nuclear stain, 23.3% (n= 7) shows good nuclear stain. 70% (n=21) show excellent cytoplasmic stain, 26.7% (n=8) shows good cytoplasmic stain, 3.3% (n=1) shows poor cytoplasmuc stain.

Out of 30 smears fixed with corn solution, 60% (n=18) shows excellent nuclear stain, 40% (n=12) good nuclear stain, 3.3 % (n=1) shows excellent cytoplasmic stain, 83.3% (n=25) shows good cytoplasmic stain, 13.3% (n=4) shows poor cytoplasmic stain.

Out of 30 smears fixed with 95% alcohol, 76.7% (n=23) shows excellent nuclear stain , 23.3% (n= 7) shows good nuclear stain, Out of 30 smears fixed with corn solution , 60% (n=18) shows excellent nuclear stain, 40% (n=12) good nuclear stain. This result shows no significant variation between corn solution and 95% ethanol alcohol in nuclear stain (P value=0.165), as showing in table (1).

Table 1: Effects of fixative in the quality of nuclear stain

Type of agents	Quality of nuclear stain		Total
	Good	Excellent	
Corn solution	12(40.0%)	18(60.0%)	30(100.0%)
95% ethyl alcohol	7(23.3%	23(76.7%)	30(100.0%)

P value=0.165

Out of 30 smears fixed with 95% alcohol 70% (n=21) show excellent cytoplasmic stain, 26.7% (n=8) shows good cytoplasmic stain , 3.3% (n=1) shows poor cytoplasmic stain, Out of 30 smears fixed with corn solution 3.3 % (n=1) shows excellent cytoplasmic stain, 83.3% (n=25) shows good cytoplasmic stain, 13.3% (n=4) shows poor cytoplasmic stain. This result shows highly significant variation between corn solution and 95% ethyl alcohol in cytoplasmic stain (P value=0.000), as showing in table (2).

Table 2: Effects of fixative in the quality of cytoplasmic stain

Type of agents	Quality of cytoplasmic stain			Total
	Poor	Good	Excellent	
Corn solution	4(13.3%)	25(83.3%)	1(3.3%)	30(100.0%)
95% ethyl alcohol	1(3.3%)	8(26.7%)	21(70.0%)	30(100.0%)

P value= 0.000

Discussion

In this study we compare between 95% ethanol alcohol and corn syrup as cytological fixative, the procedure of fixation with corn syrup is inexpensive.

From each case involved in this study two samples have been collected, one sample was fixed with 95% ethanol as control, while the other was fixed with corn syrup as test.

Out of 30 smears fixed with 95% alcohol, 76.7% (n=23) shows excellent nuclear stain, 23.3% (n= 7) shows good nuclear stain. 70% (n=21) show excellent cytoplasmic stain, 26.7% (n=8) shows good cytoplasmic stain, 3.3% (n=1) shows poor cytoplasmuc stain.

Out of 30 smears fixed with corn solution, 60% (n=18) shows excellent nuclear stain, 40% (n=12) good nuclear stain, 3.3 % (n=1) shows excellent cytoplasmic stain, 83.3% (n=25) shows good cytoplasmic stain, 13.3% (n=4) shows poor cytoplasmic stain.

All the smears in both group was satisfactory. It shows good fixation and also good staining intensity of oral squmous cells and background which agreed with previous study (5; 11; 12).

Conclusion

Study concluded that Corn syrup can be used as cytological fixative alternatively to 95% ethyl alcohol.

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Conflict of Interest

No conflict of interest

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Research Article

Practice & Opinion of Doctors in Hospitals toward Referral System in Iraq

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ABSTRACT

Background: Primary Health care (PHC) is common to be the cornerstone of a person-centered health system. While the adoption of a well-function, two-way, and organized referral system is the mainstay in the development of an efficient healthcare delivery system.
Objective: to assess hospital doctor's practice and opinion toward referral system and to determine their commitment to its instructions and guidelines.
Subjects and Methods: A cross-sectional study (with analytic elements) was conducted in nine Iraqi governorates. Eight doctors from each health directorate, resulting in a total of 72 doctors using a specially designed questionnaire. Statistical analysis was done by using SPSS, the P≤0.05 was considered statistically significant.
Results: most of the total 72 physicians, 31(43.1%) aged 40-49years, 48(66.7%) were males, and 58(80.6%) were specialists., 58 doctors (80.6%) had no coordination with the PHC-doctors, the same percentage considered the current referral system is ineffective. PHC patients-crowded, doctors-shortage were the causes of inadequately filling PHC-part of the referral form. And PHC-hospital doctors-coordination had a significant association with their age, gender, and their thinking about adequate filling.
Conclusion: despite the importance of the referral system, half of the doctors disagreed about the current referral system's effectiveness; & most of them had no coordination with the PHC-doctors. PHC crowded/doctors' shortage the main causes of inadequate filling PHC part of referral-form.

Introduction

In most countries, the national health care system provides services for three tiers of health care; primary, secondary, and tertiary. The three should work for clients' proper health care, and a good referral system is the main link between them. (1)

Good communication between the sectors becomes a crucial factor in the delivery of good quality health care. As modern healthcare systems become more complicated and more people need coordinated care from both sides of the primary/ secondary interface. (2)

Ideally, the primary health care centers (PHCC) are supposed to be the point of first contact for patients who are then referred from there to other levels of health care. Primary health care providers play a very important role in controlling the quality & cost of health care as a whole. They are the first gate to the health services and function as a gatekeeper and health care managers. They should focus on health promotion and prevention, keep the community healthy, and do the referral at the right time and to the right place/direction. (3)

By referral, a health worker transfers the responsibility of care temporarily or permanently to another health professional. (4)

The purpose of a referral is to allow two doctors with various backgrounds and specialties to consult with one another in order to solve a patient's issue and deliver the finest care possible at the ideal time and location. Referral has significant effects on patients, the healthcare system, and medical expenses. (5)

At present, little direct contact between primary care doctors and specialists, thus written communication in the form of referral and reply letters with its long history in the medical profession is the most common tool and an important aspect of quality care. (6)

In a high-quality referral, which is a data-intensive process, crucial elements of demographic and clinical data are transferred to other healthcare professionals along with clinical questions, including the reasons for the referral, a preliminary diagnosis, previous illnesses and treatments, prescriptions, drug sensitivities & habits. (7) PHC physician will gain from the opinions of hospital specialists. (8)

Incomplete referrals can result in care discontinuation, delayed diagnosis, inadequate follow-up plans, repeated and unnecessary tests, polypharmacy, and the inability of the receiving physician to recognize the need for referral, all of which lower the standard of care, increase medical errors, and drive up costs. (5) Although a limited number of patients will develop life-threatening complications, very few of these can be predicted. Therefore, the system of referring any of the patients to the next referral center needs to be improved. (9)

In any health care delivery system, an appropriate structure is essential to promote comprehensive scope, continuity, integration of components, and operational efficiency. Patients must be able to easily access health care workers and/or health centers in their communities. (10)

Thus, patients requiring further evaluation and treatment are referred to a tertiary health facility. However, the referral decision made by primary care physicians has a significant effect on the cost and quality of care that patients receive. (11)

Normally, if the initial problem cannot be managed at the level of PHC, the decision will be made to refer the patient to a specialist. All patients should be seen first by a primary health care physician who decides whether a referral to secondary care is necessary, except in an emergency. This avoids system inefficiencies such as disadvantaged groups suffering from a lack of specialist care due to specialist doctors being overwhelmed by the inappropriate self-referrals. (12)

In order to effectively meet patients' health requirements, an efficient referral system must exist between the various levels of health care delivery. (13)

In Iraq; The health system has been adopted in 1958, the referral system is applied in a limited way to provide some health services, where preventive and therapeutic services are provided in the relevant preventive centers. (14)

The application of the referral system among health institutions has been at different stages of activation and inertia since the 1970s and 1980s until the 1990s. The unified health book system was applied to the patient in 1985 and was implemented in several health departments with the mechanism of reviewing the patient to institutions (Primary, secondary, tertiary) to be documented in the patient's health book and follow-up of the patient in all stages of his life, the work was frozen under a short period. (15, 16)

The Iraqi Ministry of Health (MOH) reactivated a referral system in late 2008. Most Primary Health Care (PHC) clinics (85%) had a referral system record, however (69%) did not have an electronic archive or family inventory, and (64%) said they do not have any follow-up mechanisms for the patients who need continuous care. (17)

The primary goal of any health system reform in Iraq should include strengthening the health care delivery and the referral system with special emphasis on changing the curative hospital-based system to a decentralized PHC-based system. (18) The purpose of referral services is to guarantee that patients receive cost-effective and high-quality management so at the proper level of the healthcare facility. Additionally, referrals act as a connection between primary, secondary, and tertiary care. (19) Clinical coordination is regarded as a priority in health policy as its absence might result in ineffective care and low quality. (20)

Patients visit an ever-expanding range of professionals in a variety of diverse venues due to rising medical specialization, quickening scientific and technical advancements, and the way health care is structured. (21) thus making clinical coordination difficult & jeopardizing the quality and efficiency of health care. (22)

The foundation of feedback mechanisms is professional communication and information sharing. (23) Through communication or information sharing between two or more people, mutual adjustment mechanisms accomplish therapeutic coordination in order to address the issue at the same level at which the information was generated. Last but not least, some methods, including doctor-to-doctor clinical case conferences and virtual consultations, integrate mutual adjustment processes with skills standardization through medical training. (24)

This study aims to assess referral system practice & hospitals doctor’s opinion toward referral system and to determine commitment of them to referral system instructions and guidelines.

Subjects and Methods

A cross-sectional study with analytic elements was conducted in a sample from nine out of 15 Iraqi governorates after excluding the 3

North governorates in the Kurdistan region covering 9 general Health Directorates (Al-Karkh and al-Resafa (two-side of Baghdad), holy Karbala, Maysan, holy Al-Najaf, Kirkuk, Basrah, Al-Muthana and Ninawa).

Ethical issues:

The ethical and scientific approval was taken from the Iraqi Ministry of Health/ human resources and development center, all the nine health directorates, and each hospital manager. Also, the doctor's agreement to be enrolled in the study by oral consent was obtained before being interviewed by a tested questionnaire.

Sampling method: Two hospitals were selected randomly from each Health Directorate, and two doctors; who present in these counselling clinic at day of interviewer visit; were enrolled from four specialties (medical consultant ward, surgical consultant ward, gynecological/obstetric consultant ward, and pediatrics consultant ward).

The study was conducted from the first of February to the 30th of June 2019, with the data collecting taking place over a two-month reference period in March and April 2019. Eight medical professionals from each of the following health directorates: Al-Karkh, al-Resafa, holy Karbala, Maysan, holy Al-Najaf, Kirkuk, Basrah, and Al-Muthana, for a total of 72 medical professionals. branches.

Study Tool: A special questionnaire form was developed by the researchers and the opinion of four experts (two community physicians, and two family physicians) was taken into consideration. The questionnaire consisted of four parts. First part consists from 5 questions: Age, gender, specialization of the doctor, the governorate and the sector. The second part consists from three questions: "How many your duties in the counselling clinic per month averagely?", "How many patients per clinic you seen averagely?", "How many referred patient per all patients attending the counselling clinic in your duty averagely?". The third part consists from: "Did you asking each patients about if he/she have referral form?", "Did you respond to each referred forms by writing your notes in the hospital part? ", "Did you encourage referred patients to return to PHCs for follow up", "What is your opinion about coordination between PHC & hospital", "What is your think about causes of partial/not adequate referral form - PHCs doctor partial filling", "What is your opinion about the importance of hospital feedback", "What is your opinion about Effective current referral system". The fourth part was about doctor's suggestion

Pilot study: To assess the feasibility, time, cost, and effect size of the study, ten doctors who were not included in it underwent a pretest utilizing the study questionnaire as the pilot study's instrument (statistical variability). reliability testing = 6.1, and take four expert opinion taken; two community physician and two family physician. Every idea was taken into account.

Statistical analysis: SPSS version 23 was used for the analysis. Frequencies and percentages were used to express qualitative data. Mean and standard deviation were used to express quantitative data (SD). A p-value of less than 0.05 was regarded as statistically significant when using the Chi-Square test, yate correction or fisher exact test when it needed for statistical analysis.

Results

The mean age ± SD of the participants was 44.87 ± 8.52 and 31(43.1%) were at {40-49 years} age group, 48(66.7%) were males and 58(80.6%) were specialists, and most of the doctors 45(62.50%) have 3-6 duties in consultant clinics/month followed by 12(16.7%) have 7-10 duties, and 21(29.2%) had 26-50 Patient /clinic as shown in table 1.

