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Executive Editor
Borneo Journal of Medical Sciences
Faculty of Medicine and Health Sciences
Universiti Malaysia Sabah
Jalan UMS
88400 Kota Kinabalu
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Tel: +60 88 320000 Ext 5620
Fax: +60 88 320928
Email: bjms@ums.edu.my

PUBLISHER

Penerbit Universiti Malaysia Sabah (UMS)
Ground Floor, Library
Universiti Malaysia Sabah
Jalan UMS
88400 Kota Kinabalu
Sabah, Malaysia
Tel: +60 88 320 789
+60 88 320 462
Fax: +60 88 320 446
Email: penerbit@ums.edu.my

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Editorial

Diabetes: 'Poverty amidst Plenty' and 'Hunger – in the Modern World of Plenty' in the Twenty First Century

Urban John Arnold D'Souza, Aza Sherin Mohamad Yusoff, Sadia Choudhury Shimmi
Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

According to the latest report of the United Nations (UN), about 20 million people are facing famine with around 1.4 million children are at a risk of dying. Secretary General Antonio Guterres of the United Nations has made it clear to the world that, parts of Nigeria, Somalia, Yemen and south Sudan are at a higher risk of the famine and a possible catastrophe that may be highly dangerous to the mankind. Diabetes mellitus is a metabolic disorder, wherein plenty of sugars or glucose in the blood circulation, keep the cells and tissues hungry because it is an insulin related disorder. Similarly, in the modern World of plenty, where a greater number of World populations are filled with enormous wealth and food and amidst this population, a less fortunate population is dying because of hunger! Shortage of food, malnourishment and few people dying on a daily basis are the outcome of the famine. According to the UN, lack of money in the UN treasury, donor countries, conflicts, problems of delivery of relief are few of the concerns which are looming the UN.

On the other-hand nutrient deficiency is another major problem that mainly affects the children in the world. Micronutrients, vitamins –A, folate, minerals –iodine, iron and zinc deficiencies are due to a lack of quality of food due to the lack of awareness which affect both the physical and mental health. Billions of people are possessing one or the other micronutrient deficiencies such as folate, vitamin A, iodine, etc., which results in weakening the immune system and vulnerable to a variety of diseases. Around 30 – 35% of the children in the developing world are vitamin A deficient with a high grade of health problems. Anaemia among the women, iodine deficiencies, though they eat plentiful is a masked hidden hunger. Inadequate diet or its quality need to be made aware to the parties. Micronutrient deficiency is not a problem only among the poor but also among the affluent where there is no proper awareness among the public. A variety of food in moderation such as eggs, fish, legumes, meat, fruits and vegetables are in daily need for the body for a normal physical and mental growth. Southeast Asia being mostly a green belt, luckily has a wealth of blessing but need the responsibility of educating the public at large on the need of micronutrients on a day-to-day regular basis is a priority. Poverty amidst plenty as stated above also applicable in terms of micronutrients, and hunger only is not the cause for lack of food, but plenty of food without proper awareness also may be a kind of 'poverty amidst plenty'. If proper awareness and nutritional education is made available at large to the growing population of the world along with generosity of affluent may make a difference in the world. Let us join hands in alleviating the hunger of the world!

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1. https://www.nytimes.com/2017/02/22/world/africa/why-20-million-people-are-on-brink-of-famine-in-a-world-of-plenty.html: A version of this article appears in print on February 23, 2017, on Page A8 of the New York edition with the headline: 20 Million Face Famine in a 'World of Plenty'

Review Article

Immunotherapy for Treatment of Cancer: A Review

Che Ismail Che Noh[1]*, M. Shamsur Rahman[1]
[1]Department of Biomedical Sciences and Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia
*Corresponding author's email: ismailnoh83@ums.edu.my
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ABSTRACT

Cancer is the leading cause of morbidity and mortality worldwide and has put heavy burden on public resources. The incidence of cancer and cancer related deaths are both increasing in trend. The conventional treatment for cancer includes surgery, radiation therapy and chemotherapy. However, the use of radiotherapy and chemotherapy, though effective, can be limited for their toxicities. Better understanding of human immunological system has enabled researchers to develop novel immune-based therapeutic agents for cancer. The effectiveness of immunotherapy, either as a single or combination therapy with conventional treatment has been proven through numerous studies. Immunotherapy also has the advantages over radio-chemotherapy for being less toxic, and more target-specific. There are many types of immunotherapies established for treatment of cancer. These include monoclonal antibodies, prophylactic vaccines, immune adjuvants, and cytokines. Beside the existing therapy, various investigational immunotherapy candidates are currently undergoing active development, such as therapeutic cancer vaccines and CAR-T cell therapy, providing better option for treatment of cancer in the near future.

Keywords: cancer, immunotherapy, monoclonal antibodies, vaccines, cytokines, chimeric antigen receptors

INTRODUCTION

Cancer is one of the leading cause of morbidity worldwide and associated with high fatality rate. It affects the overall quality of life and substantially burden the public health care expenditure. The incidence of numerous type of cancers shows an increasing in trend. In 2012, 14 million new cases of cancer have been documented. However, the incidence is expected to increase up to 21 million by 2030.[1, 2] Despite the advances made in development of cancer treatment, it remains as one of the major cause of death. About 8 millions of people died due to cancer in 2012, and it is expected to rise to 13 million by 2030.[3]

Breast, lung, cervix, stomach and colorectal cancers are among the most common cancers in women. While for men; lung, prostate, colorectal, stomach and liver cancers are the commonest. Tobacco use contributes about 20% of cancer death, and in developing countries, 20% of infection-associated cancers are contributed by HBV/HCV and HPV infection.[3]

There are varieties of treatment modalities available for cancer, with indication depends on the type and stage of cancer, general well-being of patient, as well as socioeconomic factors. Conventional treatments for cancer include surgery, radiotherapy and chemotherapy. The use of cancer chemotherapeutic agents often limited for their toxicities.[4]

Advance research made in immunological field has enable a breakthrough discovery of development of novel cancer immunotherapy. This therapy works through either manipulation of human own immune system or by administrating exogenous laboratory-made immune cells into human body to fight against cancers.[5] The advantages of immunotherapy compare to conventional treatment include, it causes less adverse effect, more target-specific and some immune cells have the 'long-term memory' to prevent cancer recurrence.[6]

In history, William Coley is the first person who realised the potential use of immune cells to fight against cancers.[7] He noticed that, some of his patients who acquired post-surgical infection, showed better improvement in their cancer progress. He continued the discovery by treating his cancer patients through provoking immune system by using specific

cultured bacteria, known as *Coley toxins*.[2] Since then, more researches have been carried out and discoveries being made in developing immunotherapy. In recent decades, there is increase in number of immunotherapy receiving approval for cancer treatment. It has become a vital component of treatment regimen for certain types of cancer.[8]

UNDERSTANDING CANCER IMMUNOLOGY

Tumour-associated antigen (TAA) is a specific antigen expressed by most cancer cells. TAA can come from various sources which elicits different immune responses. The examples include TAA derived from oncogenic viruses, overexpression of cellular proteins, and mutated genes and onco-suppressor by-products.[9]

Activation of cytotoxic T-cells (CTL) is the key element of immunological reaction towards cancer cells. CTL is produced by cancer infiltrating lymphocytes. It recognizes TAAs which present on the MHC Class I molecules on tumour cell surface. Subsequently the Fas/FasL pathway will be activated by CTL and initiate the programmed-cell death of the malignant cells.

However, cancer cells can develop mechanism to escape human immune attack to be manifested clinically. This can be achieved by several means include by reducing number of MHC Class I on surface to avoid immune cells recognition, inhibitory signalling, and activation of immunosuppressive activity.[9] Thus, the principal mechanism of cancer immunotherapy is through improving the ability for cancer cells recognition or by introducing the missing immune system components. .

DIFFERENT MODALITIES OF IMMUNOTHERAPY

Cancer immunotherapy can be "passive" or "active" therapy. "Passive" immunotherapy includes treatment with monoclonal antibodies, tumour adjuvant, and delivery of cytokines which directly initiate anticancer activity.[10] "Active" immunotherapy refers to the use of vaccination to stimulate patient own immune system; as a treatment, itself or as a cancer prophylaxis.[11] However, this is an ambiguous term, as some immunotherapy can be both active and passive therapy (e.g. monoclonal antibodies therapy).

Immunotherapy can be further classified into specific; which triggers T-cells responses against tumour-associated antigens and non-specific therapy; which not targeting to any specific antigens (Table 1).[10, 12]

Table 1 Classification of cancer immunotherapy[12]

Immunotherapy	Active	Passive
Specific	Cancer vaccines; tumour-associated or viral antigens	Injection of monoclonal antibodies
Non-specific	Immune adjuvants Cytokines therapy	Chimeric Antigen Receptor T-cell therapy (CAR T-cell)

I. Monoclonal Antibodies

Monoclonal antibodies therapy is one of the most successful forms of immunotherapy for both solid and haematological cancers. The production of monoclonal antibodies is based on the selection of specific antigen for specific tumour growth (Table 2).

Table 2 Tumour-associated antigen targeted by monoclonal antibodies[13]

Antigen category	Examples of antigen	Tumour types expressing antigen
Cluster of differentiation (CD) antigens	CD20	Non-Hodgkin's lymphoma
	CD30	Hodgkin's lymphoma
	CD33	Acute myelogenous leukaemia
Glycoproteins	EpCAM	Epithelial tumours (breast, colon, lung)
	CEA	Epithelial tumours (breast, colon, lung)
	gpA33	Colorectal carcinoma
Glycolipids	Gangliosides	Neuroectodermal tumours
Carbohydrates	Lewis-Y[2]	Epithelial tumours (breast, lung, prostate)
Vascular targets	VEGF	Tumour vasculature
	VEGFR	Epithelium-derived solid tumours
Growth factors	ErbB1/EGFR	Glioma, lung, breast head and neck tumours
	ErbB2/HER2	Breast, colon, lung, ovarian
Stromal and extracellular antigens	FAP	Epithelial tumours (colon, lung, pancreas)
	Tenascin	Glioma, epithelial tumours

Monoclonal antibodies can be produced in the form of murine, chimeric, humanized or human antibodies (Table 3). Murine monoclonal antibody is the first generation of antibodies produced by hybridoma technology. It is prepared in the laboratory by injecting human cancer cells or its antigen protein into mice. This will activate immune reaction and production of antibodies. These antibodies will then be fused with laboratory-grown cells to form hybridomas, which allows massive production of antibodies.[10] Nevertheless, the use of murine form of antibodies can be limited due to the risk of immune activation against these antibodies.

Table 3 Examples of different forms of monoclonal antibodies approved for treatment of cancer[14]

Monoclonal antibodies	Target	Type	Indication(s)
Cetuximab	EGFR	Chimeric IgG	Colorectal cancer
Panitumomab	EGFR	Human IgG	Colorectal cancer
Trastuzumab	HER2	Humanized IgG	Breast cancer, gastric cancer
Pertuzumab	HER2	Humanized IgG	Breast cancer
Alemtuzumab	CD52	Humanized IgG	Chronic lymphocytic leukaemia
Rituximab	CD20	Chimeric IgG	Chronic lymphocytic leukaemia
Ipilimumab	CTLA-4	Human IgG	Melanoma
Nivolumab	PD-1	Human IgG	Melanoma
Denosumab	RANKL	Human IgG	Breast cancer, prostate cancer
Ibritumomab	CD20	Murine IgG	Non-Hodgkin's lymphoma

Improvement in efficiency of immune reaction while at the same time reducing immunogenicity can be achieved through production of chimeric, humanized and fully human antibodies. The humanized monoclonal antibody developed by replacing Fc and Fv regions with human germline amino acid and production of fully human antibodies achieved through transgenic mice and phage display technique.[13]

Monoclonal antibody can cause direct cell deaths by inducing apoptosis of cancer cells through inhibition of signalling pathway for cells growth.[13] It also indirectly induces cells death by recruiting cytotoxic cells such as monocytes and macrophages, and mediates cancer cell death through antibody-dependant cell mediated cytotoxicity (ADCC) or by binding to complement and mediate cancer cell death through induction

of complement dependent cytotoxicity (CDC).[13] Another mechanism action of monoclonal antibody is through vascular and stromal ablation thus retarding the tumour growth and vascularisation.[15] Monoclonal antibodies also can be conjugated with radioactive substances, toxins or chemotherapeutic drugs targeting specific cancer cells improving its efficacy.

Various Types of Monoclonal Antibodies

A. Naked Monoclonal Antibodies

It is the most common type of monoclonal antibodies used for cancer treatment. It acts without being conjugated with other material. In the body, it will attach to antigens on tumour cells or some non-cancer cells or can be free floating (Table 4).

Table 4 Examples of naked monoclonal antibodies[16]

Example	Mechanism of action	Description
Alemtuzumab	Attaches and recruits immune cells to kill tumour cells	• For chronic lymphocytic leukaemia • Target CD52 protein
Trastuzumab	Attaches and inhibits signalling pathway for tumour growth	• For HER2-positive breast cancer • Target HER2 protein

Trastuzumab is used as adjuvant chemotherapy for breast cancer patients with HER2-positive subtype (account for 20 – 30% for overall breast cancer incidence). Studies have shown that trastuzumab significantly contributed towards improvement in patient's outcome and cost-effective in long term. Valachi et al. in their study demonstrated that treatment regimen consisting of trastuzumab and chemotherapy for HER-2 positive breast cancer, gave higher therapeutic outcome in term of pathological response rate (38%) compared to chemotherapy alone (21%).[17] Although, initially, this treatment regimen could increase the treatment cost, however in the long run it is proven to be more cost-effective.[18]

B. Conjugated Monoclonal Antibodies

Conjugation of monoclonal antibodies with active substances such as chemotherapy drugs, radioactive particles or toxins provides transport mechanism for the drug to reach the specific target. It increases the efficiency of drug delivery avoiding toxic effects on normal cells (Table 5). Conjugated monoclonal antibodies are also useful for study of distribution of specific tissue in the body. For example, monoclonal antibody-213-immunoreactive (Mab 213-I) has been used to detect the details distribution of immuno-reactive olfactory and glomeruli cells in the rat olfactory system.[19]This is based on the Mab 213-I immune reaction against TGFα; an antigen that also highly expressed in variety of cancer cells. Thus, it could potentially provide a basis for better detection of cancer cells distribution.

Table 5 Different forms of conjugated monoclonal antibodies[10, 16]

Type	Example	Description
Chemolabelled	Brentuximab vedotin	• Conjugated to chemotherapy • Target CD30 protein • For refractory Hodgkin's Lymphoma
Radiolabelled	Ibritumomab tiuxeta	• Attached with small radioactive particles • Target CD20 antigen • B cells carcinoma, Non-Hodgkin Lymphoma
Immunotoxins	Moxetumomab pasudotox	• Conjugated with anti-CD-22 exotoxin • Target specific antigens on surface cancer cells • Under clinical trials for B-cell malignancies

C. Bi-specific Monoclonal Antibodies

Two distinct types of monoclonal antibodies were bind together to two different types of cancer surface antigens. Blinatumomab as example, targets CD19 protein on leukaemic/ lymphoma, while cells with another antibodies target CD3 on T-cells. Direct target for both proteins initiates a greater immune response attack against the tumour cells.[20]

Adverse Effects of Monoclonal Antibodies

Generally, monoclonal antibodies cause less adverse effects in comparison to conventional treatment such as chemotherapy. The potential adverse effects of monoclonal antibodies associated with the possibility of triggering immunological reaction following the therapy. It is relatively uncommon. However, monoclonal antibodies may prompt type I immune reaction (anaphylactic), mediated by IgE antibodies. Immediate immunologic reaction may affect specific organ and present with symptoms of allergic rhinitis, conjunctivitis, angioedema, asthma, urticarial, eczema, etc. or could affect multiple organs leading to anaphylactic shock.[14] Administration of prophylactic antihistamine prior to infusion can prevent immediate hypersensitivity.

Type II hypersensitivity also can occur in treatment with monoclonal antibodies. The patient may develop depletion in number of platelets, white blood cells and anaemia due to haemolysis. Patients also risk for type III characterized by vasculitis, serum sickness and respiratory problems. In delayed hypersensitivity (type IV), tumour lysis syndrome or cytokines release syndrome may occur.

II. Cancer Vaccines

Cancer vaccines can be classified either as prophylaxis or therapeutics (Table 6).

Table 6 The classification of cancer vaccines[12]

Vaccine type	Name of agent	Indication
Preventative	Hepatitis B virus vaccine	• Hepatocellular carcinoma
	Human papilloma virus vaccines; Gardasil and Cervarix	• Cervical cancer
Therapeutic	Vitespen	• Melanoma and locally renal cell carcinoma • Prostate cancer
	GVAX	• Advance metastatic prostate cancer
	Sipuleucel-T (Provenge) ProstVac-VF	• Prostate cancer
	BiovaxID	• Non-Hodgkin's lymphoma

A. Prophylactic Cancer Vaccines

Certain chronic and persistent viral infection can predispose the development of cancers in human. For example, chronic Hepatitis B and C virus infection predispose human for development liver cancer while Human Papilloma Virus (HPV) infection contributes for development of cervical cancer.[21] Vaccination against these infections could provide protection against development of these cancers.

Another approach of cancer vaccine is to prevent the recurrence rather than occurrence of a cancer. A personalised vaccine is formulated according to patient tumour's mutant protein and indicated to patient who had received surgery and at high-risk of cancer recurrence. Several studies have demonstrated that it is effective in preventing cancer recurrence and inducing complete remission.[22]

HPV vaccines for Cancer of Cervix

Cervical cancer is one of the most common cancers among women. The incidence of cervical cancer is almost 500,000 worldwide and responsible for 250,000 casualties every year.[23] Seventy per cent of overall cervical cancer is associated with HPV 16 and 18 infections. These infections also linked to the development of cancers of anus, vulva, vagina, and penis.[24]

Gardasil and Cervarix are the two examples of HPV vaccines currently available in the market. Gardasil consists of purified proteins of 4 subtype of HPV (HPV 6, 11, 16, 18). Cervarix is made up of purified proteins of 2 subtype of HPV 16 and 18.[25, 26] Exposure to these proteins provokes the immune response and production of antibodies. These antibodies will provide protection against active infection and enhance immune attacks when active infection is present.

HPV vaccines can effectively reduce the incidence of cervical cancer in young women, especially when given at their early age or before sexually active.[27] HPV vaccines can provide up to 98% preventive efficacy against cancer of cervix caused by HPV 16 and 18.[28] Vaccination for HPV should not be limited to women. HPV vaccines is also recommended to men as it provides protection against development of genital warts, which is one of the predispose condition for genital cancers, and to reduce the risk of spread of infection from men to women.[29]

B. Therapeutic Cancer Vaccines

Therapeutic cancer vaccine is relatively a novel concept. It differs from prophylactic vaccines as therapeutic cancer vaccine is used to direct immune system attack against pre-existing tumour cells. Currently, there are many therapeutic cancer vaccines under active researches. Sipuleucel-T is the only therapeutic cancer vaccine received approval from US FDA (since 2010).

The indication of Sipuluecel-T is for treatment metastatic hormone refractory prostate cancer.[30] It mainly consists of autologous peripheral blood mononuclear cells (PMBCs). PMBCs are exposed to prostatic acid phosphatase linked to GM-CSF (PAC-GM-CSF), to provoke immune system attack against prostate malignant cells. A multicentre, double blind placebo-controlled phase 3 clinical study has shown that Sipuluecel-T significantly prolonged overall survival and death risk reduction among hormone refractory prostate cancer patients.[31]

Another potential therapeutic vaccine for prostate cancer is ProstVac, an investigational candidate currently in phase 3 study. It is a vector-based vaccine regimen consists of transgenes for prostate-specific antigen and multiple T-cell co-stimulary molecules (PSA-TRICOM). It works by disrupting the immunological tolerance to PSA and mediates a strong immune response against prostate cancer cells.[32, 33] Preliminary study has shown that this vaccine produced significant immunologic reactivity with negligible toxicity while phase 2 study has demonstrated the overall patient survival benefit.[34]

III. Immune Adjuvants and Cytokines

A. Immune Adjuvants

Immune adjuvant is a non-specific immunotherapy, which instead of targeting tumour cells directly, it works as a booster for immunological reaction against malignant cells. This will lead to reduction in tumour growth in otherwise would not regress by normal immune responses.[8, 16]

Intravesical Bacille Calmette-Guerin (BCG) is an example of effective adjuvant to surgery for superficial or carcinoma-in-situ (CIS) bladder cancer. It contributes towards reduction of cancer growth and prevention of recurrence – reducing more than 20% of cancer growth and up to 40% of recurrence rate in comparison to surgery alone treatment.[35] Other studies also support the finding, which shows that the use of adjuvant BCG increases 10-year progression-free rate by 60%.[36, 37]

The mechanism of action of BCG is through induction of local immunological system, principally mediated by T-helper cells responses. The most efficient regimen of BCG as adjuvant therapy is initial 6 weeks treatment followed by one-year maintenance course. Though it is effective, the use of BCG as adjuvant therapy for bladder cancer can be limited for its potential adverse effects on urinary system (patient complained of dysuria, urgency and frequency). Thus, to optimise the treatment it is important to manage these adverse effects properly.

B. Cytokines Immunotherapy

Immune cells secrets glycoproteins known as cytokines. Cytokines are important proteins that help in regulating activity of immune cells as well as tumour growth. One of the examples of laboratory-made cytokines is Interleukins-2 (IL-2). IL-2 has been approved for treatment of melanoma and renal cell carcinoma, particularly useful when the cancer is refractory towards conventional treatment.[8, 16] It can be administered as a single or as combined therapy with interferon-alpha to improve the efficiency. Adverse effects of IL-2 include fever, chills, malaise, gastrointestinal symptoms, weight gain, and rare but serious, cardiovascular toxicity.

Interferon alpha (IFN-alpha) is the most useful interferon therapy for cancer. It is a form of cytokines which build from 150 amino acids. IFN-alpha works by binding to immune cells surface receptor and mounting an immune reaction towards malignant cells. This is achieved by several means; promoting B and T cells activity and upregulating gene like MHC Class 1, tumour antigen and adhesion molecule against cancer cells[38]. IFN-alpha also exhibits anti-angiogenic activity as well as interfering the cell division causing the shrinkage in tumour growth.

IFN-alpha is indicated for various cancers including melanoma, renal cancer, AIDS-related Kaposi's sarcoma, haematological cancer such as hairy cell leukaemia and follicular non-Hodgkin's lymphoma. Adverse effects of

interferon alpha include flu-like symptoms in initial week of therapy, gastrointestinal disturbances, headache, skin rashes, thinning hair, pancytopaenia and increased risk of autoimmunity.[39] These side effects are dose-related and can be severe, and one of the limiting factor for its usage.

IFN-alpha as adjuvant therapy particularly useful in early stage or locally infiltrating cancer. In a study conducted by Kirkwood and colleagues, it has been shown that IFN-alpha prolongs the relapse free and overall survival of patient with high-risk resected melanoma.[40]

GM-CSF is another example of cytokine therapy. It is frequently being used as a form of supportive treatment after chemotherapy. This therapy is usually done as complimentary to stem cells or bone marrow transplant to replenish the myeloid series. Study of the potential use of GM-CSF as combination therapy for melanoma also has been carried out. A multicentre, phase II trials of treatment of metastatic melanoma with GM-CSF and ipilimumab vs ipilimumab alone has found that the combination therapy had significantly prolonged overall survival rate of the patient.[41] Among side effects of GM-CSF include fever, nausea, vomiting, skin rash and bone pain.

IV. CAR T-cell Therapy

Chimeric antigen receptors (CAR)-T cell therapy is a newer and promising approach of immunotherapy currently under development. It involves a procedure called adoptive cell transfer (ACT). In this procedure, the patient's blood will be withdrawn and filtered for T-cells. These T-cells then will undergo genetic modification to be attached with chimeric antigen receptors (CARs) to specific cancer. These new genetically engineered T-cells will be multiplied before re-administered to the patients' circulation. Inside the circulation, these cells will proliferate and further amplify immune response, thus providing better clinical outcome than conventional therapy.[42]

One potential disease studied with CAR T-cell therapy is acute lymphoblastic leukaemia (ALL). A series of clinical trials of an investigational immunotherapy CD19-specific CAR-T cells (CTL019) demonstrated that paediatric and young adult with relapse/refractory ALL achieved complete remission, prolonged persistence and sustained response after the treatment.[43] In July 2017, CTL019 had received recommendation from US Food and Administration Advisory Committee for approval; set to become the first commercially available CAR-T cell therapy.[44]

CHALLENGES OF CANCER IMMUNOTHERAPY

The ability of cancer cells to evade immune attack; either through intrinsic or extrinsic mechanism is one of the biggest obstacles in cancer immunotherapy. The intrinsic mechanism includes causing antigen or MHC loss, release of immunosuppressive cytokines, or expressing marker that can interrupt T-cell function such as programmed-death-receptor-1 (PD-L1). Whereas, the extrinsic factors which help cancer cells to survive include formation of physical barrier for the drug in reaching the target and existence of regulatory immune cells such as regulatory (Treg) in tumour microenvironment which able to reduce the immune responses against cancer cells.[45]

One approach to overcome this challenge and improving the treatment outcome of immunotherapy is through inhibition of immune-inhibitory pathway activated by cancer cells, known as "checkpoint blockade". Anti-CTLA-4 antibodies (ipilimumab) act by down-regulating the initial stages of T-cell activation, an initial target for checkpoint antibodies[46] whereas, anti-PD-1antibodies (pembrolizumab) inhibit the expression PD-1 which responsible for downstream signalling to inhibit T-cells proliferation[47]. These drugs have received FDA approval for treatment of metastatic melanoma and other various cancer conditions.

CONCLUSION

Better understanding of human own immunological system has led to discovery of immune-based therapy for cancer. Recruiting and manipulating human own immune system has become the basis of development of immunotherapy against cancer cells. Various kinds of immunotherapy have received market approval over the years; monoclonal antibodies, prophylactic and therapeutic tumour vaccines, immune adjuvants and cytokines are among the examples. The effectiveness of immunotherapy in treating cancer has been established through many clinical studies. Immunotherapy improves the overall survival rate of the patient and reduces cancer recurrence. Immunotherapy superior to conventional treatment for being more target-specific, cause less adverse effects, better tolerability to the patients and cost-effective for long-term usage.

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Comparison of Knowledge about Voluntary Blood Donation among the Medical and Non-medical Students of Universiti Malaysia Sabah

M. Tanveer Hossain Parash[1*], Thiruchelvam Ayudurai[2], Krishna Dilip Murthy[1], Sadia Choudhury Shimmi[1], ABM Tofazzal Hossain[3], Bablu K Ghosh[4]

[1] Department of Biomedical Science & Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

[2] Department of Pathobiology & Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

[3] Department of Surgery Based Discipline, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

[4]Electrical & Electronics Engineering, Faculty of Engineering, Universiti Malaysia Sabah, Sabah, Malaysia

*Corresponding author's email: parash_cmc@ums.edu.my

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ABSTRACT

The huge population of persuadable, active, healthy and young students is the potential as blood donors to meet safe blood donation. This study focused on the existing level of knowledge regarding voluntary blood donation among medical (Faculty of Medicine and Health Science) and nonmedical students with science background (Faculty of Engineering) at the Universiti Malaysia Sabah. Four hundred and fifty-five students consisting of medical (231) and nonmedical (224) were selected through stratified random sampling to participate in the study. Data was collected using validated structured questionnaire. Statistical analysis was performed by structural equation modelling using SPSS AMOS Graphics version 22 and SPSS version16. The results showed a significant ($p<0.05$) difference between the students of medical and nonmedical faculty in their knowledge about voluntary blood donation. The medical and nursing students are more aware about blood donation than the nonmedical students.

Keywords: voluntary blood donation, medical students, non-medical students.

INTRODUCTION

Availability of safe-blood and blood products is a critical component in improving health care.[1] The demand for blood and blood products in most countries continues to increase because of the rise in human lifespan expectancy and the implementation of new aggressive surgical and therapeutic methods requiring copious quantities of blood and blood products.[2] To meet up the ever increasing clinical requisite for whole blood and blood derivatives, and to sustain self-sufficiency, continuous effort needs to be made.[3]

Regular blood donation by the voluntary donors can assure an adequate and reliable supply of blood. As the prevalence of blood-borne infections is lowest among this group, so they are considered to be the safest group of donors. World Health Assembly resolution (WHA 63.12) urges all member countries to develop their national blood systems depending on voluntary donation and work to achieve the goal of self-sufficiency.[4]

Compared to the international standards that, 5% of a country's population should be blood donors, it was found to be only 2.5% of Malaysians in 2014.[5] The Malaysian National Blood Centre recognized that, most of the states faced the difficulty in getting blood donors. The problem even worsen during the festivals. Although blood donation activities were organized everywhere, minimal participation was recorded.[6] Thus, there is a definite need for taking various initiatives to increase the awareness on blood donation among Malaysians.