Table 1: Distribution of studied doctors according to their age, gender, specialty degree, and load of work

		Frequency N=72	Percent %
Age (years) Mean=44.87 SD= ±8.52	30- 39	21	29.2
	40-49	31	43.1
	50-59	20	27.7
Gender	Male	48	66.7
	Female	24	33.3
Specialty degree	Specialist	58	80.6
	Not specialist	14	19.4
Clinic duties / month (average) (3.78 ±1.778)	1-2	9	12.5
	3-6	45	62.5
	7-10	12	16.7
	>10	6	8.3
Number of Patients/ clinic duty	≤ 25	19	26.4
	26-50	21	29.2
	51-75	15	20.8
	≥ 76	17	23.6

Table 2: Distribution of studied doctors according to the referral response & their opinion about referral and feedback

		Frequency N=72	%
Asking about referral form	Every time	11	15.3
	Most time	14	19.5
	Some time	32	44.4
	Never	15	20.8
Referred patient/ all patients	Most of them	22	30.6
	Some of them	48	66.7
	Never	2	2.8
Respond to referred forms	All	24	33.3
	Most of them	14	19.5
	Some of them	15	20.8
	Never	19	26.4
encourage patients to return to PHCs for follow up	Yes	22	30.6
	No	29	40.3
	Some time	21	29.2
coordination between PHC & hospital	Yes	5	6.9
	No	58	80.6
	Some time	9	12.5
Referral form - PHCs doctor part filling	Adequate	11	15.3
	Partial adequate	30	41.7
	Not adequate	31	43.1
Opinion about important of hospital feedback	Important	64	88.9
	Not important	3	4.2
	I don't know	5	6.9
Effective current referral system	Strongly agreed	6	8.3
	Agreed	17	23.6
	Disagreed	36	50.0
	Strongly disagreed	13	18.1

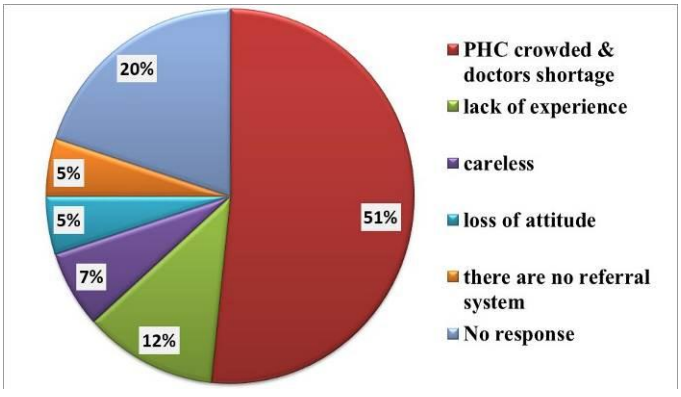

Figure 1: Distribution of Causes of "Partial or not Adequate Referral form PHCs Part (total=61)

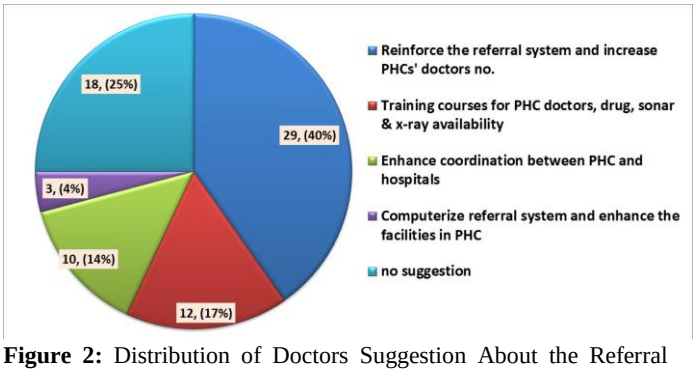

Figure 2: Distribution of Doctors Suggestion About the Referral System

Table 3: Association between PHC-hospital doctor's coordination and the doctors' characteristics:

		PHC-hospital doctor's coordination						
		yes		No		some time		P-value
		N	%	N	%	N	%	
Age	30-39	3	6.30	13	61.90	5	23.80	0.033*
	40-49	0	0.0	30	96.77	1	3.22	
	≥50	2	1.0	15	75.0	3	24.0	
Gender	male	2	4.17	43	89.58	3	6.25	0.023
	female	3	12.50	15	62.50	6	25.0	
specialty degree	specialist	4	7.27	48	82.76	6	10.34	0.534
	Not specialist	1	7.14	10	71,43	3	21.43	
Respond to PHCs referred forms	all	2	8.33	18	75.0	4	16.67	0.583*
	most of them	2	9.09	15	68.18	5	22.73	
	some of them	0	0.0	7	100	0	0.0	
	never	1	5.26	18	94.74	0	0.0	
filling PHC part in the referral form	adequate	4	36.364	4	36.364	3	27.272	< 0.001*
	partial adequate	0	0.0	25	83.33	5	16.67	
	not adequate	1	3.23	29	93.54	1	3.23	
Total 72(100%)		5	6.94	58	80.56	9	12.5	

* = yate correction

Only 32(44.4%)) of the doctors were asking patients attending the clinic during their duty, about the PHCC's referral form, while, 22 (30.6%) of the doctors mentioned that most of the patient was referred, and 48(66.7) of doctors had some referred patient per their duties. According to the current study result, 40.3% of doctors did not encourage patients to return to PHCs for follow up, and most of them 58(80.6%) had no coordination with the PHC doctors, even most of the referral form -PHCs doctor part filling were not adequate 31(43.1), or partial adequate 30(41.7%). Most participants 64(88.9%) consider the hospital referral feedback is important, and 36(50%) of them believed that current referral system is ineffective. As seen in table 2.

Table 4: Association between feedback PHCs referred forms & demographic characteristic:

		Feedback PHCs referred forms				Total	P-value
		All	Most	Some	Never		
Age\ years	30-39	5	7	2	7	21	0.318
	40-49	11	5	6	9	31	
	50-59	6	2	5	1	14	
	≥ 60	2	0	2	2	6	
Gender	Male	14	8	11	15	48	0.406
	Female	10	6	4	4	24	
Department	Surgery	5	2	4	7	18	0.443
	Medicine	3	4	6	5	18	
	Obstetric/Gynecologist	7	5	2	4	18	
	Pediatric	9	3	3	3	18	
specialty	Specialist	19	11	14	14	58	0.532
	Not specialist	5	3	1	5	14	

Table 5: Association between feedback PHCs referred forms and clinic features

		Feedback PHCs referred forms				Total	P-value
		All	Most	Some	Never		
Clinic duties per month	1-2	3	1	3	2	9	0.643
	3-6	15	9	9	12	45	
	7-10	5	1	3	3	12	
	≥11	1	3	0	2	6	
The patient was seen per clinic duty	1-2	3	1	3	2	9	0.187
	≤ 25	5	7	3	4	19	
	26-50	9	4	6	2	21	
	51- 75	4	1	4	6	15	
	≥ 76	6	2	2	7	17	
Asking each patient about the PHCCs referred form	Every time	7	1	1	2	11	0.055
	Most time	1	5	6	2	14	
	Sometime	10	7	6	9	32	
	Never	6	1	2	6	15	
Referred patient/all patient	Most of them	4	8	8	2	22	**< 0.001**
	Some of them	13	4	6	2	25	
	Rarely	7	2	1	13	23	
	Never	0	0	0	2	2	
Advice patient to return to the PHC	Yes	8	6	2	6	22	0.062
	No	7	2	9	11	29	
	Sometime	9	6	4	2	21	
PHC – hospital coordination	Present	2	2	0	1	5	**0.015**
	No present	18	7	15	18	58	
	Sometime	4	5	0	0	9	
Adequate filling PHC referral part	Adequate	4	5	0	2	11	**0.048**
	Partial adequate	7	7	9	7	30	
	Not adequate	13	2	6	10	31	
Hospital feedback to PHCCs important	Important	22	13	13	16	64	0.399
	No important	0	0	2	1	3	
	I don't know	2	1	0	2	5	

As it appeared in figure (1) the most cause of partial/not adequate filling of PHC referral form part, was PHC crowded with patients and doctors' shortage 31(51%), followed by lack of experience, doctors carless, loss of attitude toward the referral system, and there are 12(20%) had no response to this question.

Nearly One-third of the doctors suggest reinforcing the referral system and increasing PHCs' doctors' number, the other 12 doctors suggest Training courses for PHC doctors to increase the experience,

availability of drug, sonar & x-ray, 10 doctors suggest the coordination between PHC and hospital which now very limited, others suggest computerize referral system like others countries, and 18 doctors give no suggestion. As shown in figure 2.

According to doctors' opinion about the presence of PHC-hospital doctors' coordination; had a significant association with their age, gender, and their thinking about Adequate filling PHC part in the referral form, but with no significant association with their specialty degree, and their Respond to PHCs referred forms as appeared in the table (3).

The study revealed a significant association between doctors applying feedback to PHCs referred-forms and the percentage of referred patient/all patients, presence of coordination between their hospitals and refereeing PHC, and Adequate filling PHC referral part, while there is no significance with doctors' age, gender, department, specialty, clinic duties per month, asking each patient about the PHCCs referred form, advice patient to return to the PHC and their opinion about the importance of hospital feedback to PHCs as shown in table (4) and (5).

Discussion

In Iraq, the healthcare system is divided into various tiers, from primary health care (PHC) to tertiary levels of care, which offer the most advanced services. The secondary and tertiary levels of care are expected to be referred to patients with non-urgent issues via letter. This referral letter describes the rationale for the referral and serves as a permission slip to allow the patient simple access to treatment by a specialist at the secondary or tertiary service level. Feedback should be communicated back to the primary level as soon as the issue for which a patient was referred is resolved or under control. In order to ensure that mutual referrals go well, the MOH supervision of referrals is essential. Although the government supports the implementation of mutual referrals, no specific incentives for good system performance have been identified. Similarly, the MOH has not developed any sanctions against hospitals that purposefully keep patients in stable conditions, so there is no incentive for the hospital to actively strengthen their coordination with PHC, according to a 2014 Albattal study conducted in Saudi Arabia. (4)

The most common cause of partial/not adequate filling of PHC Referral form part, was PHCC crowded with patients and doctor's shortage 31(51%). This is not surprising because, in Iraq, the physician rate is only 9.25 for each 10000 populations, (Total without Kurdistan region), according to an annular statistical report of the ministry of health 2020. (25), adding to the shift in distribution where the majority are working in hospitals rather than the PHC sector, further, this is agreed with Saadian study 2018. (1)
One-third of the doctors suggest reinforcing the referral system and increasing PHC-doctors' number, the other 12 doctors suggest training courses for the PHC doctors to increase their experience, and also suggested the availability of drugs, sonar & x-ray, while 10 doctors suggest the coordination between PHC & hospital which is now very limited, others suggest computerize referral system like others countries, and 18 doctors give no suggestion. A systemic review study done in 2019, found the effectiveness of e-Referral Systems positive evidence includes reducing wait times and enhancing primary care-specialist communication. (26) And agreed with a Saudian study 2018. (1)

According to doctors' opinions about the current PHC-hospital doctor's coordination; had a significant association with their age, gender, and their thinking about adequate filling PHC part in the referral form, it's part of good PHC-hospital doctors coordination, filling all parts of the referral form, to give good information, reporting all results of the investigations & imaging done to about the referred case, and decrease the time to redo the full examination and redo the investigations, also it will be logically respected more. Also, this is agreed with the Saudian study 2018, and another study based on the guidelines and systematic review. (1, 6)

While the presence of the PHC-hospital doctor's coordination; had no significant association with their specialty degree, and their response to PHCCs referred forms, which does not go with other studies done in Beijing, The PHC doctors recommended creating a distinct organization that coordinates referral to the hospital because a referral system is not adequately established. (27- 29).

The current study revealed a significant association between doctors applying feedback to PHCs referred-forms and the percentage of referred patients, presence of coordination between their hospitals and refereeing PHC, and adequate filling PHC referral part, while there is no significance with doctors' age, gender, department, specialty, clinic duties per month, a patient is seen per month, asking each patient about the PHCCs referred form, advice patient, to return to the PHC and their opinion about the importance of hospital feedback to PHCs. Mutual respect results in feedback to fully completed PHC referral forms, however a heavy workload may make it more likely that referral letters won't get a response, similar to a systemic review result that Inter-clinician collaboration is most likely the single most important factor to improve the letter quality and, the healthcare system. (30).

Conclusion

Despite the importance of the referral system, half of the doctors disagreed about the current referral system's effectiveness; & most of them had no coordination with the PHC-doctors. PHC patients-crowded/doctors-shortage the main causes of adequate filling PHC-part of referral-form. Based on these results it is recommended to reinforce the referral system. Improve coordination between PHCCs and hospitals and support and increase in numbers of PHCs' doctors.

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Conflict of Interest

No conflict of interest

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Research Article

The Correlation between Serum Inositol 1,4,5 Triphosphate Level and Primary Hypothyroidism

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Keywords: IP3: inositol 1,4,5-trisphosphate, thyroid- stimulating hormone, primary hypothyroidism, subclinical hypothyroidism.

ABSTRACT

Background: Most primary hypothyroidism patients also experience inefficiency and irregularity. It is possible to understand the significance of myo-inositol in treating the thyroid gland by relating it to the synthesis of thyroid hormones. Study aimed to estimate serum of inositol 1,4,5-triphosphate (IP3) in primary hypothyroidism disorder and through that level it can shed light on whether it is accused of inactivity of the thyroid gland and at the same time open the doors for the use as a treatment.

Subject and Methods: The study was taken from the analytical cross-sectional design.120 subjects were divided into three groups, the first group included 40 healthy subjects, the second group included 25 patients with subclinical hypothyroidism, and the last group had 55 patients with primary hypothyroidism. with the subjects chosen from a teaching laboratory in the medical city. thyroid hormones and serum TSH was determined using Enzyme Immunoassay by Tosoh instrument assay, while serum inositol 1,4,5-triphosphate (IP3) using (ELISA) system.

Results: primary hypothyroidism patients showed a significant ($p \leq 0.05$) decrease level of serum IP3 when compared with healthy subjects. There is significant positive correlation with serum inositol 1,4,5 triphosphate (IP3) and each triiodothyronine S.T3 ($r = 0.581$, $p \leq 0.05$), thyroxine S.T4 ($r = 0.597$, $p \leq 0.05$), and significant negative correlation thyroid-stimulating hormone S.TSH ($r=-0.820$, $p \leq 0.05$), in primary hypothyroidism Patients.

Conclusions: inositol 1,4,5 triphosphate (IP3) deficiency in primary hypothyroidism disorder may be a cause of it happening, at the same time may be useful in its treatment even if it was not studied adequately in the study, but through its effect on a thyroid hormone.

Introduction

Primary Hypothyroidism is a condition in which the thyroid gland does not produce enough thyroid hormone to meet the body's needs for regulating metabolism and energy utilization. It affects practically every organ in the body. (1)

A rise in blood TSH with normal thyroxine (T4) and triiodothyronine (T3) concentrations in the first biochemical anomaly in primary hypothyroidism (i.e., subclinical hypothyroidism). (2)

Phosphoinositide is part of the phosphatidylinositol signal transduction channel across the plasma membrane via the second

messenger 1,4,5-triphosphate, which modulates intracellular Ca+2 release, according to several studies (3).