The students are a huge proportion of persuadable, active, healthy and young population of a country. They are the potential source of blood donors to meet safe-blood requirements of the country.[7, 8] To be able to utilize this invaluable source of safe-blood, it is relevant to have baseline data about their knowledge in respect of voluntary blood donation.[9]

There is a paucity of literature on the knowledge about voluntary blood donation among Malaysian students. Hamid et al. (2013) studied factors influencing blood donation among 18 – 50 years old age groups.[6] Roshan et al. (2009) studied the response rate of Malaysian blood donors of different age groups with reactive screening test to transfusion medicine unit calls.[10] The present study focuses on the existing level of knowledge about voluntary blood donation among medical (MD medicine and Diploma nursing of Faculty of Medicine and Health Sciences) and non-medical students from science background (BSc engineering students of Faculty of Engineering) at Universiti Malaysia Sabah.

RESEARCH METHODOLOGY

A cross-sectional study was conducted between July 2015 and June 2016 at Faculty of Medicine and Health Science (FMHS) and Faculty of Engineering (FKJ), Universiti Malaysia Sabah (UMS). Ethical permission [(JKEtika3/15(7)] was obtained from the ethical committee of UMS.

A total number of four hundred and fifty-five students (FMHS = 231 and FKJ = 224) who fulfilled the inclusion criteria (students of medical faculty, students of non-medical science faculty, age ranged from 18 – 22 years) were selected through stratified random sampling to participate in the study. For the objective, comparing knowledge of two diverse groups of students (two means), we required a sample size of 220 (n_1) for the FMHS students and a sample size of (n_2) for the FKJ students to detect the mean difference of 0.20 with a power of 0.85 (85%) and an alpha of 0.05. The mean difference of 0.20 was considered smallest significant difference to be detected. The SD of (the variable of interest) was estimated as 0.50. This calculation was done using ScalexMean version 1.0.2.[11]

After obtaining informed consent from each participant, data was collected using standardized structured questionnaire. The questionnaire was constructed after review of the literature on similar studies. The framework was based on the World Health Organisation (WHO) manual Methodological guidelines for socio-cultural studies on issues related to blood donation 2005[12] and transfusion practice guidelines for clinical and laboratory personnel by National Blood Centre, Ministry of Health, Malaysia, 3rd edition, 2008.[13] Briefly, the questionnaire consisted of five sections with 42 multiple choice questions regarding subject demographics, knowledge, attitude, experience and practice of blood donation.

Statistical analysis was performed by structural equation modelling using SPSS AMOS Graphics version 22[14] and SPSS version 16[15].

RESULTS

Among the participants, 310 were female, and 145 were male. Of the 50.77% participants from the students of FMHS, 40% were from different years of the MD Medical Course and 10% were from Diploma of Nursing. Of the rest 49.23% from Faculty of Engineering, with Electrical Engineering 14%, Computer Engineering 14%, Mechanical Engineering 8%, Chemical Engineering 8% and Civil Engineering 4%.

The medical students could answer more correctly compared to non-medical students in response to questions number 1 to 7 and the non-medical medical students could answer more correctly in response to question number 8 (Figure 1).

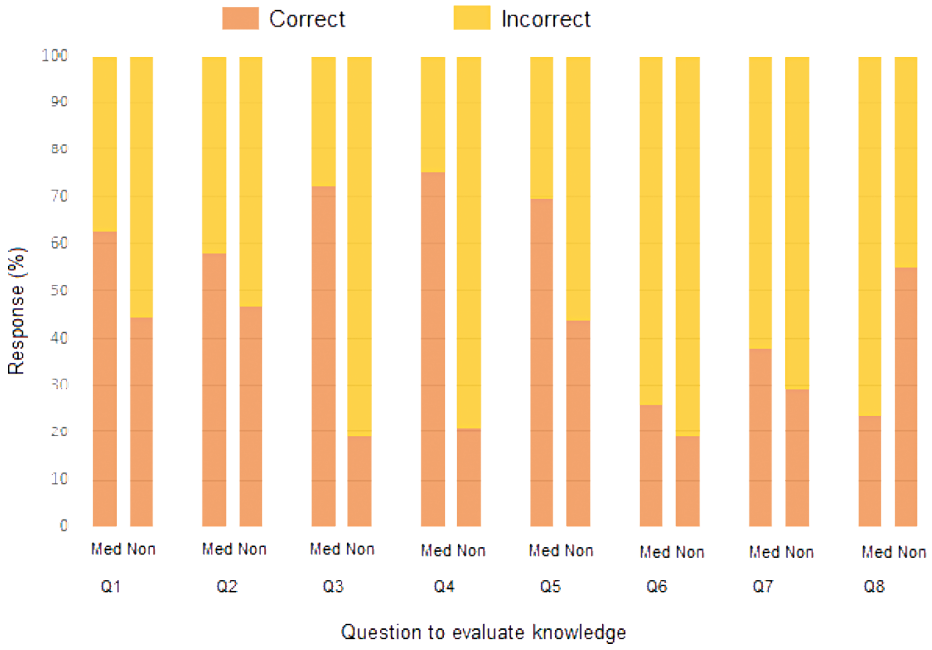

Figure 1 Distribution of responses of the medical and non-medical students in respect to questionnaire to evaluate their knowledge regarding voluntary blood donation (n = 455)

There were apparent differences between the medical and non-medical students with respect to knowledge regarding the lowest age, lowest body weight, lowest haemoglobin level and ideal blood pressure of a blood donor, the frequency of donating blood, criteria for not being eligible for donating blood and volume of blood collected during each blood donation (Table 1).

The sources for their information were their school, college, and universities (33% for non-medical students, 43% for medical students), followed by the internet (23% for non-medical students, 13% for medical students); while 5% non-medical students, 10% medical students knew from all possible sources (Figure 2).

Table 1 Comparison of knowledge about voluntary blood donation between the participants of different faculties (n = 455)

Item	Faculty	n	Mean (± SD)	Mean difference	95% CI		t	df	p-value
					Lower	Upper			
The lowest age for donating blood (Q1)	Medical	231	1.62 (± .49)	0.18	.09	.27	3.93	451.61	<.001**
	Non-medical	224	1.44 (± .50)						
The lowest body weight of a blood donor (Q2)	Medical	231	1.58 (± .50)	0.11	.02	.20	2.39	452.20	0.017*
	Non-medical	224	1.47 (± .50)						
The lowest level of haemoglobin of a blood (Q3)	Medical	231	1.72 (± .45)	0.53	.45	.61	13.42	448.83	<.001**
	Non-medical	224	1.19 (± .40)						
The recommended blood pressure of blood donor (Q4)	Medical	231	1.75 (± .43)	0.54	.47	.62	13.80	452.69	<.001**
	Non-medical	224	1.21 (± .41)						
The frequency of donating blood (Q5)	Medical	231	1.70 (± .46)	0.26	.17	.35	5.77	447.86	<.001**
	Non-medical	224	1.44 (± .50)						
The volume of blood collected during each donation (Q6)	Medical	231	1.26 (± .44)	0.07	-.01	.14	1.73	450.33	0.084ns
	Non-medical	224	1.19 (± .40)						
The duration of a blood donation process (Q7)	Medical	231	1.38 (± .49)	0.08	-.00	.17	1.95	452.53	0.052ns
	Non-medical	224	1.30 (± .46)						
Criteria for not donating blood (Q8)	Medical	231	1.24 (± .43)	-0.31	-.40	-.22	-7.24	438.18	<.001**
	Non-medical	224	1.55 (± .50)						

*Significant at .05 level, **Significant at .001 level, ns = Non-significant at .05 level

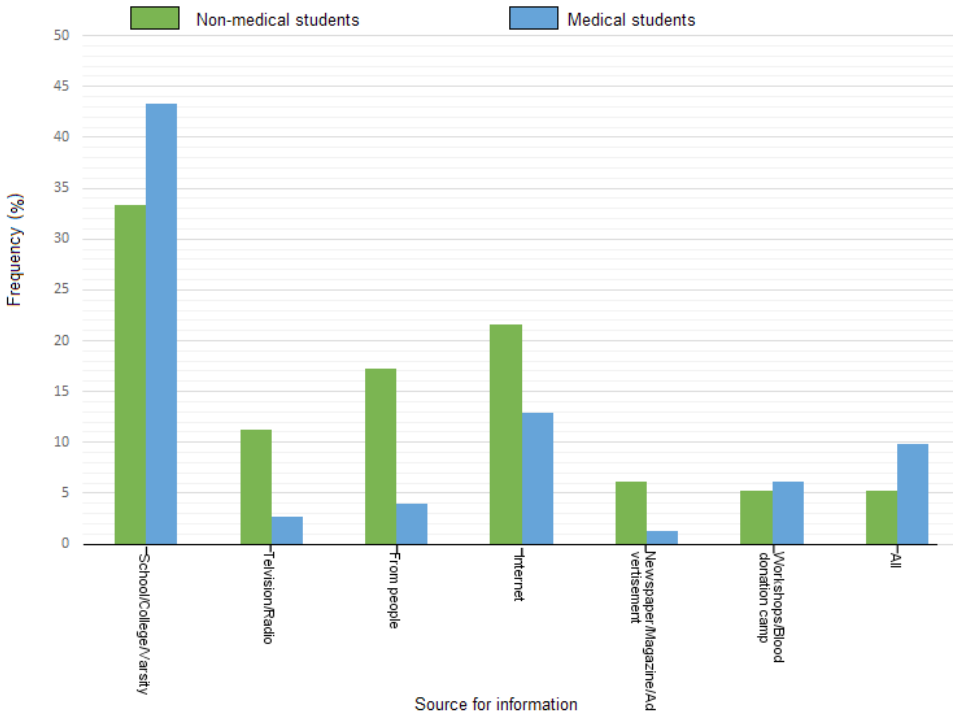

Figure 2 Sources for information about voluntary blood donation ($n = 455$)

DISCUSSION

Findings of the study show that the medical students had significantly ($p < 0.05$) more knowledge about knowledge regarding the lowest age, lowest body weight, lowest haemoglobin level and ideal blood pressure for a blood donor and the frequency of donating blood than the non-medical students. The non-medical students had significantly ($p < 0.05$) more knowledge about the criteria for not being eligible for donating blood than the medical students. There was no significant ($p > 0.05$) difference in knowledge regarding volume of blood collected during each blood donation and duration of blood donation process.

Kumari and Raina explored the knowledge, attitude and practices of the college students of Jammu, India regarding voluntary non-remunerated blood donation.[8] Of the 1520 college students, 210 were blood donors.[8] In this study 81.57 % of students were aware of voluntary blood donation; 62.5% of the students had awareness regarding spread and transmission of HIV/AIDS, and 76.68% of the students had knowledge that blood donation has medical benefits.[8]

Sabu et al. conducted a cross-sectional study to determine the knowledge and attitude about blood donation among science students from different faculties in a University Campus of South India.[7] Of 410 students, the overall knowledge on blood donation was good, but majority (62%) of students never donated blood. Knowledge level of health science students (53.1%) was the highest.[7]

Thus, the findings of this study correspond to those of Kumari and Raina (2015) and Sabu et al. (2011).

CONCLUSION

The medical and nursing students study blood in their curriculum. It is predictable that their knowledge would be more about blood donation than the non-medical students. Knowledge

about blood donation should not be confined to medical students only. Everyone should know his own blood group and some basic information about blood donation. Measures for increasing knowledge regarding blood donation should be considered for non-medical students. The measures might vary from talks on blood donation, poster exhibition, pamphlet distribution, organizing blood donation campaign on a regular basis under an organization. Since the study was confined to only two science-based faculties, this study should further be extended to all the faculties of Universiti Malaysia Sabah to get the complete scenario of the awareness and knowledge regarding voluntary blood donation. This would be a step towards attaining self-sufficiency in safe blood donor for Sabah.

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Research Article

Emergence of ST131 H30-Rx Subclones among Uropathogenic *Escherichia* coli Isolates from Two Large Hospitals of Kota Kinabalu, Sabah, Malaysia

Yun Mei Lai, Myo Thura Zaw, Nor Amalina Emran, Zaw Lin*

Department of Pathobiology and Medical Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.

*Corresponding author's email: zawlin@ums.edu.my

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ABSTRACT

***Escherichia coli* sequence type 131 (ST131) carries multiple drug resistance (MDR) genes as well as virulence genes. Drug resistant characteristics give a management problem to health care personnel. Four MDR *Escherichia coli* ST131 H30-Rx subclones were identified among 80 Uropathogenic *E. coli* (UPEC) isolates by using 4 allelic-specific Polymerase Chain Reactions (PCR) in two hospitals of Kota Kinabalu, Sabah, Malaysia. There is emergence of multidrug resistant *E. coli* in Kota Kinabalu.**

Keywords: Uropathogenic *E. coli*, ST131 H30-Rx subclones, multiple drug resistance, hospitals of Kota Kinabalu

INTRODUCTION

E. coli ST131 is a worldwide pandemic clone of *E. coli*, causing multiple antimicrobial-resistant infection.[1] Multi-locus sequence typing (MLST) examines the nucleotide sequences of several (i.e. 6 – 10) housekeeping genes for the molecular epidemiological typing of bacterial pathogens.[2, 3] MLST identifies the pandemic spread of CTX-M-15 extended-spectrum beta-lactamase (ESBL)-producing *E. coli* from three continents in 2008. Previous studies have confirmed the worldwide prevalence of ST131 and it carried a broad range of virulence and resistance genes on a transferable plasmid.[1] Regarding *fim*H gene responsible for adhesion of *E.coli* to host cells, three types of *fim*H alleles are well known for ST131 clones. H30 is associated with O25b serotypes while H41 is associated with O16 serotypes and H22 is sensitive to ciprofloxacin and the serotype is O25b.[4] The term H30-R is used for ciprofloxacin resistant isolates.[5]

Hundred per cent of $bla_{CTXM-15}$ producer *E.coli* isolates were included in the H30Rx subclones and these can give rise to healthcare associated (HCA) episodes.[6] In this $bla_{CTX-M\ 15}$ nomenclature: '*bla*' is the beta-lactamase gene, 'CTX-M' is the sub-family and cefotaximase from Munich and '15'is the specific variant.[7] H30-Rx is resistant to at least third generation cephalosporin (3GC) and ciprofloxacin.

Allelic-specific PCR is the method applied for selected amplification of one allele among many alleles of one gene. Although there are seven house-keeping genes to be amplified in MLST to identify each ST, the two genes (*mdh* and *gyr*B) have 2 or 3 single nucleotide polymorphisms (SNPs) in ST131. Using these SNPs, forward primers and reverse primers are designed so that ST131 can be identified by adjusting the PCR conditions.[8] The same concept can be usable in H30 allele and CTX-M15 to be amplified out of various fimH alleles and CTX-M alleles respectively.

In this study, we tried to detect ST131, H30-Rx subclones by the principle of allelic-specific PCR so that the physicians of hospitals of Kota Kinabalu were aware of these highly pathogenic bacteria.

MATERIALS AND METHODS

Samples

Eighty isolates of UPEC from two main hospitals of Kota Kinabalu, Sabah, Malaysia namely Hospital Queen Elizabeth (47 isolates) and Hospital for Women and Children (33 isolates) were included in this study. Sample collection

was done from the period of 1 January to 30 April of 2016.