Inositol triphosphate is a group of somewhat distinct molecules generated from the C6 sugar alcohol. It is composed of an inositol ring with three phosphate groups bound at the 1, 4, and 5 carbon positions, and three hydroxyl groups bound at positions 2, 3, and 6. it is the nine types of isomers, Other inositols (epi-, allo-, muco-, cis, l-chiro-, d-chiro-, scyllo-and neo) are less well known, except d-chiro-inositol, which plays an important insulin resistance and insulin signal transduction (4).

Inositol derivatives are important components of the cell's structural lipids, and they regulate cell proliferation, morphogenesis, glucose metabolism, and cytoskeleton reorganization, among other things. (5).

MYO Inositol-containing phospholipids serve as precursors for the manufacture of several signaling intermediates, including inositol1,4,5 triphosphate (IP3), phosphatidylinositol (PI), inositol phosphates (IP), phosphatidylinositol-phosphates (PIPs), inositol-phosphoglycans (IPGs), glycosyl Phosphoinositides are made up of myo-I, which are mediators in the phosphatidylinositol (PIP2) signal transduction pathway. (6,7)

Phosphatidylinositol 4,5biphosphate (PIP2), the precursor of diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3), modulates intracellular Ca+2 released by hydrolysis, which is involved in the (phospholipase C) PLC dependent inositol phosphate Ca+2 / DAG, acting as second messengers of many hormones such as FSH, TSH, insulin and LH (3)

Inositol plays a role in the signaling of various hormones, including, thyroid-stimulating hormone (TSH), gonadotropins, and insulin. In specific, inositol metabolism impairment can negatively affect secretion in mammalian thyroid, hormone storage, and biosynthesis. (8)

MYO Inositol derivates and dietary MYO Inositol are absorbed in the gut via sodium-dependent transporters called sodium-myoinositol channels type 2 (SMIT2) and type 1 (SMIT1), which are found in the jejunum and duodenum, respectively. (9).

Inositol homeostasis involves three distinct processes: 1) absorption and excretion through the intestines; 2) transport from plasma to the interstitial fluid of cells via specific carriers; 3) endogenous synthesis and catabolism. Exogenous MYO is generally -well tolerated in daily doses ranging from 4 to 30 g for up to 12 months. Mild adverse effects such as nausea and diarrhea may occur only when daily dosages exceed 12 g.(10).

Myo Inositol is produced endogenously from glucose in two enzymatic steps. A hexokinase converts glucose to glucose-6-phosphate, which is ultimately converted to myoinositol-1-phosphate. In humans, this endogenous synthesis produces up to 2 g MYO per day in each kidney, totaling 4 g per day. (11).

Myo Inositol is found in fresh fruits, vegetables, cereals, legumes, and nuts, and is the most widely dispersed in nature. Myo-inositol is a crucial component of cell membrane structural lipids (12)

The Aim of this study is to estimate serum of inositol 1,4,5-triphosphate (IP3 in primary hypothyroidism disorder and through that level it can shed light on whether it is accused of inactivity of the thyroid gland and at the same time open the doors for the use as a treatment.

Subjects and Methods

The study was taken from the analytical cross-sectional design. The Scientific of Committee Biochemistry Department, College of Medicine, University of Baghdad, Iraq, provided ethical permission. Between December 2021 and January 2022, the research was conducted, and the individuals were picked from a teaching lab in the medical city. At the consultation at the Baghdad Teaching Hospital, a thyroid specialist made the clinical diagnosis. Participants were asked to complete questionnaires to indicate their consent for the study's data collection on both sick groups and healthy people. The study involves 72 females and 48 males. The study involved 120 subjects divided into three groups, First group: was 40 healthy individuals in age range (25 -69 years) 22 female, 18 male. were examined by laboratory tests for their serum TSH, Total T4, and Total T3) match the normal reference range of thyroid hormones. Second group: 25 patients with subclinical hypothyroidism have a serum TSH level of 4.6 to 10 mIU/L with age range (25 -69 years) 15 female, 10 male. Third group: 55 patients35 female, 20 males. who had the same specifications for the diagnosis of primary hypothyroidism with a TSH level higher than 10mIU/L. the clinical diagnosis by thyroid specialist and laboratory investigations. the Third group was divided into two sub-groups First newly diagnosis15 patients were 9 females, and 6 males in the age range (28 -63 years). the second group was diagnosed with hypothyroidism in the treatment of 40 patients 26 female, 14 male in age range (27 -68 years). Each serum sample was tested for TSH, Total T4, Total T3, and IP3.Inclusion criteria Hypothyroidism patient matching selection of study specification. Exclusion criteria Patient thyroidectomy. Patients with Radioactive Iodine (Radioiodine) Therapy for Thyroid Cancer (RAI). Patient with secondary hypothyroidism. thyroid hormones and serum TSH was determined using Enzyme Immunoassay, while serum inositol 1,4,5-triphosphate (IP3) using enzyme-linked immune sorbent assay (ELISA) system.

Results and Statistical Analysis

In the study, there are 48 men and 72 women. The successful matching of groups in terms of age and gender by matchings of subjects' ages and genders (non-significant difference with P> 0.05). Both results clarified in table 1.

Primary hypothyroidism patients showed a significant (with $p \leq 0.05$) decrease level of serum IP3 when compared with two other studied groups Subclinical and healthy subjects

Subclinical Subjects also show a significant (with $p \leq 0.05$) decrease level of serum IP3 when compared with the healthy subjects. Both results clarify in table 2 Figure (1).

There is significant positive correlation with serum inositol1,4,5 triphosphate (IP3) and each S.T3 (with $r=0.581$, $p \leq 0.05$), S.T4 (with $r=0.597$, $p \leq 0.05$), and significant negative correlation S.TSH (with $r=-0.820$, p 0.000, $p \leq 0.05$), in primary hypothyroidism Patient. In figure (2,3,4).

Table 1: Mean ± Standard deviation SD of Age and sex number in healthy, subclinical subjects and hypothyroidism patients

Studied parameter	Studied groups	Mean ±SD	P-Value	LSD P-Value
Age (years)	Healthy subject (A) NO.40	42.38 ±12.26	P>0.05 N.Sig.	AVS B P>0.05 N.Sig.
	Subclinical Subject(B) NO.25	45.36 ±12.41		A VS C P>0.05 N.Sig.
	Hypothyroidism Patient (C) NO.55	42.49 ±11.31		B VS C P>0.05 N.Sig.
Studied parameter	Studied groups	No.%	Chi-square test p-value	
Gender No%	female% in Healthy subject	55%	P >0.05 N.Sig	
	female% in Subclinical Subject	60%		
	female% in hypothyroidism Patient	65%		
	male % in Healthy subject	45%		
	male % in Subclinical Subject	40%		
	male % in Hypothyroidism Patient	35%	P >0.05 N.Sig	

sig. = significant, N.sig. = non-significant, LSD = fisher least significant difference.

Table 2: Mean ± Standard deviation SD of serum IP3 in primary hypothyroidism patients, Subclinical subjects, and healthy subjects

Studied marker	Studied groups	Mean ±SD	P-Value	LSD P-Value
S.IP3 (ng/ml)	Healthy subject (A) No.40	9.16 ±4.72	P ≤0.05 Sig	B VS A P ≤0.05 Sig
	Subclinical Subject (B) No.25	3.40 ±0.72		C VS A P ≤0.05 Sig
	hypothyroidism Patient (C) No.55	2.02 ±0.57		C VS B P ≤0.05 Sig

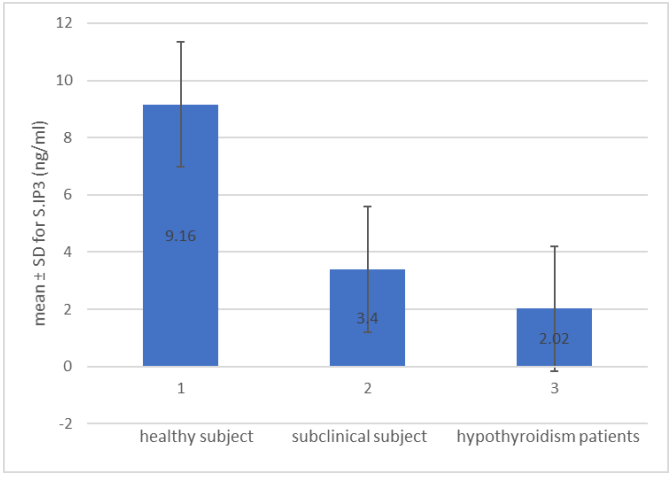

Figure 1: Mean ± Standard deviation (SD) of S. IP3 in healthy subjects, Subclinical subjects, and primary hypothyroidism patients

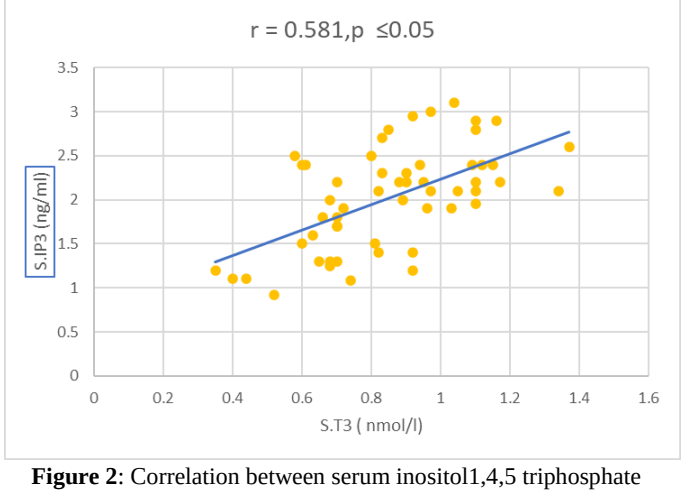

Figure 2: Correlation between serum inositol1,4,5 triphosphate (IP3) and S.T3 in primary Hypothyroidism Patients

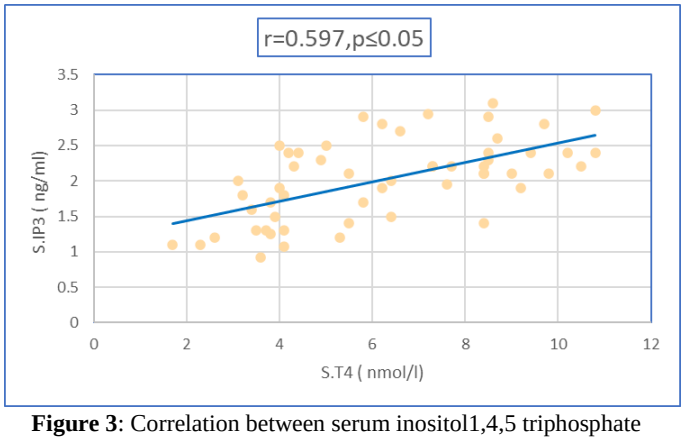

Figure 3: Correlation between serum inositol1,4,5 triphosphate (IP3) and S.T4 in primary Hypothyroidism Patients

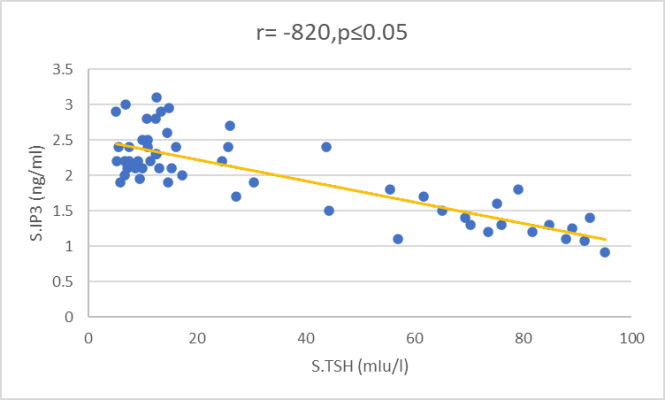

Figure 4: Correlation between serum inositol1,4,5 triphosphate (IP3) and S.TSH in primary Hypothyroidism Patients

Using receiver operator characteristic analysis (ROC) curve, to examine the diagnostic efficiency of serum IP3 level about primary hypothyroidism Patients. ROC curve is a graphical representation of the relationship/tradeoff between clinical Specificity and Sensitivity for each cut-off test. The results are tabulated in table (3) and figures (5) according to primary hypothyroidism Patients.

Table 3: Sensitivity and Specificity, the area under the curve and Cut-off value of serum IP3 in primary Hypothyroidism Patients

parameter	Sensitivity	Specificity	AUC	Cut-off value
S.IP3	82.5%	100%	0.95	5.25(ng/ml)

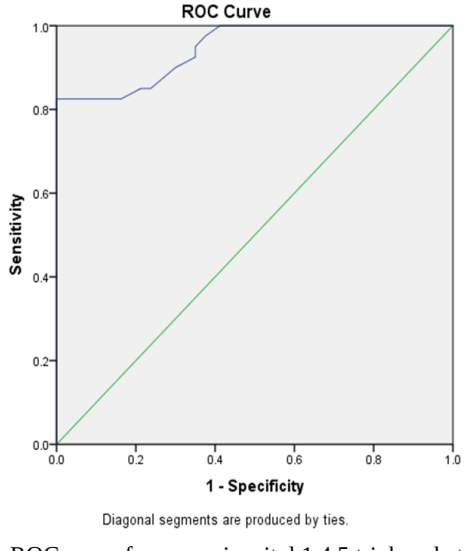

Figure 5: ROC curve for serum inositol 1,4,5 triphosphate (IP3) in primary Hypothyroidism Patients

Discussion

This study was conducted to match the age and gender No (male, female) and that was documented through (a non-significant difference with P ≥0.05) in table1.

Primary hypothyroidism individuals had the lowest levels of inositol 1,4,5 triphosphate (IP3) Once TSH attaches to its receptor on the thyroid cell surface, it stimulates cell proliferation and differentiation as well as thyroid hormone production. When TSH receptors bind, two post-receptor cascades are activated: one involves adenylyl cyclase, which results in increased intracellular cyclic AMP and protein kinase A phosphorylation, as well as activation of cytosolic and nuclear target proteins; the other is inositol-dependent and involves the phospholipase C-dependent inositol phosphate Ca+2/diacyl In addition, the inositol-dependent system controls H2O2-mediated thyroglobulin iodination, whereas the cAMP pathway is more involved in cell proliferation, differentiation, and thyroid hormone (T4, T3) release.(3,5,13).