Antibiotic Susceptibility Tests

The isolates were studied for the antibiotic susceptibility tests by disc-diffusion method with 10 antibiotics discs namely ciprofloxacin, cefotaxime, cotrimethoxazole, gentamicin, cefuroxime, imipenem, ceftazidime, cephalothin, piperacillin and ampicillin. Antibiotic susceptibility test was performed as mentioned in the Clinical and Laboratory Standards Institute guidelines.[9]

Allelic-specific PCRs for Detection of ST131

Those isolates with drug resistant patterns to at least two antibiotics ciprofloxacin and third generation cephalosporin (3GC) were investigated for *mdh* and *gyrB* allelic-specific PCR. The primer sequences for forward and reverse primers and expected amplicon size together with PCR conditions were mentioned in Table 1.[8] The bacterial DNA from bacterial suspension in L-broth incubated overnight at 37°C was denatured by a boiling method for 10 min in boiling water bath, 5 ***µl*** of template DNA was added to PCR reaction mixture containing 2 ***µl*** of 100 pmol each primer, 1 ***µl*** of dNTPs 20 mmol, 2.5 ***µl*** of 10× buffer, 1.25 unit of Taq polymerase (Takara Bio Inc, Shiga, Japan) and PCR was done in a thermocycler (Applied Biosystems, Foster City, USA). The size of PCR product was checked by gel documentation apparatus Alpha Imager® HP System after it was run in 1.5 % agarose gel and stained by florosafe.

Table 1 Primer sequences, PCR conditions, Amplicon sizes of four allelic specific PCR for investigations of ST131 H30-Rx

Target gene	Primer sequences (References)	PCR conditions	Amplicon size
mdh	F:5'– GTT TAA CGT TAA CGC CGG T-3' R:5'– GGT AAC ACC AGA GTG ACC A-3'[9]	94°C – 5m 30×: 94°C – 30s, 58°C – 30s 72°C – 10m, 4°C –∞	275 bp
***gyr*B**	F:5'– CGC GAT AAG CGC GAC -3' R:5'– ACC GTC TTT TTC GGT GGA A -3'[9]	94°C – 5m 30×: 94°C – 30s, 58°C – 30s 72°C – 10m, 4°C –∞	132 bp
fim*H *30	F:5'– CCG CCA ATG GTA CCG CTA TT -3' R:5'– CAG CTT TAA TCG CCA CCC CA -3'[11]	95°C – 8m 37×: 94°C - 20s, 65°C – 45s 72°C – 5m, 4°C –∞	354 bp
***bla*$_{\text{CTX-M 15}}$**	F:5'– ATA AAA CCG GCA GCG GTG G -3' R:5'– GAA TTT TGA CGA TCG GGG -3'[7]	95°C – 8m 37×: 94°C –30s, 65°C –45s 72°C –5m, 4°C –∞	483 bp

Confirmation by MLST using Achtman Scheme

Confirmation of ST131 was done for positive isolates by Achtman Scheme using PCR of seven house-keeping genes *adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A and *rec*A and sequencing of these amplicon as described in Wirth et al.[10]

Allelic-specific PCR for *fim*H30 Allele

The procedure and ingredients of PCR were same as above. The primer sequences, PCR conditions and expected amplicon sizes for *fim*H30 allele and $bla_{CTX\text{-}M15}$ allele were mentioned in Table 1.[7, 11]

Allelic-specific PCR for $bla_{CTX\text{-}M\ 15}$

For the H30-Rx subclones, $bla_{CTX\text{-}M15}$ was investigated by fourth allelic specific PCR. Ingredients of PCR were the same as mentioned in *mdh* and *gyr*B PCR.

RESULTS

Four isolates were positive for both alleles (*mdh* and *gyr*B) in the identification of ST131, *fim*H30 and $bla_{CTX\text{-}M15}$ allelic specific PCRs. The characteristics of four isolates which were observed to be ST131 H30Rx including antibiotic resistance patterns, results of four allelic specific PCR, zone of inhibition in ciprofloxacin resistance were shown in Table 2. The gel electrophoresis picture of two allelic-specific PCRs for *fim*H30 and $bla_{CTX\text{-}M15}$ is shown in Figure 1 and Figure 2 respectively.

The significant observation in this study was that all the ST131 H30Rx positive cases were female patients and age of over 60. Of four positive isolates, three were from Hospital for Women and Children where the number of old women patients hospitalized in this institution is more than Hospital Queen Elizabeth.

Table 2 Characteristics of four UPEC isolates observed to be ST131 H30-Rx in this study

Isolate No.	Resistant Antibiotics	*mdh*	*gyr*B	H30 allele	H30R (determined by ciprofloxacin resistant)	H30-Rx allele
Q5	*CIP, CTX TMP-STX, GM, CXM, CAZ, KF, PRL, AMP	+	+	+	Inhibition zone size in ciprofloxacin susceptibility test = 6 mm -	+
W19	CIP, CTX TMP-STX, CXM,CAZ, KF, PRL, AMP	+	+	+	ll -	+
W43	*CIP, CTX TMP-STX, CXM, CAZ, KF, PRL, AMP	+	+	+	ll	+
W47	*CIP, CTX TMP-STX, CXM, CAZ, KF, PRL, AMP	+	+	+	ll	+

*Short terms of antibiotics studied in the disc-diffusion method - ciprofloxacin = CIP, cefotaxime = CTX, cotrimethoxazole = TMP-STX, gentamicin = GM, cefuroxime = CXM, imipenem = IMI, ceftazidime = CAZ, cephalothin = KF, piperacillin = PRL and ampicillin = AMP

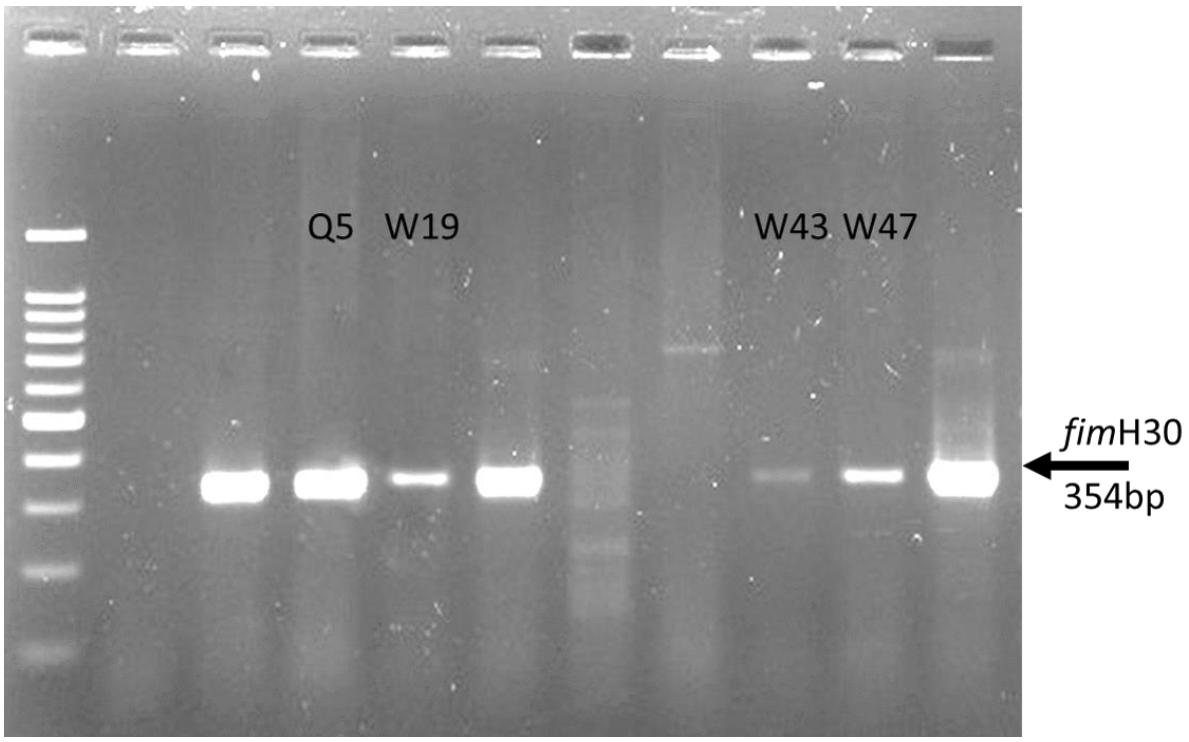

Figure 1 The gel electrophoresis picture of allelic-specific PCR for *fim*H30
Seven isolates including Q5, W19, W43, W47 were positive for PCR product showing 354bp band. The molecular marker used in the study was 100 bp ladder.

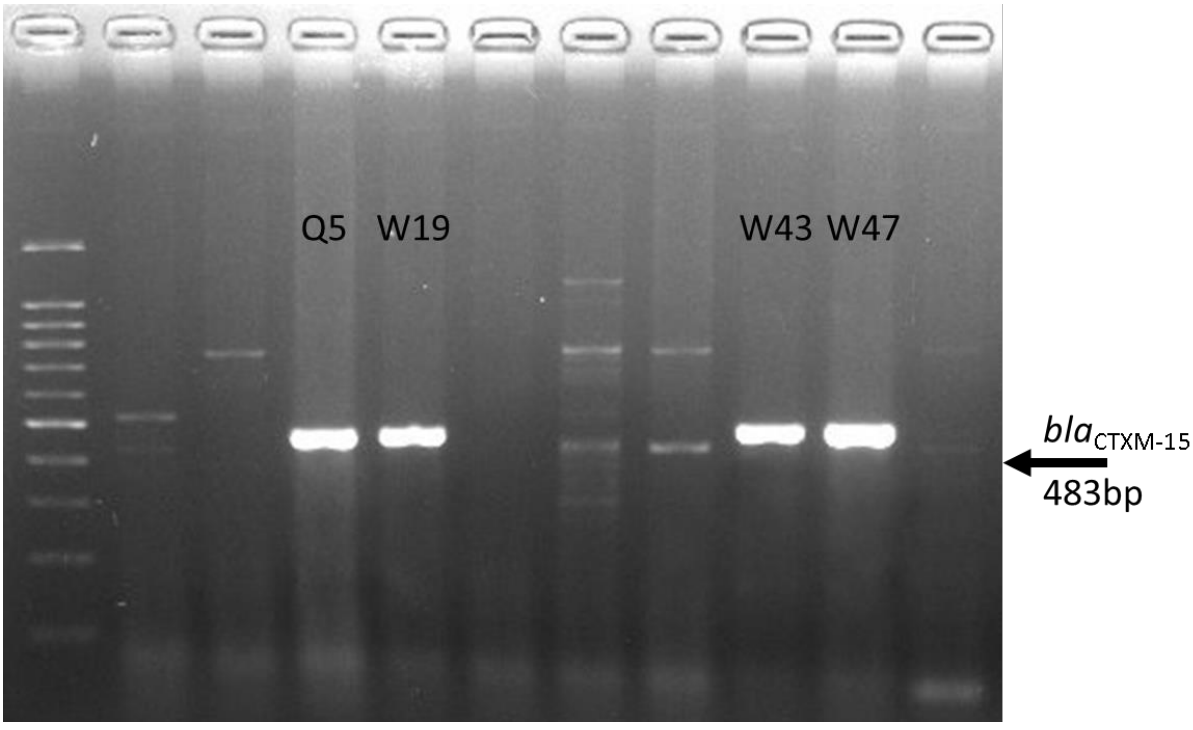

Figure 2 The gel electrophoresis picture of allelic-specific PCR for $bla_{\text{CTX-M 15}}$
Isolates no.Q5, W19, W43, W47 were positive for PCR product showing 483bp band. The molecular marker used in the study was 100 bp ladder.

DISCUSSION

Pulsed field gel electrophoresis (PFGE) is considered the gold standard for the outbreak investigations of the *E. coli* pathotypes.[12,13] This method is based on principle of restriction fragment length polymorphisms after digestion of whole genome by restriction enzymes. PFGE is excellent for molecular typing of *E. coli* responsible for recent outbreaks. However, it is time consuming, labour-intensive and requires technical experts.[13] In addition, there is a problem of comparison of data generated in different laboratories. MLST is an ideal method for the study of molecular epidemiology of antimicrobial resistant bacteria including *E. coli* pathotypes. Sequence types (STs) can indicate common ancestry lineages among bacteria. It is possible to compare data generated in different laboratories.[2, 3] However, MLST, according to Achtman Scheme, needs to do seven PCR and seven DNA sequencing for testing each isolate.[10] Due to this technical workload, researchers tried to find the allelic specific primers after comparison of DNA sequences specific to ST131 and other STs. The results pointed out *mdh* gene and *gyr* B gene were consistently specific in SNPs and consequently allelic specific PCR of these two genes were recently applied for detection of ST131 clones.[9]

Information on ST131's geographical distribution is incomplete. Analysis on previous studies has shown it has distributed as a human infection in Europe, North America, Canada, Japan and Korea. There was limited data from Asia, the Middle East and Africa[1]. The clone is also detected in companion animals, non-companion animals and foods. Urinary tract infection was predominant in human infections and life threatening sepsis was a serious complication[1]. Phenotypic detection of the ST131 clone is not possible and Genotyping was done by DNA-based techniques including MLST and PCR to identify known single nucleotide polymorphisms, which are the method of choices[1]. Whole genome sequencing is the latest method for the detection of this pathogen.

In Malaysia including Sabah state, there was no previous study on this globally disseminated pathogen, *E. coli* ST131 multi-drug resistant strains although there were two studies from University of Malaya which studied on samples from Pune, India.[14, 15] The other study was done on Malaysian isolates but the researchers have investigated extended-spectrum beta-lactamase (ESBL) producing *E.coli* and not multi-drug resistant strains including H30-R.[16] The current study is the first attempt to detect highly resistant H30-Rx subclones of ST131.