Thyroid Peroxidase (TPO) is an enzyme that uses hydrogen peroxide H2O2 to oxidize and integrate iodide in the tyrosyl groups of Thyroglobulin Tg. (14).

It has recently been found to be a very effective and safe treatment of IP3 and selenium for those who have subclinical hypothyroidism due to Autoimmune Thyroiditis. (15). In a study published in 2013, researchers discovered that providing myo-inositol + selenium to patients with subclinical hypothyroidism for six months reduced TSH levels by 31% compared to a control group given only selenium. These findings were then verified in a different clinical investigation by the same authors. Another study looked at TSH levels in Hashimoto patients with subclinical hypothyroidism who were given myo-inositol + selenium for six months and found that they dropped significantly after three months and even more after six months. TSH levels did not alter appreciably in the control group, which got only selenium. (16).

In a study in 1993 a year. The tissue levels of inositol 1,4,5-trisphosphate isoform were found to be significantly higher in hyperthyroid Rats' hearts and lower in hypothyroid Rats' hearts than in euthyroid ones (17). Where the results are identical to our results, but our study was on humans. where the results were to the levels of inositol 1,4,5-trisphosphate in Subclinical subjects were lower than in healthy subjects while primary hypothyroidism Patients very lower than in the healthy subject.

The results showed a substantial correlation between inositol 1,4,5-trisphosphate and negative TSH and positive T3, and T4 in primary hypothyroidism patients, and the effect has been previously explained.

serum IP3 showed high Sensitivity of 82.5% and 100% Specificity in diagnosis patients with primary hypothyroidism, this result is withdrawn the serum IP3 as a diagnostic tool for hypothyroidism by (ROC) analysis the reference range of serum IP3 was determined represented by the Cut-off value of 5.25 (ng/ml).

Conclusion

A decrease in the level of serum inositol 1,4,5 triphosphate (IP3) may be one of the reasons for the occurrence of the primary hypothyroidism disorder. Through its effect on thyroid hormone, it can suggest its use in the treatment of the thyroid gland.

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Conflict of Interest

No conflict of interest

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Research Article

Alterations in some Physiological and Inflammatory Markers in Iron-Deficient Obese Adults in the Kurdistan Region, Iraq

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ABSTRACT

Background: Iron homeostasis is crucial to many physiological functions in the human body, such as cellular activity, erythropoiesis, and the innate immune response. Iron deficiency anemia may occur from obesity's ability to disturb iron homeostasis. Obesity may be seen as a pre-inflammatory condition with mild, ongoing systemic inflammation. Additionally, an increase in hepcidin levels by chronic inflammation causes iron insufficiency in obese people. For this reason, this current experiment is designed to investigate the iron profile and some hematological and inflammatory parameters in obese adults in the Kurdistan region-Iraq.

Subjects and Methods: The cross-sectional study was designed within the context of a medium private laboratory with participants being common people involved, 200 adults participated in this study and were allocated into two groups according to BMI (control group (BMI ≤ 29.9): N=100 and obese group (BMI >30): N=100). Oxygen saturation (SpO2) and pulse rate were assessed. Blood sera (once) was obtained for iron profiles (s. Iron, Ferritin, Hepcidin) and inflammatory levels (c-reactive protein (CRP), interleukin 6 (IL-6)).

Results: Our findings highlighted that all inflammatory markers increased significantly in the obese groups in both sexes and a positive correlation with BMI and a significant decrease in iron in the obese group.

Conclusions: This research reveals that hepcidin levels in obese adult people contribute to the development of iron deficiency anemia due to increased inflammation.

Introduction

Over the past 50 years, obesity has become a global epidemic in terms of prevalence. It is one of the most critical public health issues of the twentieth century, according to public health professionals. Most of the world is now stigmatizing obesity (particularly in the western world) (1). In addition, Iron deficiency anemia is a condition in which the body is unable to store enough iron, leading to reduced red blood cell production (2), One-third of the global population suffers from anemia, with IDA being the main cause (3). In the 1960s, the first account of a probable relationship between the iron status of obese people and obesity occurred (4), Four decades later, cross-sectional research completed in 2003 revealed that overweight and obese children and adolescents had a high prevalence of iron insufficiency, According to a research that used data from the National Health and Nutrition Examination Survey (NHANES III), American children who were overweight had a double the likelihood of being iron deficient than children of normal weight (5), Similar to this, Yanoff et al. (2007) showed that the prevalence of iron deficiency increased among obese people, who had considerably lower blood iron levels and higher levels of soluble transferrin

receptor than non-obese persons (6), In another study, Menzie et al. (2008) discovered that when obese people were compared to non-obese adults, the level of serum iron and transferrin saturation was considerably lower in obese adults (4). The study aimed to investigate the associations between iron parameters, hepcidin and inflammation markers in obese adults in Kurdistan region-Iraq.

The endocrine organ adipokine, which is secreted by adipokine-producing adipose tissue, plays a role in inflammatory processes. As a result, obesity might be seen as a pre-inflammatory condition with mild, ongoing systemic inflammation. This inflammatory state, particularly iron deficiency, may be crucial in the etiology of illnesses linked to obesity. Additionally, a strong negative predictor of serum iron content was discovered to be fat mass (7). Homeostasis of iron, an essential micronutrient, is crucial to many physiological human functions, such as cellular activity, erythropoiesis, and innate immunological response (8). So Iron deficiency anemia may occur from obesity's ability to disturb iron homeostasis. Increased hepcidin levels caused by chronic inflammation may be the cause of the link between obesity and iron insufficiency. A little peptide hormone called hepcidin controls the intestinal absorption of iron negatively. Significant body weight loss improves iron status by increasing iron absorption in overweight and obese people by reducing chronic inflammation and serum hepcidin levels. To verify this impact, more randomized controlled studies are necessary (4). Interleukin-6 (IL-6) produced by macrophages in response to inflammatory stimuli encourages hepatocytes to generate acute-phase proteins such as C-reactive protein (CRP) and hepcidin. This inflammatory condition could result in prolonged hypoferremia and anemia (9).

Subjects and Methods

The cross-sectional study was carried out from Nov 2021 to Feb 2022. In this study, 200 adults were included and allocated in to two groups according to BMI (control group (BMI ≤ 29.9): N=100 and obese group (BMI >30): N=100). The ages of the participant started from 25 and above, and both males and females were included. The physiological markers like oxygen saturation (SpO2) and pulse rate were measured by using a pulse oximeter (American Diagnostic Corporation, China). Techniques are commonly used clinically, whether in focused thought, or a medical procedure. Blood samples (10ml) were collected from each participant and divided into EDTA tubes for hematological parameters and Gel tubes for serological tests. Blood was gathered in gel container tubes, centrifuged at 15000 rpm for 5 min then serum separated. A fully automated chemical analyzer (Roche Cobas Integra 400 plus (Germany) has been used to estimate the Iron and CRP parameters. A microplate reader (Lab, China) has been used to estimate serum IL-6 and Hepcidin. Data were analyzed using the statistical package for Social Sciences (SPSS, version 21). In the study, a one-way analysis of variance (ANOVA) was used. A P-value of < 0.05 was considered statistically significant.

Results:

The results of the current study showed that the level of oxygen saturation (PO2) was significantly reduced in the obese group for both males and women compared to the control in the male and female groups, respectively.

The rate of heartbeat per minute was increased non-significantly in the obese group when compared with those of the control groups in both sexes as shown in Table 1.

Table 1: Physiological parameters comparison in control and obese groups about sex

Gender		Male			Female		
BMI		Control	Obese	p-value	Control	Obese	p-value
Statistics		Mean± S.D	Mean± S.D		Mean± S.D	Mean± S.D	
physiological marker	O2 (%)	98.41±2.47	94.71±2.45	0.047	98.65±2.27	95.96±2.73	0.044
	Pulse (beat/minute)	84.41±11.15	87.6±10.39	0.147	85.09±9.58	86.73±8.58	0.724

Table 2 demonstrates a significant increase ($p<0.05$) in white blood cell counts and a significant decrease in hemoglobin levels in the obese groups when compared with those of the control groups in both sexes, while other hematological parameters showed non-significant changes in the obese group when compared to the control group in both sexes.

Table 2: Hematological parameters comparison in control and obese groups about sex

Gender		Male			Female		
BMI		Control	Obese	p-value	Control	Obese	p-value
Statistics		Mean± S.D	Mean± S.D		Mean± S.D	Mean± S.D	
hematological tests	RBC	4.95±0.43	5.1±0.61	0.164	4.38±0.46	4.51±0.35	0.091
	WBC	6.93±1.69	7.8±1.98	0.021	7.02±2.31	8.64±1.17	0.026
	HGB	14.21±1.16	12.79±1.5	0.000	12.38±1.19	11.87±1.34	0.046
	HCT	43.45±3.37	44.33±4.39	0.262	37.21±4.08	38.56±3.45	0.074
	MCV	84.57±15.51	87.15±7.74	0.313	82.27±14.38	79.97±17.4	0.476
	MCH	31.78±12.37	28.42±3.11	0.05	30.12±12.39	32.8±17.87	0.392
	PLT	223.65±63.58	228.06±51.92	0.709	271.84±94.29	263.8±62.9	0.61

Table 3 revealed a significant decrease in serum iron level in the obese group in comparison to the control group in both sex groups. Regarding the Ferritin level, the obese group showed nonsignificant increases ($P \geq 0.05$) in Ferritin levels compared to the control group in both male and female groups.

The level of hepcidin increased significantly in the obese group compared to the control group in both male and female groups.

Table 4 showed a significant increase in inflammatory markers in the obese groups compared to the control group, the highest level of C-reactive protein and interleukin-6 was found in the obese group in both male and female groups compared to these control groups, respectively.

Table 3: Iron profile markers in the control and obese groups about sex

Gender		Male			Female		
BMI		Control	Obese	p-value	Control	Obese	p-value
Statistics		Mean± S.D	Mean± S.D		Mean± S.D	Mean± S.D	
Iron Profile	S.IRON	108.73±31.25	93.68±27.04	0.025	94.83±21.83	79.75±31.78	0.014
	FERRITIN	145.1±103.02	165.09±107.08	0.347	163.94±64.77	161.34±79.88	0.846
	Hep	402.57±194.52	475.98±158.82	0.043	402.26±124.06	487.32±128.1	0.037

Table 4: Inflammatory markers in obese groups regarding sex

Gender		Male			Female		
BMI		Control	Obese	p-value	Control	Obese	p-value
Statistics		Mean± S.D	Mean± S.D		Mean± S.D	Mean± S.D	
Inflammatory markers	CRP	3.48±2.9	5.77±7.44	0.04	4.23±4.68	6.47±6.37	0.049
	IL-6	47.37±10.22	52.36±13.48	0.035	65.9±37.04	77.94±37.24	0.002

Discussion

If the BMI is over 30, hypercapnia and hypoxia in arterial blood can happen in someone who is dangerously overweight. 90% of people with a BMI greater than 30 have sleep problems and have lower nighttime oxygen saturation (10). The effects of obesity on gas exchange in adults may be underappreciated in comparison to other clinical entities that are frequently associated with hypoxemia (such as smoking, heart failure, and obstructive lung disease) (11). Some previous studies confirmed that hypoxia and a decrease in heartbeat per minute have been recorded in obese people (12).

Leukocytes are thought to have a significant part in the low-grade inflammation that has been defined as the state of obesity. According to Salma et al. (13), obese people with a high WBC count have insulin resistance. In obese people, platelets, red blood cells (RBC), and hemoglobin are associated with cardiorespiratory disorders (13) and the association between BMI and hematological parameters is mediated by their associations with abdominal fat and insulin resistance markers (14). Previous studies found the alteration of hematological parameters in obese individuals with an increase in leukocyte count (15).

A link between obesity and iron status is suggested by the high prevalence of obesity along with the incidence of iron deficiency seen across different age and sex groups (16). Iron deficiency anemia may occur from obesity's ability to disturb iron homeostasis. Increased hepcidin levels caused by chronic inflammation may be the cause of the link between obesity and iron insufficiency (4). Previous studies showed that the level of hepcidin increases with BMI in the obese population, which leads to some issues in the body such as iron deficiency anemia (4, 17).

The present study showed a significant increase of both inflammatory markers CRP and IL-6 in the obese group when compared to the control group in both sexes. These results conducted by previous studies that obesity, particularly visceral obesity, is increasingly thought of as a low-grade inflammatory illness because of raised serum levels of a variety of inflammatory markers (IM), such as C-reactive protein (hs-CRP) and interleukin-6 (IL-6) (18-20). Another study supported our finding that there was a significant relationship between weight, BMI, waist-hip ratio, hip circumference, and serum CRP, TNF, and IL-6 concentrations. CRP and IL-6 are substantially linked with visceral adipose tissue, waist circumference, and BMI in obese people (19). CRP was strongly correlated with BMI according to multiple regression analysis, but IL-6 was significantly correlated with visceral adiposity in obese participants. Because obesity and visceral adiposity are positively correlated with higher cytokine levels, lowering these risk factors is crucial for preventing cytokine level increases (18). Obesity most likely plays a major role in the etiology of chronic diseases by causing the establishment of low-grade chronic inflammation both locally and systemically in adipose tissue. The molecular processes that initiate obesity-lined inflammation are different from those that initiate the traditional inflammatory response caused by infections and involve different signaling pathways. Lack of nutrition leads to quantitative and qualitative changes in the lipid content of adipose tissue, as well as the production of several chemicals that act as endogenous ligands to activate immune cells, which in turn starts the inflammatory process in obese people (20).

Conclusion:

This research revealed that hepcidin levels in obese adult people contribute to the development of iron deficiency anemia due to increased inflammation because obesity and visceral adiposity are positively correlated with higher cytokine levels; lowering these risk factors is crucial to prevent increases in cytokine levels . Also, an increase in obesity-associated anthropometric measurements (BMI) is associated with relative leukocytosis within the physiological range. Physiologically, the decrease in oxygen saturation significantly with a nonsignificant increase in a heartbeat in the obese group may be due to other clinical features. Further experiments on physiological parameters could support the finding of the correlation between obesity and iron deficiency anemia.

Ethical Clearance

This work was supported by Sulaimani Polytechnic University (Iraq). Experimental Protocol was approved by the Ethics Review Committee of Health and Medical Technology College in Sulaimani, Iraq. (Approval no: MLD00075).

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Conflict of Interest

No conflict of interest

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Al-Kindy College Medical Journal (KCMJ)

Research Article

Gingival Pigmentation Pattern in Correlation to Skin Color in a Group of Kurdish People in Sulaimani City

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Keywords: Depigmentation, Fitzpatrick scale, gingival pigmentation, Interdental papilla, Skin color.

ABSTRACT

Background: Melanin pigmentation of the gingiva appears in all ethnicities. Excessive pigmentation is an esthetic concern that has increased awareness about depigmentation procedures. This epidemiological study aims to find the correlation between skin color and gingival pigmentation in Sulaimani Governorate, Kurdistan/Iraq.

Subjects and Methods: A total of 820 apparently healthy and non-smokers, including 338 males and 482 females with healthy gingiva, aged between (18-40 years old) were enrolled in this study. Clinical examination on the participants' gingivae was performed to assess color, and the distribution of pigmentations. Afterward the of participants skin color were visually examined and divided into six groups according to the Fitzpatrick scale.

Results: Skin color of (53%) of our participants was medium to light brown. Most of the pigmentations were observed in the attached gingiva and interdental papilla (26.83%) and least being in the marginal gingiva and interdental papilla (9.27%). A statistically significant correlation was found between the participants' skin color, and the intensity of their gingival pigmentations

Conclusion: Darker-skinned subjects have heavier gingival pigmentation compared to fair-skinned people in Sulaimani Governorate, Kurdistan/ Iraq. The intensity of gingival pigmentation increases with age with the highest rate of gingival pigmentation was in the upper and lower second premolar to premolar area.

Introduction

The gingiva is a fundamental part of the periodontium that hides the alveolar processes of the jaws and the cervical part of the teeth (1).

Esthetics has become a significant point of dentistry, and clinicians must balance reaching acceptable gingival esthetics with addressing physical and functioning issues (2).

The gingiva's color is critical to overall esthetics (3). The color of the gingiva varies between individuals and is believed to be related to cutaneous pigmentation (2).

The color of healthy gingiva varies from pale pink to a dark bluish purple. Between these two points of normalcy, a wide variety of colors exists (4). Clinically, it varies between individuals in various mouth areas and correlates with skin color (3). Moreover, the color of gingiva is determined by various factors, including differences in the number, size, and distribution of melanosomes, activity of the melanocytes in the basal cell layer of the epithelium, dissimilarities in the type of melanin, and the masking influence of the heavily keratinized epithelium (4).

Melanin hyperpigmented gingiva is an esthetic concern for many individuals, primarily if the hyperpigmentation occurs on the facial aspect of the gingiva and is visible during smiles and speech, especially in cases with a gummy smile (5).

The gingiva is the most frequently pigmented intraoral tissue and the most visible during inspections. While melanin is the most common cause of gingival pigmentation, other pigments such as carotene, oxyhemoglobin, and reduced hemoglobin are also found in the masticatory mucosa and contribute to the average color of the integument (6).

Melanin is the primary pigment that gives tissues their color. It appears in the oral tissues as early as three hours after birth and is sometimes the only sign of pigmentation on the body (4).

Melanin is a non-hemoglobin-derived pigment produced by melanocytes, dendritic cells of neuroectodermal origin found in the gingival epithelium's basal and spinous layers. Melanin granules are phagocytosed and contained within melanophages or melanophores, the epithelium, and connective tissue cells (7). Pigmented areas are thought to form only when melanin granules synthesized by melanocytes are transferred to keratinocytes.

In some populations, gingival hyperpigmentation is considered a genetic trait and is more appropriately referred to as physiologic or racial gingival pigmentation (8). However, the gingiva may also exhibit pigmentations due to other etiologies. Benign and malignant lesions, intentional cultural tattooing, drugs, heavy metal ingestions-poisonings, iatrogenic, smoking, and systemic problems can all cause gingival pigmentation (9).

The skin plays an essential role because it acts as a physical barrier against mechanical, chemical, and microbial factors that may affect the body's physiological status (10). Melanin on the epidermis has significant evolutionary and physiological consequences, particularly for unclothed humans. Thus, through its optical and chemical filtering properties, a high melanin content (racial pigmentation) protects the skin against ultraviolet (UV)-induced skin damage (11).

There are considerable variations in the intensity of melanin pigmentation between persons of different ethnical/racial groups and between persons of the same ethnical/racial group, and these variations are normal (12). Physiological/ethnical melanin pigmentation of the oral mucosa is common in black individuals (8). Moreover, it is more frequent in darker-skinned whites (Caucasians) than lighter-skinned whites (4).

Fitzpatrick and Breathnach designated this close relationship between melanocytes and keratinocytes as the epidermal-melanin unit.

The Fitzpatrick scale (also Fitzpatrick skin typing test or Fitzpatrick photo typing scale) is a numerical classification schema for human skin color. It describes a way to classify the skin by its reaction to exposure to sunlight (13).

Previous studies have demonstrated the link between gingival pigmentation and skin color; for example, the study done by Ponnaiyan et al demonstrated the link between gingival pigmentation and skin color, which is that distribution of gingival pigmentation is greater in dark skinned populations (14).

This epidemiological study aimed to find the correlation between gingival color and skin color in Sulaimani Governate, Kurdistan/Iraq.

Subjects and Methods

This cross-sectional study was carried out in Sulaimani Governate, Kurdistan/Iraq from November 2021 to March 2022. A total of 820 participants, male and female, non-smoker healthy subjects with healthy gingiva were included, as well as subjects in the age range from 18-40 years old. A clinical examination of the gingiva from the second premolar of the right side to the second premolar of the opposite side was performed to assess the color of the gingiva and anatomic distribution of gingival pigmentation. The intensity of gingival pigmentation was also observed. Afterward, the skin color was visually examined and assessed as white, medium white, olive (medium brown), or dark brown. The exclusion criteria were participants with systemic disease, those using certain drugs that cause gingival pigmentation, and females with hormonal disturbance. In addition, pregnant and lactating women, participants with amalgam tattoos, smokers, and subjects with periodontal disease, and any gingival pathology that might cause gingival pigmentation were excluded. Ethical approval was obtained from the Ethics Committee of the College of Dentistry, University of Sulaimani. number (79/21) at (9/11/2021).

This study used the Dummett-Gupta Oral pigmentation Index (DOPI) for evaluation of physiologic gingival pigmentation intensity (9). The examination of gingival color was performed under the light of a dental chair from 9 am to 11 am.

This index shows the assignment of a composite numerical value to the total melanin pigmentation manifested on clinical examination of various oral tissues. The criteria are as follows:

0=Pink tissue (no clinical pigmentation)

1=Mild, light brown tissue (mild clinical pigmentation)

2=Medium brown or mixed pink or brown tissue (moderate clinical pigmentation)

3=Deep brown or blue/black tissue (heavy clinical pigmentation)

Subsequently, the skin colors were assessed by visual examination under natural light and were divided into six groups according to the Fitzpatrick scale. Skin color was classified by evaluating the color of the inner aspect of the upper arm, which is relatively unexposed to sunlight. These findings were recorded in a specially designed case sheet for each participant.

The criteria of the Fitzpatrick scale are as follows: (13)

Type I: Pale white skin, red or blond hair, blue eyes, freckles

Type II: white or fair skin, red or blond hair, blue, hazel, or green eyes

Type III: medium white or fair skin, any eye or hair color

Type IV: light brown skin (olive, moderate brown)

Type V: dark brown skin

Type VI: deeply pigmented dark brown (very dark brown to black)

Statistical Analysis:

Data analysis was performed using the statistical package for social sciences (SPSS) program version 22. Frequency and percentages with the mean and standard deviation were used for both qualitative and quantitative data respectively. Chi-square test was used for determining associations between categorical data. P-values equal to or less than (0.05) were regarded as statistically significant.

Results

The mean age and standard deviation of the study participants were (25.26 ± 6.23).

Regarding the participants' sociodemographic characteristics, 41.2% were males while 58.8% were females. In terms of skin color, the lowest percentage (5%) of the participants had light skin color, while 53% had medium to light brown colored skin. The remaining sociodemographic characteristics are shown in Table (1).

Table 1: Sociodemographic characteristics of the participants

Sociodemographic Parameters		Frequency	Percent
Gender	Male	338	41.2
	Female	482	58.8
Color of skin	Light pale, white	4	.5
	White, fair	54	6.6
	Medium, white to light brown	435	53.0
	Olive, moderate brown	287	35.0
	Brown, dark brown	40	4.9
Total		820	100.0

Regarding the participants' gingival pigmentation, grade 0 was the most frequent grade of pigmentation, while the least frequent was grade 2, as shown in Figure (1).

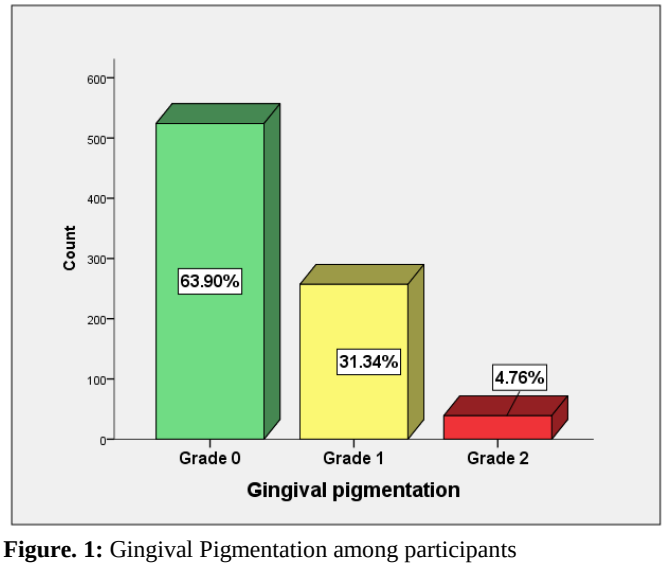

Figure. 1: Gingival Pigmentation among participants

Regarding distribution of pigmentation, the majority (63.9%) of participants had no distribution of pigmentation, while in the remainder it was distributed in the marginal or attached gingiva and interdental papilla, as shown in Figure (2).

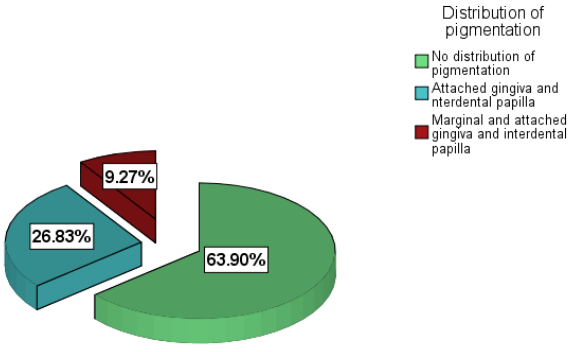

Figure. 2: Distribution of Pigmentation among participants

Concerning the area of pigmentation, the results ranged from 63.9% who had no area of pigmentation to just 0.12% who had pigmentation in the lower right second premolar-to-premolar, with these and the remaining percentages shown in Figure (3).

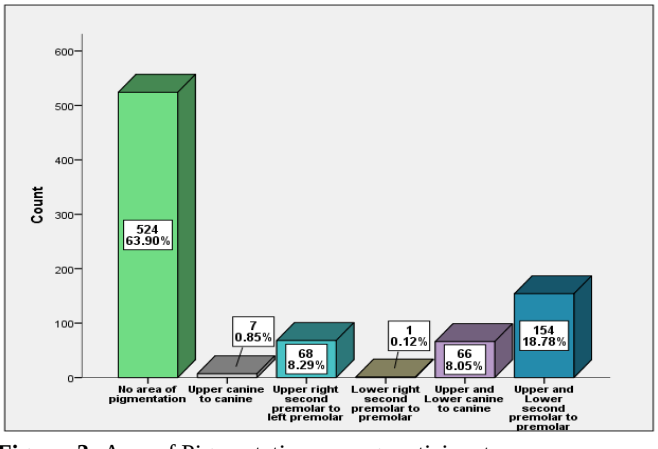

Figure. 3: Area of Pigmentation among participants

Table 2: Association between Gingival Pigmentation and Skin Color

Color of skin		Gingival pigmentation Grade 0	Grade 1	Grade 2	Total	P Value
Light pale, white	Count	4	0	0	4	
	%	.8%	0.0%	0.0%	.5%	
White, fair	Count	54	0	0	54	
	%	10.3%	0.0%	0.0%	6.6%	
Medium white, to light brown	Count	423	12	0	435	
	%	80.7%	4.7%	0.0%	53.0%	< 0.001
Olive, moderate brown	Count	43	243	1	287	
	%	8.2%	94.6%	2.6%	35.0%	
Brown, dark brown	Count	0	2	38	40	
	%	0.0%	.8%	97.4%	4.9%	
Total	Count	524	257	39	820	
	%	100.0%	100.0%	100.0%	100.0%	

In addition, a strong association was found between gingival pigmentation and skin color, as the p- value was highly significant

(< 0.001). For example, only 2.6% of participants with olive or moderate brown skin color had Grade 2 gingival pigmentation, while about 97.4% of those with brown or dark brown skin color had Grade 2 gingival pigmentation; meanwhile, those with white or fair skin color had no gingival pigmentation, these and the remaining results being shown in Table 2.

Regarding association of gender with variables, we found a strong association between gender and the rest of the variables as the p- values in all of the associations were highly significant (< 0.001). For example, while 55% of the males had Grade 0 pigmentation, about 70% of the females had grade 0 pigmentation, also 11.8% of the males had pigmentation, compared to 4.8% of females, these and the remaining results being shown in Table 3.