This kind of molecular epidemiological study is necessary to be done in the future as a timely manner because spread of ST131 is increasing year by year. The physicians should be aware of this pathogen which causes treatment failure, prolonged stay in hospital, bacteraemia of urinary origin and fatality due to disseminating sepsis.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Newly-developed De Cordova's Formula for Calculation of LDL Cholesterol in Bangladeshi Population

Nadia Nasrin[1], Md. Mozammel Hoque[2]
[1]Sir Salimullah Medical College, Dhaka, Bangladesh
[2] Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
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ABSTRACT

Raised serum LDL cholesterol is an important modifiable risk factor for the development of atherosclerosis and cardiovascular disease. So the management of dyslipidaemia is mostly based on the concentration of LDL cholesterol. Friedewald's formula is commonly used method to estimate LDL cholesterol in most clinical laboratories. However, this formula cannot reflect the actual figure of LDL cholesterol. In 2013, de Cordova developed a new simple formula to calculate LDL cholesterol without using serum TG which is said to be more accurate than Friedewald's formula. The present study was designed to compare the formula-based calculated LDL cholesterol (Friedewald's formula and de Cordova's formula) with direct homogenous estimation. The objective of the present study was to evaluate of applicability of de Cordova's formula for calculation of LDL cholesterol. By using non-probability sampling technique, 460 individuals were enrolled in the study who were attending in the one-point collection centre of BSMMU for lipid profile estimation. Subjects were categorized as normolipidaemic individuals and dyslipidaemic patients. Serum TC, TG, HDL cholesterol and LDL cholesterol were measured by direct automated method. LDL cholesterol was also calculated by Friedewald's formula and de Cordova's formula. Results were expressed as mean ± SD. Comparison was done by Pearson's correlation test, agreement was done by Bland-Altman agreement test between measured and calculated LDL cholesterol. The mean ± SD of measured LDL cholesterol was 132.99 ± 36.65 mg/dL. LDL cholesterol calculated by Friedewald's formula and de Cordova's formula were 121.39 mg/dL and 116.81 mg/dL respectively. The limits of agreement between measured LDL cholesterol (direct method) and calculated LDL cholesterol by de Cordova's formula were lowest and agreement was better for all dyslipidaemic subjects. de Cordova's formula showed better agreement with measured LDL cholesterol (direct method) than Friedewald's formula for approximate calculation of LDL cholesterol without using triglycerides.

Keywords: LDL cholesterol, de Cordova's formula, Friedewald's formula

INTRODUCTION

The concentration of serum low-density lipoprotein cholesterol (LDL-C) is an independent risk factor for the development dyslipidaemia as well as coronary heart disease.[1, 2] Determination of the circulating level of LDL (low-density lipoprotein) cholesterol is important for the diagnosis and risk assessment for atherosclerosis and coronary artery disease (CAD).[3] Studies have shown the importance of blood lipids in the management and monitoring of patients with cardiovascular risk.[4, 5] As LDL cholesterol is the primary lipid agent for CAD risk prediction and therapeutic target, an accurate and precise determination of LDL cholesterol is very important for early identification of patients at risk.[6] Ultracentrifugation-polianion precipitation/ Beta Quantification (ßQ), is the reference method for measurement of LDL cholesterol concentration, which is expensive, laborious and not available everywhere.[7] The direct methods are costly and require expensive automation and are not affordable by most of the laboratories in the developing countries.[8] Several direct methods have been developed but all are expensive and not suitable for developing countries like ours, that is why Friedewald's formula is most commonly used for determining LDL cholesterol in the clinical laboratory.[9] This formula estimates LDL cholesterol from measurements of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) cholesterol [LDL cholesterol = TC – TG/5 – HDL cholesterol].[10] Also in Bangladesh, Friedewald's formula is the most commonly used procedure in clinical practice.[8] This formula

has several limitations and cannot be applied in hypertriglyceridemia (TG level > 400 mg/dL), in hyperchylomicronemia, patients with type III hyperlipoproteinemia.[11 – 14] Friedewald's formula should be used with precaution in several pathologic states (diabetes, hepatopathy and nephropathy), even if the TG concentrations are between 200 – 400 mg/dl.[1, 15] de Cordova et al. recently published a new, simpler and less expensive formula (LDL-C = ¾ [Total cholesterol- HDL-C]) independent of serum TG after analyzing lipid profiles of a large cohort of Brazilian population.[16] It opens a new door to calculate LDL cholesterol in non-fasting state.[17]

de Cordova's formula accurately estimates LDL cholesterol avoiding some of the limitations of currently published formulas, and it is an attractive alternative when direct estimation is not possible.[16] Direct measurement of LDL cholesterol is costly and Friedewald's formula cannot give accurate result.[18] Friedewald's formula is invalid when serum TG level is > 400 mg/dl and fasting blood sample is needed to calculate LDL cholesterol by Friedewald's formula.[11, 18] So we need to search out more accurate formula for calculation of LDL cholesterol for correct diagnosis and management of dyslipidaemia. This study was done to assess the applicability of de Cordova's formula for calculation of LDL cholesterol in Bangladeshi population. If this formula-based calculated LDL cholesterol is found to be more valid, this formula can be proposed to be used clinically for correct estimation of LDL cholesterol with minimum cost and time.

MATERIALS AND METHOD

This cross-sectional, analytical study was conducted in the department of Biochemistry, BSMMU, Shahbagh, Dhaka, during the period from January 2014 to December 2015. Fasting blood samples were collected from 460 study subjects who were attending in blood collection point centre of BSMMU for lipid profile estimation (serum triglyceride, HDL cholesterol, total cholesterol and LDL cholesterol). With all aseptic precautions, 5 ml venous blood was drawn from anticubital vein after overnight fasting (about 10 – 12 h) in a disposable plastic syringe and delivered immediately into a clean dry tube. Then serum was prepared after centrifugation and stored in ultra freezer at −20°C and serum triglyceride, HDL cholesterol, total cholesterol and LDL cholesterol were measured by using the ARCHITECT auto analyzer System (Abbott Diagnostics, USA) at the department of Biochemistry, BSMMU. All kits, calibrators and quality control materials were obtained from Abbott Diagnostics, USA through local distributor. LDL cholesterol was also calculated by Friedewald's formula and de Cordova's formula. Subjects were categorized as normolipidaemic subjects, dyslipidaemic patients according to the definition of dyslipidaemia which was taken from third report of the National Cholesterol Education Program Adult Treatment Panel III.[19] Patients having TG ≥ 400 mg/dL were excluded when LDL cholesterol was calculated by Friedewald's formula. Statistical analysis will be performed by statistical package for Social Science (SPSS) Version 22. Results were expressed as mean ± SD. Comparison was done by Pearson's correlation test between estimated LDL cholesterol and formula-based LDL cholesterol. Agreement between estimated LDL cholesterol and formula-based LDL cholesterol was done by Bland-Altman agreement test.[20, 21] A p-value of < 0.05 was considered as statistically significant.

RESULTS

A total of 460 subjects were included in the study, the mean age of the study subjects was 45.32 ± 12.5, with 71 were normolipidaemic and 389 were dyslipidaemic. Out of 71 normolipidaemic subjects, 47 (66.2%) were male and 24 (33.8%) were female. Out of 389 dyslipidaemic subjects, 226 (58.1%) were male and 163 (41.9%) were female. The mean concentrations of TC, TG and HDL cholesterol were 129.11 mg/dL, 111.99 mg/dL and 47.87 mg/dL respectively in case of normolipidaemic and 204.06 mg/dL, 198.21 mg/dL and 37.26 mg/dL respectively

in case of dyslipidaemic subjects. The mean values of LDL cholesterol measured by direct method, Friedewald's formula and de Cordova's formula were 132.99 mg/dL, 121.39 mg/dL and 116.81mg/dL respectively. Patients having TG ≥ 400 mg/dL were excluded while calculating Friedewald's formula. Correlation of measured LDL-C (direct method) with calculated LDL-C in all normolipidaemic and dyslipidaemic subjects showed significant positive correlation between measured and calculated methods (see Table 1).

Table 1 Correlation of measured LDL-C (direct method) with calculated LDL-C

Calculated method	Total subjects (*n* = 460)		Normolipidaemic (*n* = 71)		Dyslipidaemic (*n* = 389)	
	***r* value**	***p* value**	***r* value**	***p* value**	***r* value**	***p* value**
Friedewald's formula	0.749[#]	<0.001	0.696	<0.001	0.635[#]	<0.001
de Cordova's formula	0.804	<0.001	0.652	<0.001	0.719	<0.001

[#]Patients having TG ≥ 400 mg/dL were excluded.

Bland-Altman agreement plot was done to see the agreement between the measured LDL-C (direct method) and calculated LDL-C by Friedewald's formula and de Cordova's formula for all (see Table 2 and Figure 1), dyslipidaemic (see Table 3 and Figure 2) and normolipidaemic subjects (see Table 4 and Figure 3) within 95% limit. For all study subjects, de Cordova's formula showed better agreement as limits of agreement were 102.01 and 87.75 respectively for Friedewald's formula and de Cordova's formula.

Table 2 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for all study subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula)[#]	7.19	26.02	58.19	−43.82	102.01
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	16.18	22.38	60.05	−27.70	87.75

[#]Patients having TG ≥ 400 mg/dL were excluded.

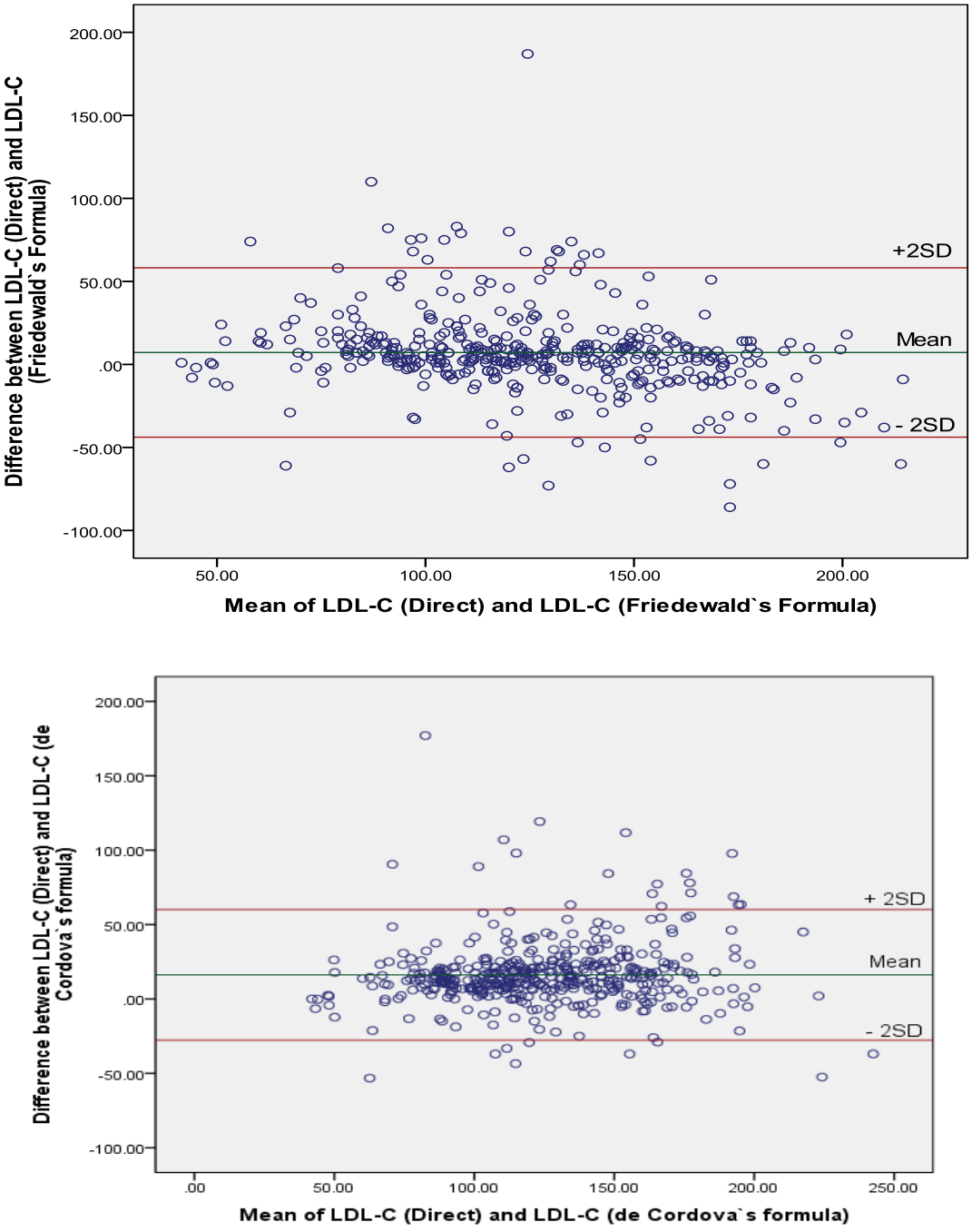

Figure 1 Bland-Altman agreement plots between measured LDL-C and calculated LDL-C for all study subjects. (Patients having TG ≥ 400 mg/dL were excluded in case of Friedewald's formula.)

Bland-Altman plot showed better agreement of the de Cordova's formula than Friedewald's formula in dyslipidaemic subjects as limits of agreement was lower in case of de Cordova's formula (62.73, −29.56 vs 60.81, −47.95) within 95% limit (see Table 3 and Figure 2).

Table 3 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for dyslipidaemic subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula) [#]	6.43	27.75	60.81	−47.95	108.76
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	16.59	23.54	62.73	−29.56	92.29

[#]Patients having TG ≥ 400 mg/dL were excluded.

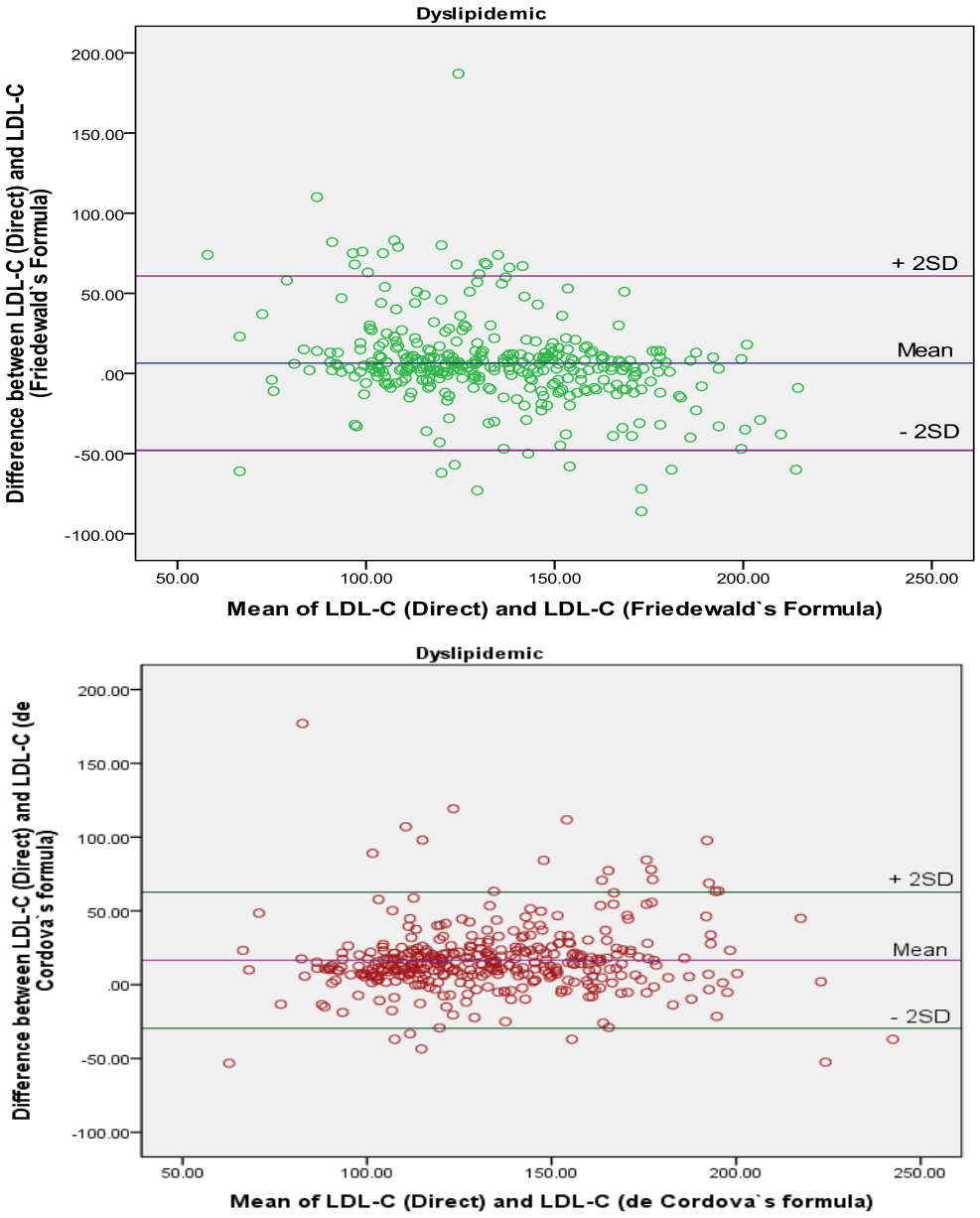

Figure 2 Bland-Altman agreement plots between measured LDL-C and calculated LDL-C for dyslipidaemic subjects. (Patients having TG ≥ 400 mg/dL were excluded in case of Friedewald's formula.)