Table 3: Association between Gender and other parameters

Parameters			Gender		Total	P-Value
			Male	Female		
Gingival pigmentation	Grade 0	Count	187	337	524	< 0.001
		%	55.3%	69.9%	63.9%	
	Grade 1	Count	128	129	257	
		%	37.9%	26.8%	31.3%	
	Grade 2	Count	23	16	39	
		%	6.8%	3.3%	4.8%	
		%	11.8%	4.8%	7.7%	
	No	Count	298	459	757	
		%	88.2%	95.2%	92.3%	
Color of skin	Light pale, white	Count	3	1	4	< 0.001
		%	.9%	.2%	.5%	
	White, fair	Count	5	49	54	
		%	1.5%	10.2%	6.6%	
	Medium white to light brown	Count	165	270	435	
		%	48.8%	56.0%	53.0%	
	Olive, moderate brown	Count	141	146	287	
		%	41.7%	30.3%	35.0%	
	Brown, dark brown	Count	24	16	40	
		%	7.1%	3.3%	4.9%	
Distribution of pigmentation	No distribution of pigmentation	Count	187	337	524	< 0.001
		%	55.3%	69.9%	63.9%	
	Attached gingiva and interdental papilla	Count	105	115	220	
		%	31.1%	23.9%	26.8%	
	Marginal and attached gingiva and interdental papilla	Count	46	30	76	
		%	13.6%	6.2%	9.3%	
Area of pigmentation	No area of pigmentation	Count	187	337	524	< 0.001
		%	55.3%	69.9%	63.9%	
	Upper canine to canine	Count	5	2	7	
		%	1.5%	.4%	.9%	
	Upper right second premolar to left premolar	Count	22	46	68	
		%	6.5%	9.5%	8.3%	
	Lower right second premolar to premolar	Count	0	1	1	
		%	0.0%	.2%	.1%	
	Upper and Lower canine to canine	Count	26	40	66	
		%	7.7%	8.3%	8.0%	
	Upper and Lower second premolar to premolar	Count	98	56	154	
		%	29.0%	11.6%	18.8%	
Total		Count	338	482	820	
		%	100.0%	100.0%	100.0%	

Regarding association between age group and other parameters (gender, gingival pigmentations, color of skin…… etc.), a significant association was found between gender and age group (p-value of 0.004), while the remaining parameters had highly significant associations with age (all p-values were less than 0.001). For example, only 36.3 % of the males were aged 18-24 years, while 63.7% of the females were in this age group. Moreover, 70% of participants within this age group had grade 0 pigmentation, compared to the age group of 32-39 years, in which only 49% had grade 0 pigmentation, these and the remaining results being shown in Table 4.

Table 4: Association between age group and other parameters

Parameters			Age Group			Total	P-Value
			(18 - 24) Years	(25-31) Years	(32-39) Years		
Gender	Male	Count	170	87	81	338	0.004
		%	36.3%	48.6%	46.8%	41.2%	
	Female	Count	298	92	92	482	
		%	63.7%	51.4%	53.2%	58.8%	
Gingival pigmentation	Grade 0	Count	331	107	86	524	< 0.001
		%	70.7%	59.8%	49.7%	63.9%	
	Grade 1	Count	122	66	69	257	
		%	26.1%	36.9%	39.9%	31.3%	
	Grade 2	Count	15	6	18	39	
		%	3.2%	3.4%	10.4%	4.8%	
Color of skin	Light pale, white	Count	1	2	1	4	< 0.001
		%	.2%	1.1%	.6%	.5%	
	White, fair	Count	48	5	1	54	
		%	10.3%	2.8%	.6%	6.6%	
	Medium white to light brown	Count	267	90	78	435	
		%	57.1%	50.3%	45.1%	53.0%	
	Olive, moderate brown	Count	135	76	76	287	
		%	28.8%	42.5%	43.9%	35.0%	
	Brown, dark brown	Count	17	6	17	40	
		%	3.6%	3.4%	9.8%	4.9%	
Distribution of pigmentation	No distribution of pigmentation	Count	331	107	86	524	
		%	70.7%	59.8%	49.7%	63.9%	
	Attached gingiva and interdental papilla	Count	108	52	60	220	< 0.001
		%	23.1%	29.1%	34.7%	26.8%	
	Marginal and attached gingiva and interdental papilla	Count	29	20	27	76	
		%	6.2%	11.2%	15.6%	9.3%	
Area of pigmentation	No area of pigmentation	Count	331	107	86	524	< 0.001
		%	70.7%	59.8%	49.7%	63.9%	
	Upper canine to canine	Count	7	0	0	7	
		%	1.5%	0.0%	0.0%	.9%	
	Upper right second premolar to left premolar	Count	26	17	25	68	
		%	5.6%	9.5%	14.5%	8.3%	
	Lower right second premolar to premolar	Count	1	0	0	1	
		%	.2%	0.0%	0.0%	.1%	
	Upper and Lower canine to canine	Count	30	20	16	66	
		%	6.4%	11.2%	9.2%	8.0%	
	Upper and Lower second premolar to premolar	Count	73	35	46	154	
		%	15.6%	19.6%	26.6%	18.8%	
Total		Count	468	179	173	820	
		%	100.0%	100.0%	100.0%	100.0%	

Discussion

Gingival hyperpigmentation is a condition of major concern, and many patients present to the periodontist with the unesthetic condition of dark gingiva, which is genetically present in some populations and known as physiologic or racial pigmentation (14).

Melanin pigmentation in different populations has been reported to vary between 0% to 89% and to be affected by ethnic factors and smoking habits (15).

This present study is the first study carried out among the Kurdish population in Sulaimani city that has established a correlation between gingival pigmentation and skin color, and which included both participants with gingival pigmentation and those without gingival pigmentation (Grade 1). For example, a previous study carried out in India included only participants who had gingival pigmentation (16). In the present study, the majority of cases had no gingival pigmentation, while among those with pigmentation, it was mostly grade 1 pigmentation (solitary unit(s) of pigmentation in papillary gingiva without extension between neighboring solitary units), and the lowest percentage had Grade 2 pigmentation. This finding is in agreement with a study done at the Islamic Azad University in Tehran (17), but contrasts with the finding of the study done in India by Ponnaiyan et al. (14). This could be due to the majority of the present study's participants having type 3 or 4 skin color according to the Fitzpatrick scale, ranging from medium, light brown, to moderate brown (13). Meanwhile, the majority of participants in the Ponnaiyan et al study had type 5 or type 6, ranging from dark brown to black according to the Fitzpatrick scale (13).

Regarding the distribution of pigmentation, the present study discovered that pigmentation was most commonly found in the attached gingiva and interdental papilla, which agrees with a study done on a south Indian population (16) and a study done in Pakistan (18). However, this result differed from that produced in a study done in Nigeria, where they found attached gingiva was the most common category of pigmentation (19). This indicates that there are ethnical variations in the pigmentation of the gingiva.

The color of gingiva has been correlated to skin color in the present study, and the association was highly significant. It was observed that darker-skinned subjects had heavy gingival pigmentation, whereas fair-skinned subjects had mild gingival pigmentation (those recording type 4 or type 5 skin color according to the Fitzpatrick scale) (13). These findings are similar to the previous studies on Indian populations, where the incidence of pigmentation of the gingiva was found to increase with complexion changes to the darker shades (14) (16) (20).

In the present study, a strong association was found between gingival pigmentation patterns and gender, with gingival pigmentation being more frequent among males than females. However, a study done in Brazil showed that physiological pigmentation of the oral mucosa affected males and females equally (21). This finding is contradictory to a study done among a Nigerian population which found no correlation between gingival pigmentation and gender (19).

The present study found that the intensity of gingival pigmentation increased with age and that gingival hyperpigmentation is more common in adults aged 32-39 years. This finding agrees with the study done in Pakistan (18). Nevertheless, another study (20) found that gingival hyperpigmentation is more frequent in younger adults (18-25 years). This could be due to the size of keratinocytes and thickness of the epidermis, since older adults have larger keratinocytes and thicker epidermis (22).

The highest rate of gingival pigmentation identified in this study was in the upper and lower second premolar to premolar, in contrast to the study of (14), in which the highest rate of gingival pigmentation was found in the area of the incisors, and the rate decreased considerably in the posterior areas. This could be due to racial variation, different anatomic distribution of gingival pigmentation in the mouth, or greater exposure to sunlight.

Conclusion

Gingival pigmentation has become a significant esthetic concern among patients today. Because of this, depigmentation procedures have emerged as a mainstay of periodontal treatment. It can be concluded from the findings of the present study that the majority of the population in Sulaimani city have type 3 and type 4 skin color (medium, white to light brown and olive, moderate brown) according to the Fitzpatrick scale. The majority of our participants also had no pigmentation, and among those who had pigmentation, the majority was in the attached gingiva and interdental papillae. This study concludes that it has statistically established an association between the intensity of gingival pigmentation and skin color. In addition, the intensity of gingival pigmentation was found to increase with age, and the highest rate of gingival pigmentation was in the area of upper and lower second premolar to premolar.

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Conflict of Interest

No conflict of interest

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Case Report

The Youngest Palestinian Case of Multisystem Inflammatory Syndrome in children (MIS-C)

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ABSTRACT

The multisystem inflammatory syndrome in children (MIS-C) considers a post-infectious immunological response to coronavirus illness (COVID-19) that was originally identified in the United Kingdom and later identified in other countries. A previously healthy 3-month-old boy was admitted to hospital context with -5-day history of fever, gastrointestinal symptoms [diarrhea, vomiting of normal gastric contents], hypoactivity, and poor oral intake, but so far no history of covid-19 active disease. The infant was dehydrated, with macular non-blanching skin rash everywhere over his body and widespread non-pitting edema. With supportive measures, methylprednisolone and IV immunoglobulin, the child improved, with his fever, skin rash, and laboratory tests returning to normal. On the seventh day of hospitalization, he was discharged. This is identified as the youngest reported case of MIS-C since the beginning of the COVID-19 pandemic.

Introduction

SARS-CoV-2-related Multisystem Inflammatory Syndrome in Children (MIS-C) is becoming ever more frequent. It might be a life-threatening illness that affects previously healthy children and adolescents 2–6 weeks following Covid 19 disease (COVID-19). Although the precise cause of this illness is unclear, immunological processes and vasculopathy have been suggested. Fever, scientific indications of inflammation (including elevated ferritin and IL-6), and clinically severe sickness needing hospitalization with multisystem (>2) organ involvement mark this disorder (cardiac, renal, respiratory , dermatologic, hematologic, gastrointestinal, or neurological); Furthermore, no other possible diagnoses should be considered, and a marker for current and recent SARS-CoV-2 infection (RT-PCR, serology, or antigen test) or COVID-19 exposure must have occurred through the four weeks preceding the onset of symptoms (RT-PCR, serology, or antigen test). (1, 2). Both diagnosis and therapies, including resuscitation, have been difficult due to the syndrome's non-specific combination of symptoms and lab results (and the requirement of a positive COVID-19 test).

Symptoms of MIS-C are highly similar to those of Kawasaki disease, toxic shock syndrome (TSS), and also macrophage activation syndrome (MAS), a pattern of secondary haemophagocytic lymphohistiocytosis. Because several clinical signs were similar to Kawasaki's illness, MIS-C was first labeled as Kawasaki-like (KD) (3). Current statistics, on the other hand, show that there are certain differences between these two situations, such

as the time of presentation: KD affects the number of kids before they reach the age of five, while MIS-C begins to affect older children, with such an average age of eight years. (4). It is obvious that MIS-C tends to affect kids of all ages, having 70 percent of papers reporting a median age of seven to ten years. There appear to be proportionally fewer instances reported among kids and young adults 16 and older compared to COVID-19 infection rates in the same categories, however. this might be related to the fact that many of the papers are based on studies done in pediatric hospitals. In contrast to what has been published regarding MIS-C rates thus far, old-aged teenagers and young adults are much more likely to become infected (or examined and diagnosed as cases) than children (5).

Case Presentation

Here we report a case of SARS-COV-2 related multisystem inflammatory syndrome of an infant observed on 15\10\2021. A previously healthy 3 months old male infant without a history of COVID-19 symptomatic disease, was admitted to the pediatric ward with 5 days history of fever, gastrointestinal symptoms [diarrhea, vomiting of normal gastric contents], hypoactivity, and poor oral intake.

On physical examination, the baby was dehydrated with generalized non-pitting edema and macular non-blanching skin rash all over the body figure (1). His abdomen was distended, tympanic on percussion but without organomegaly or ascites. Otherwise, he was afebrile, alert, and conscious with normal vital signs, regular heart rate without any murmurs, good air entry bilaterally with harsh breathing sound, good muscle tone, and strength.

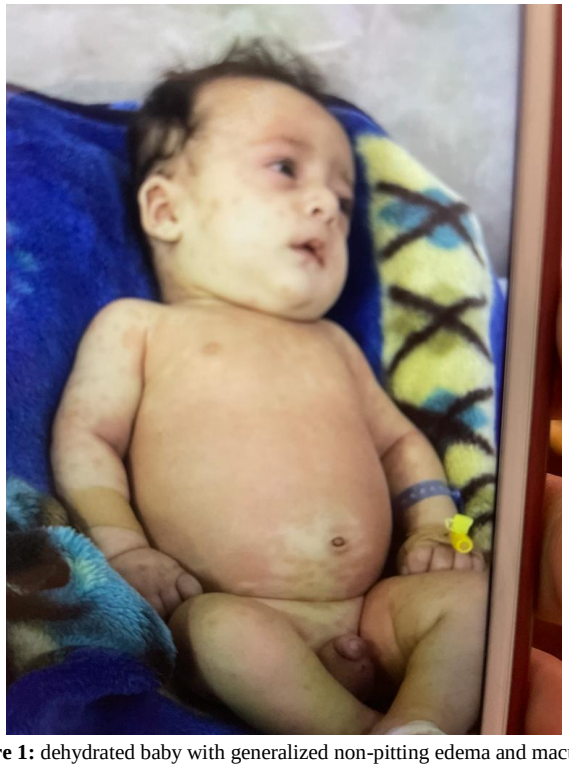

Figure 1: dehydrated baby with generalized non-pitting edema and macular non-blanching skin rash all over the body

Laboratory tests showed anemia, leukocytosis with lymphopenia, and normal neutrophil count. Troponin and COVID-19 antigen PCR were negative but COVID-19 IgG was positive. Ferritin, d-dimer, CRP, and LDH were elevated. Coagulation profile components including PT, aPTT, and INR were elevated, but platelets were still normal. Ultrasound for abdominal distention was done and intussusception was ruled out. An echocardiogram and blood culture were done and were normal.

Since admission the patient received 10 grams of IVIG twice daily for one day, 6mg of methyl-prednisone twice daily for 6 days, 300 mg of ceftriaxone twice daily for 5 days, 6mg of IV Esomeprazole twice daily for 6 days, 30 mg of albumin 20% over 1 hour on the 2nd day of admission since it was found to be decreased (2.7 g/dl) and 50 mg of IV paracetamol as needed.

On the 4th day of admission, his Ferritin was decreased to 728 and platelets increased to 113. On the 6th day of admission, his d-dimer was decreased to 849, CRP fall to 8.4, and platelets normalized. With appropriate management and supportive measures, the child evolved with resolution of fever, skin rash, and normalization of his laboratory findings. And discharged on the 7th day of admission.

Table 1: Lab values after starting treatment

Laboratory test		Result	Normal range
CBC	HB	7.9	
	HCT	23.32	40-52%
	WBC	27.56 (neutrophils :21) (lymphocytes :63.7)	(5-10) *10^6 45-65% 25-45%
	PLTs	15.61	150-400
CRP		138.4	Up to 6 mg\l
Troponin		7.5	Healthy patient : <30 ng\l AMI patient : >30 ng\l
ESR		5	0-15
COVID-19 antigen		Negative	Negative
Ferritin-serum		1600	Males:21.8-275 ng\ml Children:7-140 ng\ml
D-dimer		2561	0-250
LDH		530	125-220 U\L
SGOT		49	0-37
COVID-19 IgG-II-spike protein		1220	Negative < 50 AU\ml Positive > 50AU\ml
Albumins		2.7	3.5-5.5 g\dl

Discussion

To the best of our knowledge, this is the first reported case of MIS-C in this age in Palestine since the onset of the COVID-19 pandemic. And it's the youngest case of MIS-C among reported cases around the world. Our case clinical presentation and laboratory evaluation were consistent with CDC, WHO, and RCPCH case definitions (2, 6, 7). As there's a fever of 5 days duration, elevated inflammatory markers, involvement of gastrointestinal organs and dermatological findings, positive IgG serology test, and negative blood culture. Furthermore, ECG, echo, abdominal ultrasound, and other diagnostic and laboratory tests excluded other potential diagnoses.

It's very difficult to differentiate between MIS-C and other pediatric inflammatory syndromes such as toxic shock syndrome (TSS) and Kawasaki disease (KD); however, they're not the same entity despite the similarities in the presentations (8). Gastrointestinal symptoms like vomiting and diarrhea are distinguishing features of MIS-C. Also, the inflammatory storm observed in MIS-C is much more intense than TSS and KD (8).

In our case, there's much more evidence of inflammatory storm (CRP of 138.4, D-dimer of 2561, albumin of 2.7) along with vomiting and diarrhea (8). The age of our case was 3 months old and since Kawasaki disease presents in children less than five years of age, we can't depend on the age to distinguish between KD and MIS-C, as it's not the only case of MIS-C in children younger than 5 years old (9).

The differentiation between severe COVID-19 and MIS-C is also important. Five days history of fever, presence of rashes and Gastrointestinal symptoms are more consistent with MIS-C, also the severe elevation of inflammatory markers previously mentioned is more consistent with MIC-S rather than severe COVID-19 infection (8).

As the treatment of MIS-C is directed against the inflammatory process they have, then IVIG and/or glucocorticoids are frequently used. IVIG is frequently used if there are KD-like features, shock, cardiac or coronary involvement, and if the patient remains persistently febrile with elevated of his inflammatory markers. Glucocorticoids are added to IVIG in the case of refractory shock, KD-like features, persistent elevation of inflammatory markers, and fever (10). However, recent studies didn't find any strong evidence that recovery rates differ with glucocorticoids alone, IVIG plus glucocorticoids, or IVIG alone (11).

Also, Immunomodulators can be used in refractory cases .low dose aspirin and should be considered in certain circumstances. However, aspirin should be avoided if the platelets count is less than 80,000/ μL. And as symptoms overlap with severe bacterial illness, then empiric broad-spectrum antibiotics should be used before blood culture is taken (12).

In our case, there are refractory KD-like features along with severe inflammatory response, so our patient received IVIG with glucocorticoids. Ceftriaxone was used as an empiric antibiotic. Antiplatelet therapy was not used as platelets count was less than 80,000 /µl (it was 15.6/µl on admission in our case). Albumin was given to our patient on the second day of admission as it was decreased. IV Esomeprazole and paracetamol were also used as ajuvent therapties.

We monitor the patient signs and symptoms along with laboratory findings including CRP to see the response to the treatment previously mentioned. Our patient symptoms and laboratory findings rapidly improve in the next 7 days following the treatment. Before discharge, he was afebrile, there was no vomiting or diarrhea and his skin rashes and edema were also resolved .his CRP, Ferritin, D-dimer, Wbcs, and platelets count were also normalized before the discharge.

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Conflict of Interest

No conflict of interest

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Al-Kindy College Medical Journal (KCMJ)

Case Report

Arrhythmia-Induced Cardiomyopathy. A Palestinian Experience

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ABSTRACT

A 20 year-old male was admitted with a history of recurrent palpitations from 5 years. Baseline ECG revealed premature ventricular contractions (PVCs) with delta waves. Stress ECG showed short non-sustained Ventricular tachycardia (VT). Echocardiography showed moderate dilation of the left ventricle with mild reduced systolic function and Ejection fraction was estimated to be 42%. Right ventricle was mildly dilated and hypokinetic. Both atria were mildly dilated. The patient referred to CVC for EP study with possible ablation. The ablation of the focus led to complete suppression of the ectopy. Post-procedure ECG and echocardiography showed normalized rhythm and systolic function.

Introduction

Arrhythmias have been long considered part of the clinical presentation of heart failure (HF) and cardiomyopathy (CM) (1) . The hallmark of this condition is partial or complete reversibility once arrhythmia control is achieved. (2). Arrhythmia Induced Cardiomyopathy (AIC) can be classified into 2 categories: one where the arrhythmia is the sole reason for ventricular dysfunction (arrhythmia-induced), and another where the arrhythmia exacerbates ventricular dysfunction and/or worsens HF in a patient with concomitant heart disease (arrhythmia-mediated) (2) . Arrhythmia-induced cardiomyopathy is a reversible form of left ventricular (LV) systolic dysfunction caused by a high burden of atrial or ventricular arrhythmias (3). Tachycardiomyopathies (TCMP) are an important cause of left ventricular (LV) dysfunction that should be recognised by physicians as they are potentially reversible and have a significant impact on morbidity and prognosis (4).

Case Presentation

A 20-year-old male patient with a history of recurrent palpitations from 5 years. These palpitations only appear during slight activity and not during rest. The patient is not a smoker, not alcoholic, has had free personal and familial medical history and was previously not under any medical treatment.

On admission, his respiratory rate was 18 breaths per minute with an oxygen saturation of 98% on room air. His blood pressure was 130/70 mmHg with normal jugular venous pressure. On

auscultation, he had dual heart sounds with no murmurs. However, the heart rate heard was irregularly regular. Baseline ECG showed premature ventricular contractions (PVCs) with delta waves. Short non-sustained Ventricular tachycardia (VT) was detected on stress ECG. By measuring the distance between interventricular septum and anterior wall Echocardiography showed moderate dilation of the left ventricle with moderately reduced systolic function and Ejection fraction was estimated to be 42%. Right ventricle was mildly dilated and hypokinetic. Both atria were mildly dilated. Estimated PAP was 37mmHg (normally <20 mmHg)[5]. In addition, there is mild mitral and tricuspid regurgitation. A tachycardia-induced cardiomyopathy related to ventricular arrhythmia was suspected although a primitive dilated cardiomyopathy complicated by ventricular arrhythmia was initially not excluded. So, the patient was referred to Cardiovascular center (CVC) for electrophysiological (EP) study with possible ablation.

SPGT, SGOT, INR, BILIRUBIN, BUN and CREATININE were tested, and all of them were in the normal range.

During the EP study, detailed mapping of the right ventricular outflow tract (RVOT) and left ventricular outflow tract (LVOT) during PVCs using the Ensite precision 3-D mapping system was done. the earliest evaluation site (>300 ms ahead of the QRS) was reported between the right and left coronary cusps. Several Radiofrequency(RF) lesions were delivered at the earliest site with transient effect. The successful ablation attempt was done by curving the ablation catheter into the aortic root, this maneuver resulted in a good contact. After the ablation, the Patient was monitored on ECG in Coronary care unit and discharged the next day since his ECG was normalized. He was discharged on Ramipril 5mg (1/2x1) and Bisoprolol 5mg (1x1) for one month.

During the follow up after 1 month, his palpitations were progressively improved. Baseline and stress ECG were performed, that showed no abnormalities. Echocardiography was also performed, and revealed an improvement of both LVF and EF; the EF was increased from 42% to 52%. when the EF was 42 , the LV diastolic diameter was more than 65 mm ,

when it was 52, the LV diastolic diameter was less than 50 mm when it was 55, the LV diastolic diameter was approximately 45 mm So he discharged on the same dose of Ramipril (5 mg (1/2/1)), but taper the dose of Bisoprolol to 2.5 mg (1x1) for 11 months.

After 12 months, his palpitations nearly disappear, with no new changes on the normal baseline and stress ECG from the previous visit. Also, his LVF and EF further improved; in which EF was increased from 52% to 55%. So he discontinued both Ramipril and Bisoprolol without any complications.

Stress and abnormal ECG shown in figure1 and 2.

Discussion

This case report exhibits that PVCs can lead to arrhythmia induced cardiomyopathy (AIC). Serious arrhythmia that causes cardiomyopathy can initiate different organ dysfunction, so it is significant to manage it as soon as possible. [5] The diagnosis of AIC was replaced by "idiopathic dilated cardiomyopathy" because of the absence of awareness about AIC.[6] Numerous theories presented the pathophysiology of AIC such as introducing cellular and molecular processes, cellular processes likely related to myocardial power consumption, cellular calcium metabolism changes, oxidative tension, ischemia, or apoptosis. [7]

Cardiomyopathy is largely associated with long-term PVCs such as in our patient where there was a long-term presence of PVCs. If there was no congenital cardiac dysfunction, PVCs are quite admitted harmless. [7] However, recurring PVCs can prompt a decrease in LV capacity, specifically PVC-initiated cardiomyopathy. [7]

There are several features related to cardiomyopathies, such as PVCs' long-term presence, the absence of symptoms, PVC with an epicardial source, and longer PVC QRS duration. [7]

As PVC ablation repairs the regular LV capacity of the healthy heart, a practical procedure in those situations is completely to control the patient's symptoms of LV impairment and estimate ablation. Furthermore, separate PVCs have a great prognosis in healthy hearts with protected capacity, therefore there is no intention to intercede except important dysfunction occurs. Unfortunately, our patient has signs of LV impairment with an ejection fraction (EF) of 42%, so the ablation was mandatory. [4] Moreover, our patient followed a spironolactone regimen with a selective beta-blocker for several weeks before the ablation but without any improvement.

Frequently, outflow tract VT or persistent PVCs happen without any congenital heart disease. [8]

Radiofrequency ablation is broadly applied in the management of outflow tract VT. Regardless of the way that it might be related to serious coronary collateral damage and constant AV conduction harm. [9] It was used in our patient and improved his symptoms without any complications.

The appearance of myocardial scar showed by cardiovascular magnetic resonance imaging (CMR) may recognize patients who have constant essential cardiomyopathy and may not profit by ablation management. [10] Our patient did not do CMR, although the ablation was effective.

According to the scientific literature, whenever left ventricular pump work is repaired, the survival prognosis is good, as we can see in our patient. Though, one research demonstrated that even the long time after AIC and standardization of left ventricular systolic dysfunction (LVSD) on MRI, moderate left ventricular dilation and ultra-structural myocardial injuries might be shown, thus regular examination is significant regardless of whether there is no malformations in the following weeks or months of the ablation procedure. [11] The five weeks of follow-up for our patient showed a rapid improvement of LV function with a rapid increase in the EF.

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Statement of Human and Animal Rights

This article does not contain any experimental studies with human or animal subjects.

Statement of Informed Consent

Informed consent was obtained from the individual participant included in the study.