For normolipidaemic subjects, limits of agreement were 53.99 and 56.41 respectively for Friedewald's formula and de Cordova's formula (see Table 4 and and Figure 3). Limits of agreement were lower and showed better agreement for Friedewald's formula.

Table 4 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for normolipidaemic subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula)	11.04	13.77	38.04	−15.96	53.99
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	13.94	14.39	42.14	−14.27	56.41

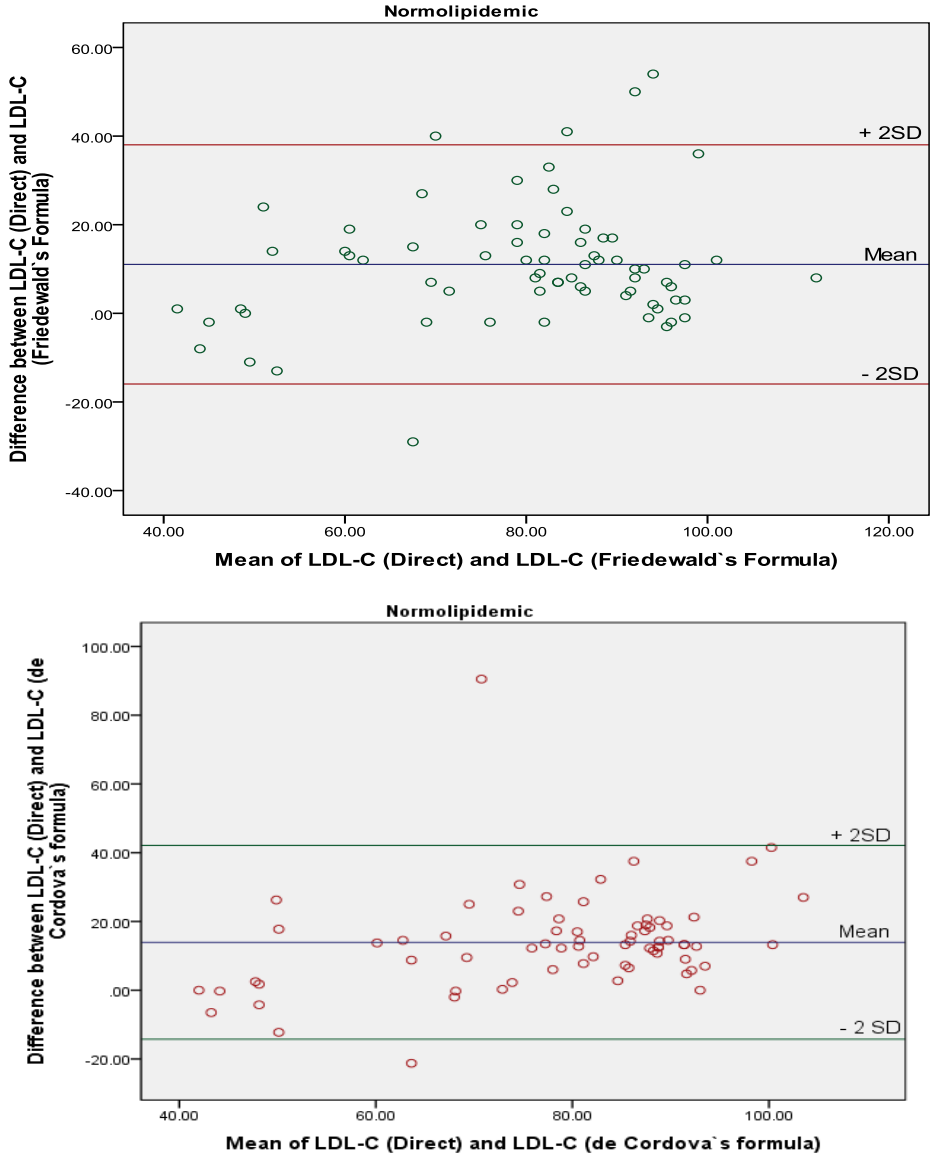

Figure 3 Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for normolipidaemic subjects

DISCUSSION

The management of dyslipidaemia is largely based on the LDL cholesterol concentration, that is why both accuracy and precision of LDL cholesterol measurement are critically important.[7, 22] Direct homogenous assays for measurement of LDL cholesterol levels have shown reasonable accuracy but all are expensive.[23] In order to improve the accuracy of Friedewald's formula, many formula had been developed.[18, 24 – 26] However, none of these formulas can replace the original formula due to less evidence.[22, 27] Therefore, Friedewald's formula is the most commonly used method although it has several limitations.[8] In this study, the mean concentration of TC, TG and HDL cholesterol were 129.11 mg/dl, 111.99 mg/dl and 47.87 mg/dl respectively in case of normolipidaemic and 204.06 mg/dl, 198.21 mg/dl and 37.26mg/dl respectively in case of dyslipidaemic subjects. Saiedullah et al. conducted their study on 644 samples and found the mean values of TC, TG, HDL cholesterol and LDL cholesterol were 218.78 mg/dl, 383.59 mg/dl, 36.11 mg/dl and 120.01 mg/dl respectively.[2] In our study, the mean values of LDL cholesterol measured by direct method, Friedewald's formula and de Cordova's formula were 132.99 mg/dL, 121.39 mg/dL and 116.81 mg/dl respectively. Studies showed remarkable underestimation of LDL cholesterol calculated by Friedewald's formula in Bangladeshi population.[2, 8, 18] Boshtam et al. revealed a highly significant correlation between direct method and Friedewald's formula.[28] However, the Friedewald's formula overestimated the LDL cholesterol value compared to the direct method.[28] The results of our study did not support Boshtam et al.[28] In our study, de Cordova's formula gave better result than Friedewald's formula. In all dyslipidaemic study subjects, the correlation coefficient of LDL cholesterol calculated by de Cordova's formula with the measured LDL cholesterol was statistically high significant and better than the correlation coefficient of LDL cholesterol calculated by Friedewald's formula with the measured LDL cholesterol (0.804 vs 0.749, 0.719 vs 0.635, $p < 0.001$). However, in normolipidaemic subjects the correlation coefficient of LDL cholesterol calculated by Friedewald's formula with the measured LDL cholesterol was statistically high significant and better than the correlation coefficient of LDL cholesterol calculated by de Cordova's formula with the measured LDL cholesterol (0.696 vs 0.652, $p < 0.001$). Siddique et al. found bias of calculated LDL cholesterol against measured LDL cholesterol −5.2% for de Cordova's formula and −9.6% for Friedewald`s formula.[17] de Cordova's formula revealed better performance than Friedewald's formula for approximate calculation of LDL cholesterol without using triglycerides and showed better agreement in Bland-Altman plot.[16] We also did Bland-Altman plot to see the agreement between measured LDL cholesterol (direct method) and calculated LDL cholesterol. Our study supported Siddique et al.,[17] we also found better agreement of the de Cordova's formula than Friedewald's formula. In all dyslipidaemic subjects, limits of agreement were lower in case of de Cordova's formula (60.05, −27.70 vs 58.19, −43.82 and 62.73, −29.56 vs 60.81, −29.56) within 95% limit (see Tables 2 and 3, Figures 1 and 2). In the case of normolipidaemic subject, limits of agreement were 53.99 and 56.41 respectively for Friedewald's formula and de Cordova's formula (see Table 4 and Figure 3) and showed better agreement for Friedewald's formula because limits of agreement were lower. Our study result supported the result of Nigam.[29] Nigam studied on de Cordova's formula and found this formula can be used in non-fasting specimen, validated in large number of Brazilian individuals with wide range of TC, HDL cholesterol and TG levels.[29] However, de Cordova's formula did not perform better than Friedewald's formula in healthy individuals.[29] Our study result differs from the result of Martin, et al.[30] They revealed that the Friedewald's formula has a better agreement with directly measured LDL cholesterol compared to the de Cordova's formula.[30]

CONCLUSION

From this study, it may be concluded that estimation of LDL cholesterol by de Cordova's formula shows better agreement with measured LDL cholesterol (direct method) than Friedewald's formula for all and dyslipidaemic subjects. de Cordova's formula can be used clinically for approximate calculation of LDL cholesterol without using triglyceride as well as in non-fasting states. However, more studies are recommended regarding the validity of this formula for calculation of LDL cholesterol both in fasting and non-fasting states in our country and neighbouring countries.

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Case Report

A Fatal Case of Metformin and Gliclazide Poisoning and its Management

Low Qin Jian[1*], Chew Soo Foong[2]
[1]Medical Department, Melaka General Hospital, Melaka, Malaysia
[2]Clinical Pharmacist, Melaka General Hospital, Melaka, Malaysia
[*]Corresponding author's email: peterlow4964@gmail.com
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ABSTRACT

Both metformin and gliclazide have been used extensively in the management of type II diabetes mellitus. Metformin and gliclazide overdose can lead to severe hypoglycaemia refractory to intravenous (IV) dextrose rescue therapy. A 21-year-old man complained of vomiting and felt dizzy after four hours of taking 70 tablets of Metformin 500 mg and 40 tablets of Gliclazide 80 mg. He had major depressive disorder and wanted to commit suicide. He was given IV Dextrose 50% 50 cc immediately. Octreotide had been used successfully to reverse the refractory hypoglycaemia caused by gliclazide overdose. Unfortunately, he developed severe lactic acidosis with acute kidney injury. Dialysis had been done by continuous venovenous haemodiafiltrationa and intravenous sodium bicarbonate 8.4% infusion was given. However, the patient succumbed due to the severe lactic acidosis and kidney failure despite of urgent dialysis. Octreotide infusion helps in preventing refractory hypoglycaemia secondary to sulfonylurea overdose by inhibit calcium-mediated insulin release. Metformin overdose causes severe lactic acidosis due to conversion of glucose to lactate. Sodium bicarbonate therapy in metformin induced lactic acidosis is also controversial. Though sulfonylurea and metformin are the most commonly-prescribed anti-hypoglycaemic agents, thus during prescribing everyone has to be careful about the overdoses and side effects of these drugs.

Keywords: metformin, gliclazide, poisoning, octreotide

INTRODUCTION

Sulfonylurea medications were first used to treat type 2 diabetes in 1954 and they remain in common use today.[1] Metformin and gliclazide overdose can lead to severe hypoglycaemia refractory to intravenous dextrose rescue therapy. The National Health and Morbidity Survey (NHMS) 2011 reported type 2 diabetes prevalence figures of 15.2% and 20.8% for adults above the age of 18 to 30 years, respectively, in Malaysia.[2] The high prevalence of diabetes in our population poses a risk of poisoning either intentionally or unintentionally.[2]

Gliclazide, a sulphonyurea oral hypoglycaemic agent, acts by inhibit potassium efflux through ATP-sensitive potassium channels in pancreatic beta cell membranes.[3] High intracellular potassium level resulted in depolarization and calcium influx, triggering insulin secretion from beta cells.[3] In addition, gliclazide also increases the sensitivity of beta cells to glucose stimulus.[2] In acute poisoning, its onset of action remains unchanged but its duration of action is extended, causing prolonged hypoglycaemia.[4, 5] Its high degree of protein binding at around 94% has deemed dialysis ineffective in treating sulphonyurea overdosed.[6] Traditional approach in sulphonylurea poisoning includes dextrose administration while glucagon and diazoxide will be added in cases with rebound hypoglycaemia.[7, 8] Octreotide, a synthetic octapeptide analogue of somatostatin, also can be used to suppress insulin secretion.[8] In symptomatic intentional overdose of sulfonylurea, both intravenous dextrose and octreotide should be administered to raise the blood glucose level acutely thereby increase glucose delivery to the brain. Octreotide is a somatostatin analogue that inhibits insulin release from the pancreatic beta-islet cells. If intravenous dextrose is being given alone without octreotide, it may cause transient hyperglycaemia that triggers insulin release, leading to recurrent episodes of hypoglycaemia. The increase in insulin release can be reduced by octreotide.[8]

Metformin is a biguanide anti-hyperglycaemic agent that promotes euglycaemia by improving peripheral glucose uptake, decreasing insulin resistance and reducing hepatic glucose production.[9] Lactic acidosis is the major toxicity from acute biguanide poisoning. There is no antidote available.[10] Management of acute poisoning includes gastrointestinal decontamination and symptomatic management with dextrose, sodium bicarbonate and haemodialysis.[11]

CASE PRESENTATION

A 21-year-old man with background of major depressive disorder committed alleged suicide by swallowing 70 tablets of Metformin 500 mg and 40 tablets of Gliclazide 80 mg (both immediate release formulation). He became unwell, started vomiting and felt dizzy four hours after taking the tablets which prompted him to seek medical assistance. On arrival, his Glasgow come scale (GCS) was full but the capillary sugar reading was noted to be 1.5 mmol/L (low). He was given IV Dextrose 50% 50 cc immediately and his sugar improved to 6.3 mmol/L. Despite that, he experienced recurrent hypoglycaemia which is refractory to the subsequent IV dextrose rescue therapy. Consultation was done with endocrinologist and the National Poison Centre (Pusat Racun Negara). Their recommendations were to start him on intravenous dextrose and octreotide. He was started on IV Octreotide infusion 50 mcg/hour infusion as part of the gliclazide poisoning treatment. During octreotide intravenous infusion together with maintenance intravenous dextrose 10%, his hourly sugar levels were in the range of 6 – 10 mmol/L. Throughout the course of admission in the intensive care unit (ICU), he developed severe lactic acidosis with acute kidney injury to the extend needing continuous venovenous haemodiafiltration (CVVHDF) and regular intravenous sodium bicarbonate 8.4% infusion. He was dialyzed via CVVHDF after a discussion with our nephrologist as he was haemodynamically unstable and required triple inotropic support. Unfortunately, his condition deteriorated and he passed away at 32 hours of admission. His family members refused to do the post-mortem examination. The cause of death given was severe lactic acidosis secondary to metformin and gliclazide poisoning. There was no other co-ingestion apart of gliclazide and metformin in this case. His serum and urine toxicology for amphetamines, salicylates, opiods, benziodiazepine and alcohol were all negative. The laboratory services were unable to perform a serum level of metformin and gliclazide.