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None

Conflict of interest

The authors declare no conflicts of interest

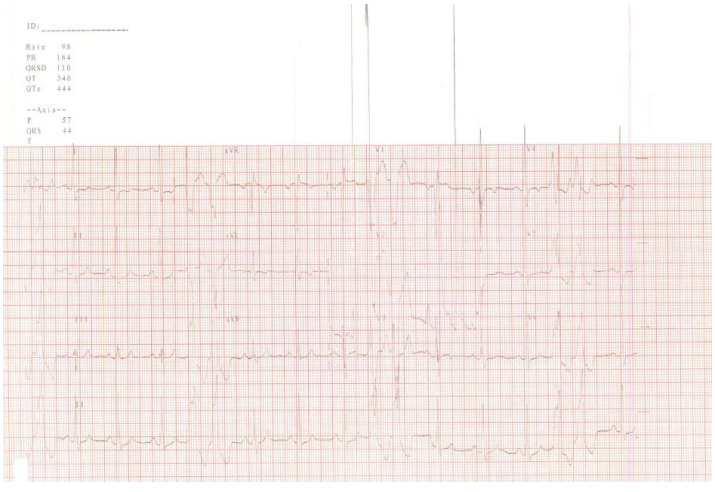

Figure 1: Stress ECG with non-sustained Ventricular tachycardia

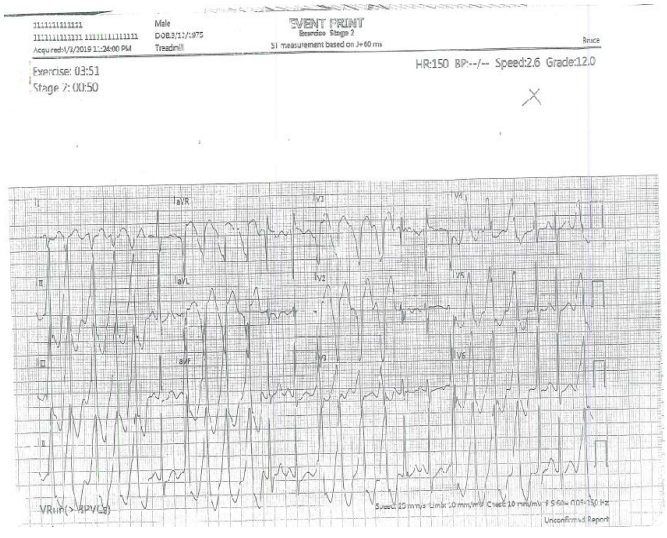

Figure 2: Abnormal ECG showing premature Ventricular contractions (PVCs) in most leads with widespread delta waves

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Al-Kindy College Medical Journal (KCMJ)

Brief Report

Prevalence of Congenital Toxoplasmosis and Congenital Rubella among Suspected Infants in Baghdad

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ABSTRACT

Congenital toxoplasmosis (CT) and congenital rubella (CR) infections are well-known causes potentially leading to devastating consequences. This report aims to address the prevalence of each of these infections among suspected infants in a pediatric hospital in Baghdad.

The study sample includes 120 blood samples of suspected infants consulting Al-Alwyia pediatric teaching hospital over one year. This report represents an extension of a previous article published in Al-Kindy College Medical Journal KCMJ about cytomegalovirus infection (CMV). Using the enzyme-linked immunosorbent assay (ELISA) method, the results show that 5.8% and 5% were positive for specific IgM antibodies for rubella and toxoplasmosis respectively. Results also show that CMV is more common than CT and CR.

Although results reported one case of co-existence of IgM antibodies for both CMV and rubella, there was a significant association of negative CMV IgG and IgM results with positive rubella results; and negative rubella results with CMV IgG and IgM results.

Conclusions: This short brief addressed the prevalence of CT and CR in Baghdad. A novel finding identified in this brief is the association of the prevalence of CR active infection with negative past and negative recent CMV infections, and the association of prevalence of CMV active and past infections with negative CR infection.

Recommendations: The study recommends increased awareness, early diagnosis and treatment for congenital infection, and consolidation of coverage of the rubella vaccine for children and girls at premarital age.

Introduction

Measuring disease frequency (prevalence or incidence) is among the most fundamental measures in epidemiology to understand the burden on the health and social care system at a particular time that influences decision-making about what public health interventions are required (1).

TORCH infections (Toxoplasmosis, Other infections, Rubella, Cytomegalovirus, and Herpes simplex virus) are a group of congenitally acquired infections that cause significant morbidity and mortality in neonates. Identifying infants with such infections by serum TORCH antibody testing is crucial for the detection of these congenital infections (2), of which the IgM antibody detection is considered the primary approach for active infection (3). Congenital rubella (CR) and congenital toxoplasmosis (CT) are well-known congenital infections which can lead to variable manifestations

including birth defects, and other serious consequences such as severe neurological sequelae and mental retardation (3,4), CT can be treatable when diagnosed at an early time (4), while CR is a mainly preventable disease through vaccination.

Congenital cytomegalovirus (CMV) can share similar manifestations to other TORCH infections such as toxoplasmosis and rubella (6). A TORCH screen panel of tests for detecting antibodies for Toxoplasma gondii, rubella, CMV, and Herpes Simplex Virus (HSV), are usually used in a variety of assay formats including the enzyme-linked immunosorbent assay (ELISA), rapid assays, and bead-based assays antibodies (Abs) for these pathogens concomitantly. Reactivity for the IgM, but not IgG, usually indicates a current infection, while IgG without IgM suggests a past infection (7,8)

During our work on the manuscript (Prevalence of congenital cytomegalovirus among suspected infants in Baghdad 2019-2020) published in Al-Kindy College Medical Journal (KCMJ) Vol. 18 No. 1 at 2022 (9) , we came across the prevalence of CT and CR among the study sample of patients. As far as the prevalence of CT and CR in Baghdad is scarce, it is crucial to address this information and shed light on it.

Subjects and Methods

According to this cohort study, blood samples were taken for 120 infants consulting Al-Alwyia pediatric teaching hospital suspected infants over one year (from 1st of October 2019 to 1st of October 2020). Males constitute 54.17 % and the 1-3 months age group infants constitute 29.17% of the sample as shown in table 1.
Criteria for inclusion included the presence of any of the following: jaundice, hepatosplenomegaly, rash, congenital malformations, hearing or ophthalmological abnormalities, and various CNS manifestations like convulsions or delayed milestones.

Serum samples were tested for rubella and toxoplasma-specific IgM Abs in addition to CMV-IgM and IgG Abs by using commercially available IgM capture ELISA kits (Bioactiva, Germany). According to the manufacturer's instructions, 1.5 - 3 ml of venous blood was obtained, samples were placed in a sterile plain tube, allowed for clotting at room temperature for half an hour, and then centrifuged at 1500 Revolutions per minute (rpm) for 5 minutes. All sera were stored in a repository at -20°C pending testing. The sample was considered positive when the ratio was >1.1, and it was considered negative if <0.9 for both IgG and IgM antibodies. Initial equivocal results were repeated in 10-14 days. CMV-IgM negative results were furtherly classified according to the presence or absence of CMV-specific IgG Abs.

The study was approved by the Al-Risafa research ethical committee and by the Ministry of Health- Iraq as part of the Al-Risafa Health Directorate research plan for 2020 which authorized the researchers to study these congenital infections in infancy. Furthermore, this paper is part of the currently implemented TORCH project for the identification of causative microorganisms attributed to congenital anomalies. This project is supported, approved, and sponsored by the public health directorate/ MOH-Iraq. Furthermore, the Training and Planning Directorate / MOH-Iraq and the Research Ethical Committee/ Baghdad Al-Risafa Health Directorate approved our research study and plan as part of the 2020 research plan. Parents' verbal approvals were obtained as far as patients involved did require routine investigations that did not involve further interventions like surgery and drugs. The statistical data analysis approaches used to analyze and assess the results of the study were done under the application of the statistical package (SPSS) version (22.0).

Results

The prevalence rates of CR and CT were 5.8% and 5% respectively. There is a significant association between rubella IgM positive results and CMV (IgG & IgM) negative results. the CMV IgM positive and negative results are associated positively with Rubella IgM negative results (with a P value of 0.028).

Table 1: Gender and age distribution of whole sample

1-Gender	No .	%
Males	65	54.17
Females	55	45.83
Total	120	100
2- Age (month)	No .	%
< 1	27	22.5
1-3	35	29.17
4-6	25	20.83
7-9	16	13.33
10-12	17	14.17
Total	120	100

Table 2: Prevalence of Rubella -IgM and toxoplasmosis-IgM antibodies during infancy distributed according to CMV IgG +ve , CMV IgM +ve, and CMV –ve with comparison significance

Finding	No. & %	CMV IgG +ve result	CMV IgM +ve result	CMV (IgG & IgM) -ve result	Total	C.S. P-value *
Rubella IgM -ve	No.	65	19	29	113	C.C. = 0.237 P=0.028 S
	%	98.5%	95.0%	85.3%	94.2%	
Rubella IgM +ve	No.	1	1	5	7	
	%	1.5%	5.0%	14.7%	5.8%	
Total	No.	66	20	34	120	
	%	100	100	100	100	
toxoplasma IgM -ve	No.	62	20	32	114	C.C. = 0.102 P=0.531 NS
	%	93.9%	100%	94.1%	95.0%	
toxoplasma IgM +ve	No.	4	0	2	6	
	%	6.10%	0.0%	5.90%	5%	
Total	No.	66	20	34	120	
	%	100	100	100	100	

Discussion

IgM Abs for rubella in our study was detected among 5.8% of infants with suspected congenital infection (table 2 and figure 1). Up to our knowledge, there was no available registered data regarding the occurrence of CR in Iraq previously (10). Reported acquired rubella cases in Iraq flared up to 197 cases in 2021. Reported cases were 10, 9, and 21 in 2020, 2019, and, 2018 respectively (11). Acquired rubella is a mild childhood illness. It has a high risk on susceptible pregnant mothers that may lead to abortions or congenital infections.

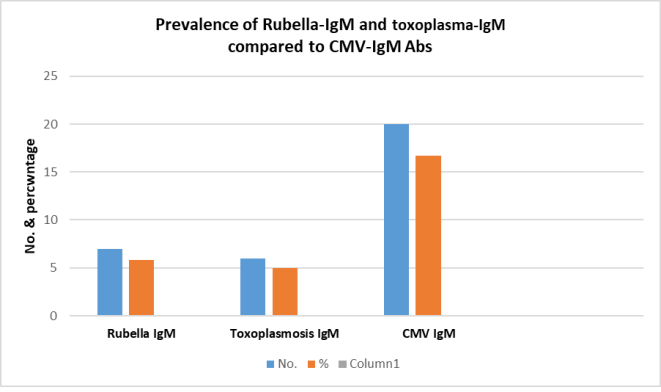

Figure 1: Prevalence of Rubella-IgM and toxoplasma-IgM compared to CMV-IgM Abs

In 2019 the coverage for the 1st dose of the rubella-containing vaccine in Iraq was 86%, this figure was increased to 92.33 in 2020. Unfortunately, it has decreased again to 84.05% in 2021 (12).
As of December 2018, 168 out of 194 countries had introduced rubella vaccine programs and global coverage was estimated at 69%. These programs include vaccinating girls (aged 10-14 years) in addition to early childhood vaccinations (13).

CR is still highly encountered in many developing countries. For example, rubella-specific IgM Abs were detected in 21.2%, of suspected infants from 2016-to 2018 in India (14). In Sudan, CR cases were attributed to 7.6 % of suspected infants in 2012 (15). Differences in the prevalence of CR are related to differences in the prevalence of rubella among pregnant mothers who can catch the infection if not vaccinated.

Toxoplasma-IgM Positive Abs in this study were found to constitute 5% of infants suspected to have a congenital infection (table 2 & figure 1). 15.9% of suspected children < 5 years of age were found to have toxoplasma-IgM Abs according to a study done in Al- Alwyia pediatric hospital in Baghdad in 2005 (16). This high figure is particularly contributed to the fact that diagnosis of CT after the first year of life is confounded by the possibility of the child acquiring infection in the postnatal period (17).
According to a meta-analysis published in 2019 , toxoplasma-IgM was positive in 4.10% of Iranian neonates suspected to have CT while toxoplasma-IgM was positive in 3.02% of suspected Mexican children according to another meta-analysis published in 2012 (18.19).

This paper as far as we know is the 1st to identify the prevalence of CR and the 2nd to identify CT among suspected infants in Iraq. A previous study done in Al-Nasiriya- Iraq tested IgG antibodies for TORCH infections (but did not test IgM prevalence) among newborn infants suffering prolonged neonatal jaundice (20). These IgG isotype antibodies reflect maternal antibodies passed tans-placentally to the fetus rather than active neonatal infection (21).
The prevalence of rubella- IgM and toxoplasma -IgM among our findings were lower than the CMV- IgM prevalence (figure 1 & table 2).

Among suspected cases, IgM Ab estimates were 16.7% (9) , 5.8%, and 5% for CMV, rubella and toxoplasmosis respectively .These findings are in concordance with other studies elsewhere as far as the transmission rate for CMV is higher for CMV infection (22). we found one case of coexistence of CMV and rubella-specific IgM antibodies. IgM multi-positive results by ELISA don't necessarily indicate co-infections of multiple TORCH pathogens. These can be caused by cross-reactions between antibodies and antigens during immunoassays (3). A true IgM multi-positive results for CMV and rubella-specific IgM antibodies indicating co infections are rare instead (3,23).

CMV IgM positive and CMV IgG results were found to be significantly associated with rubella IgM negative results in 19 out of 20 and 65 out of 66 CMV IgM positive and negative results respectively . CMV IgG positive results were found to be significantly associated with rubella IgM negative results in 19 out of 20 of CMV IgM positive results on the other hand CMV (IgG & IgM) negative results were found to be significantly associated with rubella IgM positive results in 5 out of 7 of rubella IgM positive results (P=0.028). This novel finding identified in this brief might denotes a cross-protection between rubella and CMV. This cross-protection (up to our knowledge) was not yet mentioned in the literature although the heterologous immunity and cross-reactivity of adaptive immune was traced back to the first vaccine by Edward Jenner in the late seventeenth century when he used a cowpox virus to immunize against smallpox infection. In general, heterologous immunity is relatively common within closely related species but can also be seen with unrelated agents (24). In accordance with our finding, CMV carriage was associated with delayed IgG decay over time after live attenuated rubella vaccine (p = 0.034) (25). The CMV infection was also found to prevent reactivation of EBV in humans (26).

Furthermore, murine CMV was found to cross-protect against lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PV) with different efficiencies (27). As information about cross-protection and heterogeneous immunity between CMV and rubella viruses is scarce, further studies are suggested.

Conclusion

It is concluded that CT and CR infections are next to CMV among suspected congenitally infants. We recommend increasing coverage of rubella vaccination for girls at premarital age and in the pediatric vaccination program. Pregnant women should be screened routinely and frequently for TORCH infections. Furthermore increased awareness of these congenital infections is recommended for early diagnosis and treatment for these infections.

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Conflict of Interest
No conflict of interest

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