Table 1 Investigations (during admission)

	Readings	**Normal values**
Haemoglobin	15 g/dL	14 – 18 g/dL
White blood cells	19.5 × 10^9/L	4.0 – 12.0 × 10^9/L
Platelet	129 × 10^9/L	150 – 400 × 10^9/L
Creatinine	179 µmol/L	50 – 110 umol/L
Urea	2.5 mmol/L	4 – 8 mmol/L
Sodium	148 mmol/L	135 – 140 mmol/L
Potassium	2.9 mmol/L	3.5 – 5 mmol/L
Corrected calcium	2.10 mmol/L	2.2 – 2.6 mmol/L
Phosphate	3.58 mmol/L	1.0 – 1.5 mmol/L

Table 2 Serial blood gases result

	Normal range	Arrival	6 hours later ventilated	12 hours later ventilated	18 hours later ventilated	24 hours later ventilated
pH	7.35 – 7.45	6.8	6.828	6.8	6.9	6.9
pO2 in mmHg	80 – 100	45.1	120.2	118	79	73
pCO2 in mmHg	35 – 45	34.5	41.1	45.9	38.4	49
HCO-3 in mmol/L	23 – 29	6.3	6.4	6.5	8.1	6
Base excess	−2 to 2	−27	−27.5	−27.3	−23	−25
Lactate in mmol/L	<1	5	12	15	19	18

DISCUSSION

Intravenous dextrose is used to treat hypoglycaemia and is crucial to raise the blood glucose rapidly during resuscitation in order to increase glucose delivery to the brain.[7] The brain uses glucose at a rate of 20 times that of other body tissues and cannot use free fatty acids directly since they are not transported across the blood-brain barrier. However, ketone bodies (beta-hydroxybutyric acid and acetoacetic acid) are transported across the blood-brain barrier and their metabolism can help supplant the need of glucose. Glucagon is another option to treat hypoglycaemia. Its efficacy depends on body glycogen stores. It increases serum glucose level by stimulating gluconeogenesis using hepatic glycogen. However, it induces insulin release from pancreatic beta cells. Hyperinsulinaemia may worsen the refractory hypoglycaemia.[7] Glucagon (5 mg) intramuscularly (IM) may be used as a temporizing measure while IV access is obtained but it is not a substitute for dextrose. The efficacy of glucagon is dependent upon hepatic glycogen stores which may be depleted in the setting of prolonged hypoglycaemia. The short duration of action of glucagon further limits its effectiveness.[7]

Diazoxide is an antihypertensive agent that produces arteriolar vasodilation and reduced peripheral resistance.[7] Besides that, it inhibits pancreatic insulin release. It has been used to treat sulphonylurea poisoning for over two decades but there are concerns regarding its hypotensive, tachycardia, sodium and fluid retention.[7] So, production of injection diazoxide had been stopped. Its therapy has fallen out of favour with the increased use of octreotide, which was reported to be superior compared to intravenous diazoxide.[5, 12]

Octreotide has been proposed to act by inhibit calcium-mediated insulin release through reducing calcium influx across voltage-gated channels in pancreatic beta cells.[12] Octreotide is administered intramuscularly or subcutaneously in doses of 50 – 150 mcg every 6 hours for 24 hours.[11] It can be continued for another 24 hours if there is recurrence of hypoglycaemia.[12] Octreotide can also be given as continuous infusion.[12] However, it has been reported that continuous infusion is not better than intermittent intramuscular and subcutaneous dosing.[12]

A double-blinded placebo-controlled study involving 40 patients was performed by Fasano et al. in 2007.[6] In that study, researchers concluded that serum glucose levels were consistently higher for the first 8 hours in patients treated with octreotide compared to patients treated with intravenous dextrose only.[6] This clinical picture was not seen in the present case because this patient had also developed severe lactic acidosis from overdose of metformin.

McLaughlin et al. retrospectively reviewed 9 patients with sulphonylurea overdosed (dose range 40 – 125 mg).[14] The investigators concluded that octreotide is effective in preventing recurring hypoglycaemia secondary to sulphonylurea. Risk of rebound

hypoglycaemia was 27 times lesser in octreotide group compared to placebo. In addition, the amount of 50% dextrose used was significantly lower after octreotide administration.[14]

The mechanism leading to metformin induced lactic acidosis is complicated. Metformin promoted the conversion of glucose to lactate in the splanchnic bed of the small intestine.[16] Metformin also inhibits the mitochondrial respiratory chain complex 1 leading to decreased hepatic gluconeogenesis from lactate, pyruvate and alanine. This results in additional lactate and substrate for lactate production.[17]

Sodium bicarbonate therapy in metformin induced lactic acidosis is controversial.[10] Theoretical disadvantages of prolonged sodium bicarbonate including excess sodium load, rebound metabolic alkalosis, decreased myocardial contractility and reflex vasodilation.[11] Therefore, it is recommended to limit its use to patients with severe metabolic acidosis, for example, arterial pH below 7.10. If severe metabolic acidosis is present, sodium bicarbonate may be administered but there are disadvantages with this practice. It can lead to a leftward shift of the haemoglobin dissociation curve, excess sodium load, rebound metabolic alkalosis, disturbances in serum potassium and calcium, decreased myocardial contractility increase carbon dioxide production and reflex vasodilatation after bolus injection.[11]

Extracorporal removal via haemodialysis is the preferred approach if haemodynamically stable. In patients with haemodynamic unstability, continuous venovenous haemofiltration (CVVH) can be performed.[19]

CONCLUSION

Octreotide infusion may help in preventing refractory hypoglycaemia secondary to sulfonylurea overdose. This patient unfortunately succumbed due to severe lactic acidosis from his metformin overdose despite intensive care services. This is a life-threatening condition. Though sulfonylurea and metformin are the most commonly prescribed anti-hypoglycaemic agents, thus during prescribing everyone has to be careful about the overdoses and side effects of these drugs.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

CONSENTS

Written informed consent was obtained from the patient to publish the case. A copy of written consent is available for review by the Chief Editor.

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Case Report

Submucosal Lipomatosis of Caecum with Concomitant Acute Appendicitis: A Diagnostic Dilemma

Nornazirah Azizan[1], Firdaus Hayati[2*], Pauline Yap[2], Aishath Azna Ali[3], Nor Haizura Abd Rani[4], Fazarina Mohammed[5]

[1]Department of Pathobiology and Medical Diagnostic, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

[2]Department of Surgery, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

[3]Department of Surgery, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[4]Department of Pathology, Queen Elizabeth Hospital 1, Sabah, Malaysia

[5]Department of Pathology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[*]Corresponding author's email: firdaushayati@gmail.com

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ABSTRACT

Colonic lipomatosis is relatively a rare tumour of mesenchymal origin, composed of well-differentiated adipocytes interlaced by fibrous tissues. A 59-year-old lady presented with right iliac fossa pain with positive rebound tenderness, Rovsing's and obturator signs. Investigation revealed marked leucocytosis suggestive of an acute appendicitis. Diagnostic laparoscopy revealed an inflamed appendix with concomitant caecal mass suspecting of a malignancy. Laparoscopic right hemicolectomy was proceeded following oncologic resection. However, final histopathologic examination was consistent with caecal colonic lipomatosis with concomitant acute appendicitis. Hereby, dual pathologies can be elicited in an acute abdomen.

Keywords: acute abdomen, acute appendicitis, colonic lipomatosis.

INTRODUCTION

Colonic lipomatosis is mostly asymptomatic. It can manifest occasionally as a surgical emergency namely intussusception, obstruction, or bleeding and even masquerade malignancy.[1] In contrast, presented as more benign display it has been reported to mimic an acute appendicitis in surgical specimens removed for other bowel pathologies.[2] Majority of the cases are localized in submucosa with occasional involvement of the muscularis propria, while few are located at subserosal layer. They are mainly localized in the right-side of the colon especially the caecum. Imaging plays an important role to preoperatively diagnose this condition.[3]

CASE PRESENTATION

A 59-year-old lady was referred from a private centre for acute abdomen. She presented with right iliac fossa pain associated with fever, nausea and vomiting. She denied alarming and constitutional symptoms prior to this onset. Clinically, there was rebound tenderness with positive Rovsing's and obturator signs. Biochemical investigation revealed marked leucocytosis, which was suggestive of inflammatory origin. She was posted for appendicectomy. However, peri-operative finding revealed a caecal mass. Suspecting malignancy, laparoscopic right hemicolectomy following oncologic resection was carried out.

The appendix was grossly dilated, measuring 60 mm × 15 mm × 15 mm, covered by slough with presence of faecolith at the appendiceal lumen opening. There was a thickened area at the caecum, adjacent to the appendix, measuring 40 mm × 40 mm × 12 mm (Figure 1). On sectioning, it was well-defined grey-whitish firm in nature. There was no exophytic lesion arising from the colonic mucosa. There were 15 lymph nodes isolated from the mesenteric fat; the largest lymph node measures 20 mm × 15 mm × 10 mm. Patient was discharged at day 3 after obtaining a good postoperative recovery.

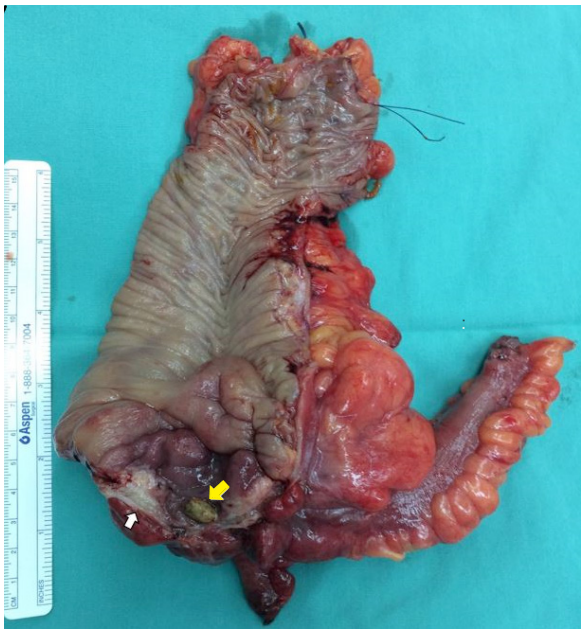

Figure 1 A thickened well-defined grey-whitish area (white arrow) at the caecum with faecolith (yellow arrow) at the appendiceal lumen opening

On microscopic examination, the submucosa layer at the caecum was thickened and composed of diffusely-distributed mature univacuolated adipocytes that are relatively uniform in size and lack of cytological atypia (Figure 2). No lipoblast or atypical stromal cells were seen. The surrounding stroma showed reactive fibroblast and infiltrated by chronic inflammatory cells. Sections of the appendix revealed a marked mucosal ulceration with dense transmural neutrophilic infiltrates. The lumen is filled with acute inflammatory exudate. The 15 lymph nodes obtained showing reactive hyperplasia without evidence of malignancy. In view of benign histology, patient was discharged from the follow-up and is symptom-free at present.

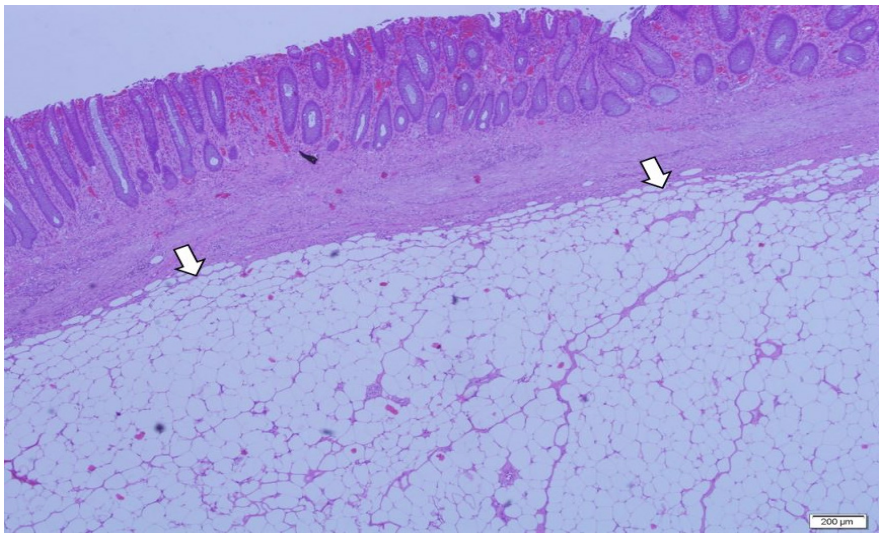

Figure 2 The submucosa layer at the caecum is composed of diffusely-distributed mature univacuolated adipocytes (white arrows)

DISCUSSION

Colonic lipomatosis is a rare entity and poses a diagnostic challenge to both surgeons and pathologists. It was first described by Hellstrom in 1906 with an incidence of less than 5% at autopsy.[1] In addition, it may be detected incidentally during colonoscopy as well as during open or laparoscopic surgery. Patients rarely present as acute abdomen. Only a quarter of cases with colonic lipomatosis develop symptoms. Abdominal pain, rectal bleeding, intestinal obstruction, changes of bowel habit or prolapse can be part of the presentations. Surgical interventions hence are warranted in such cases.

In contrast, stable patients are best managed conservatively after improvement of clinical parameters.[2] Computed tomography (CT) can be selected as a preoperative modality of diagnosis.[2] Features such as ovoid or pear-shaped lesion with densities of −40 to −120 Hounsfield units are typical of fatty composition to suggest of colonic lipoma in CT scan.[3] Other additional pathologies are required to be ruled out especially perforated diverticular disease or caecal tumour in this age group of population. Endoscopy also plays a role in preoperative diagnosis. The cushion or pillow sign (pressing forceps against the lesion results in depression or pillowing of the mass) and naked fat sign (extrusion of yellowish fat at biopsy site) are typical features in colonoscopy.[3] Other endoscopic imaging such as endoscopic ultrasound (EUS) has been used as well. EUS that exhibits a hyperechoic lesion originating from the submucosal layer is pathognomonic for lipoma.[4]

The management of symptomatic colonic lipoma can be either surgical or endoscopic techniques depending on the initial diagnostic methods. Upon endoscopy, large lesions at 2 cm diameter can safely be removed by electrosurgical snare resection after epinephrine or saline solution injection at the base.[5] This precaution is to ensure a meticulous haemostasis. EUS should be utilized concomitantly to ensure that the lipomas do not extend into the muscularis propria.

In this reported case, the patient presented with acute abdomen necessitating surgical intervention as suggested clinically and biochemically. Acute appendicitis requires surgical intervention as the risk of appendicular perforation is anticipated. In view of accidental findings of caecal mass mimicking malignancy, she was opted for right hemicolectomy anticipating malignancy as the probable diagnosis. However, the histopathological diagnosis fortunately was distinct from the initial expectation.

CONCLUSION

Even colonic lipomatosis is rare, physicians especially surgeons should be acknowledged of the existence of this entity. The decision to embark into surgical pathway shall be decided intelligently to avoid inevitable complications. This case highlighted the presence of dual pathologies in an acute abdomen. Being a benign nature, the prognosis of this case is excellent and the patient requires no follow-up in future.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

CONSENTS

Written informed consent was obtained from the patient to publish the case with its related pictures. A copy of the written consent is available for review by the Chief Editor.

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Case Report

Fat Embolism Syndrome Treated with Methylprednisolone: A Different Perception or a Misconception?

Mohd Shaffid Md Shariff[1], Hanizah Ngadiron[2], Firdaus Hayati[3], Affirul Chairil Ariffin[2]
[1]Orthopaedic Surgery, Universiti Sains Islam Malaysia, Negeri Sembilan, Malaysia
[2]Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Negeri Sembilan, Malaysia
[3]Department of Surgery, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia
[*]Corresponding author's email: firdaushayati@gmail.com
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ABSTRACT

Fat embolism syndrome is manifested by the fat globule presence in the pulmonary and systemic circulation. A 34-year-old man was involved in a motor vehicle accident with a fracture of the left femur and avulsion fracture of the left posterior cruciate ligaments. He developed signs and symptoms that suggested an early diagnosis of fat embolism syndrome. Intravenous methylprednisolone administration was administered as part of the treatment. The role of methylprednisolone in a patient with fat embolism syndrome is controversial due to unproven effectiveness. In this case, fat embolism syndrome after a femur fracture was treated successfully with methylprednisolone.

Keywords: fat embolism syndrome, methylprednisolone, femur fracture.

INTRODUCTION

Fat embolism syndrome (FES) is often seen in association with long bone or pelvic fractures. It remains a diagnostic challenge for clinicians and surgeons. It is hard to diagnose, and the severity of its consequences may vary. The incidence of FES varies and often underestimated by the physicians. Its clinical manifestation includes respiratory, cerebral dysfunction and petechial rash. Corticosteroid treatment in fat embolism is a topic of interest since 40 years ago.[1] Few studies had postulated regarding corticosteroid treatment before, but its outcome is arguable. Herein, a case of fat embolism syndrome after a long bone fracture treated successfully with methylprednisolone.

CASE PRESENTATION

A 34-year-old technician had skidded while riding a motorbike during a torrential downpour rain. He sustained closed fracture mid-shaft of left femur (Figure 1) with left posterior cruciate ligament avulsion fracture (Figure 2). On admission, his Glasgow coma scale was full. His vital sign was stable. There was no other injury noted. His left thigh was swollen but soft. Sensation of the left lower limb was present and distal pulses were palpable. Haemoglobin level on admission was 12.2 g/dl. Skin traction was applied.

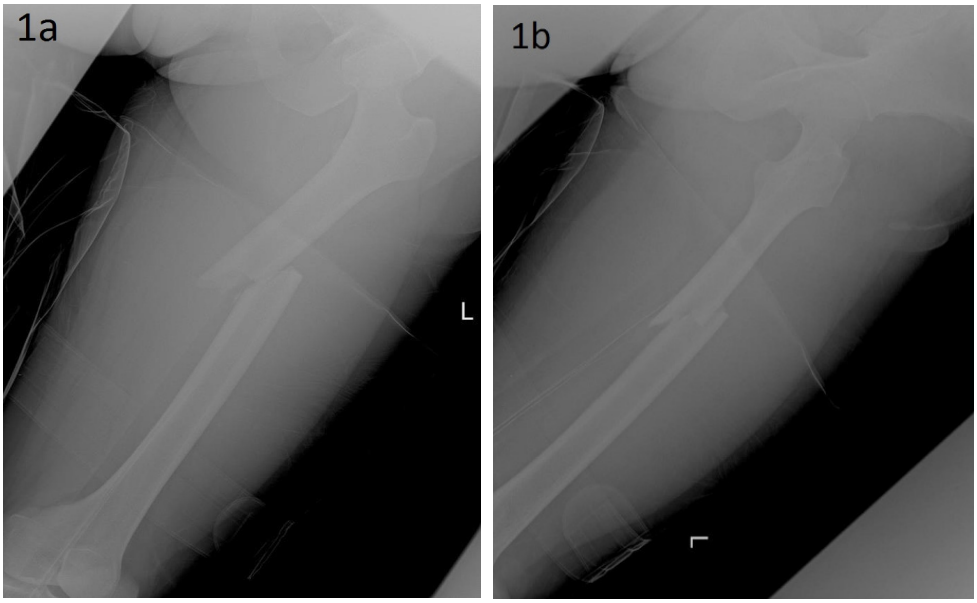

Figure 1 Radiograph AP (1a) and lateral view (1b) of left femur showing fracture of mid-shaft femur

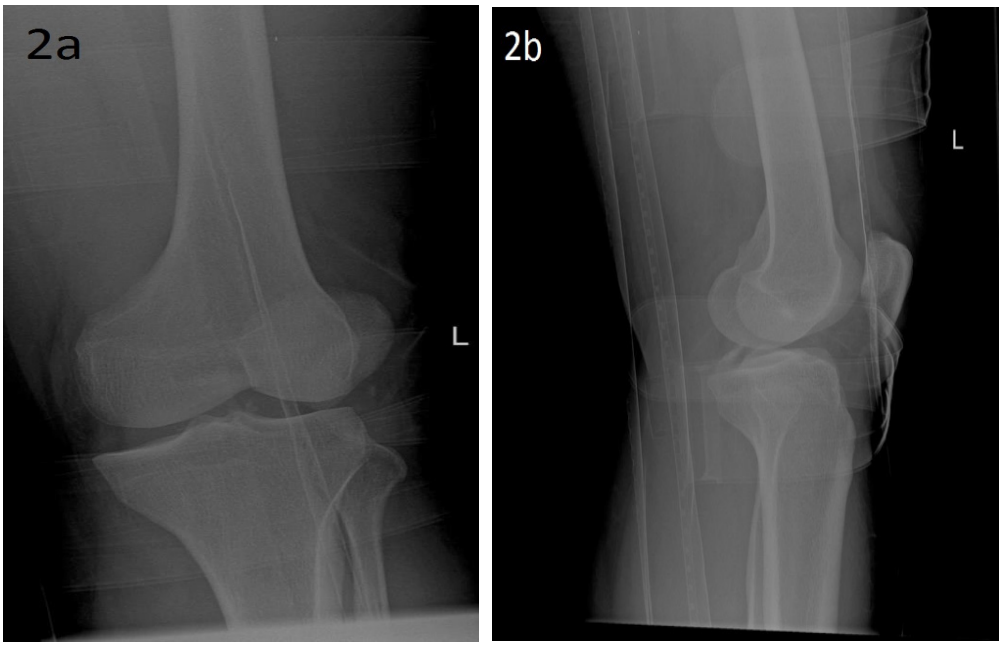

Figure 2 Radiograph AP (2a) and lateral (2b) of left knee showing avulsion fracture of the posterior cruciate ligament

A calcaneal pin was inserted after one day of trauma with a skeletal traction of 10% body weight. He was scheduled for an interlocking nail for left femur fracture and screw fixation for his posterior cruciate ligament avulsion fracture. However, at day two post trauma, he developed a spiking temperature of 38.5°C, tachycardia with a heart rate of 105 beats/minute, tachypnoea with 20 breaths/minute and low SpO_2 under room air of 94%. Arterial blood gases (ABG) air showed respiratory alkalosis with renal compensation. ECG showed pulmonary embolism type of changes (Figure 3). Chest X-ray was clear. Repeated haemoglobin was 10.3 g/dL.

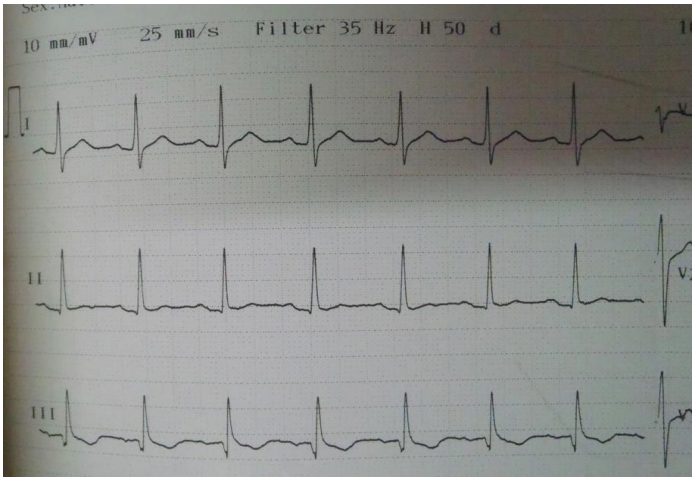

Figure 3 ECG showing S1Q3T3 changes suggestive of pulmonary embolism

Differential diagnoses at that period were fat embolism syndrome and pulmonary embolism secondary to either deep vein thrombosis or from fat globules. The patient was given 6-pint normal saline infusion, 2 pints of packed cell transfusion and oxygen 3 litres via nasal prong. Computed tomography of pulmonary angiogram (CTPA) was performed with no evidence of pulmonary embolism.

Intravenous methylprednisolone was started with a dosage of 7.5 mg/kg every 8 hours for three days. Supportive management with adequate hydration and oxygenation was continued. The symptoms resolved after completed methylprednisolone dosage three days later. The patient underwent interlocking nail of the femur and posterior cruciate ligament repair. The surgery was successful and uneventful postoperatively. He was fit for discharged three days later.

DISCUSSION

The diagnosis of FES is very challenging. Gurd's Criteria (Table 1) is the best way to the diagnosis of FES.[2] The combination of two major or one major with four minor criteria may point us to the diagnosis. FES actual incidence is undetermined due to the rarity of diagnosis.[3] Compared to the upper limb, the association between lower limb long bone fracture and FES is more significant. This feature is possible due to the larger size and easy access to the vasculature.[4] The patient presented with shortness of breath associated with fever. Few differential diagnoses may manifest with the similar signs and symptoms but given his age, injuries and symptoms, differential diagnoses were narrowed down into fat embolism syndrome and pulmonary embolism secondary to deep vein thrombosis and fat globules. Even though the ECG showed high suspicious of pulmonary embolism, however, the CTPA showed negative results.

Table 1 Criteria for the diagnosis of fat embolism syndrome according to Gurd[2]

Major Criteria	Minor Criteria
• Petechial rash: axillary or subconjuctival petechiae • Respiratory symptoms: Positive radiographic changes; hypoxaemia ($PaO_2 < 60$ mmHg; $FiO_2 < 0.4$) • Cerebral depression disproportionate to hypoxaemia • Pulmonary oedema	• Tachycardia (>110 beats/min) • Pyrexia (>38.5) • Retinal fat or petechiae • Presence of urinary fat globules • Sudden drop in haemoglobin level or platelet values • High erythrocyte sedimentation rate • Fat globules in the sputum • Urinary incontinence

The diagnosis of FES is relatively challenging and confusing. Its presentation is non-specific and posed a challenge in diagnosis. Gurd first describes this condition in 70's and was refined together with Wilson in 1974.[2, 5] Even though few authors argued regarding Gurd's criteria, however, it has become the tool of determining the diagnosis of FES in our setting. Our patient presented with respiratory symptoms, tachycardia, pyrexia, and sudden drop in haemoglobin. There were not enough criteria to fit into the diagnosis of FES. However initial presentation of FES needs to be considered.

The treatment of FES requires a close monitoring and respiratory support if indicated. Although the presence of Gurd's criteria may aid in the diagnosis, its universal usage is somewhat lacking. Despite the supportive treatment given, the patient's condition remains the same. He showed significant recovery after the administration of 12 dosages of methylprednisolone. The role of methylprednisolone in FES treatment has been debatable for the last decade. Although various studies describe the role of methylprednisolone in FES treatment, it is not used universally due

to the unverified effectiveness.[1] Its role has been investigated extensively in both animals and humans. Ashbaugh and Petty (1996) recorded a successful treatment of a massive respiratory failure secondary to fat embolism syndrome using corticosteroid.[6] They postulated that corticosteroid causes stabilization of pulmonary capillary membrane and halts inflammatory response. The treatment also suggested a stabilization of complement system and platelet aggregation.[7] One article showed a successful use of methylprednisolone as a prophylaxis against FES. The study revealed a protective effect in reducing the incidence of FES and hypoxaemia related complications.[8] However, given the unwanted side effects of corticosteroid, the practice is not widespread in some locations. Among the effects include, it can progress to peptic ulcer disease, hyperglycaemia and poor wound healing. There was a case report whereby patients with long bones fracture developed atrial fibrillation after receiving intravenous methylprednisolone.[9] Blood glucose should be monitored closely in these patients. Proton pump inhibitor would be a wise option to prevent any gastrointestinal side effects.

The recommended dosage in most literature is between 9 mg/kg and 90 mg/kg in divided dose. This dosage range is safe and postulated to provide maximum benefit. One series showed a 10-fold reduction of fat embolism syndrome episodes with methylprednisolone use.[10] These findings should convince the medical world of its beneficial role. A meta-analysis of 389 patients using corticosteroids concluded a reduction of FES in traumatic patients up to 43%. However, the sample size is not adequate to show significant differences in mortality or infections with the administration of corticosteroids.

CONCLUSION

Methylprednisolone is an enticing treatment option in fat embolism syndrome. Although the role is debatable, its usage has been shown to be safe and efficient. A randomized trial would be ideal to evaluate its true advantage.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in publishing this case.

CONSENTS

Written informed consent was obtained from the patient to publish the case. A copy of the consent is available with the Chief Editor.

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