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Editorial

Bedaquiline: An Effective Anti-tuberculous Drug with Novel Mechanism of Action

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There were 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB) and 100,000 new patients with rifampicin-resistant tuberculosis (RR-TB) in 2015. Mortality was common in Asia with 250,000 deaths in the same year.[1] Treatment was successful in 52% of the MDR/RR-TB patients whereas 17% mortality and 9% treatment failure were reported. Extensively drug-resistant TB (XDR-TB) have acquired in 9.5% of MDR-TB cases with 117 countries have reported for XDR-TB in the world.[1] Treatment success rate was only 26% in XDR-TB cases.[1] Drug sensitive strains of TB need treatment duration of 6 months whereas MDR-TB and XDR-TB requires more than 20 months of treatment.[2] The burden of MDR-TB is increasing in various regions of the world. In the last 40 years after rifampicin has been started to be used in 1970s, no new anti-tuberculous drug was introduced.[2] The research for the development of new anti-tuberculous drugs is expensive and slow because the replication rate of tubercle bacilli takes time and the pharmaceutical manufacturers which are present in well-developed countries without TB burden have no much interest.[2]

There was an increasing interest in the development of novel drugs with a different mechanism of action which can combat both drug-sensitive as well as drug-resistant strains of *M. tuberculosis* in last 10 years after global plan has been launched to stop TB.[2] Andries and co-workers at Janssen Pharmaceutical Company discovered bedaquiline, a new anti-tuberculosis (TB) drug and was approved by the US FDA in 2012 to treat MDR-TB as part of combination therapy.[2] Bedaquiline was known to have a novel mechanism of action with the effect on the metabolism of *M. tuberculosis*.[2] It inhibits mycobacterial ATP synthetase and is effective against both replicating and dormant organisms because ATP is still essential in dormant organisms for the survival. Although it has long half-life, early bactericidal activity of bedaquiline at the dose of 400 mg daily was nearly the same to 600 mg rifampicin and 300 mg isoniazid from 4th day onwards in the course of 7 days.[2] Phase II trials indicated that bedaquiline has been well tolerated by the patients and efficacy was good when it is used in combination with background regimen (BR) to treat MDR-TB.[2] Time of sputum conversion was shorter and percentage of sputum conversion was higher in both two months and six months phase trials.[2] Two black boxes were observed with bedaquiline, which are prolonged QT interval and higher mortality when compared with the placebo treatment[3]. Currently Phase III trials are on the way to verify its safety and effectiveness.[2, 3]

Drug resistance mechanisms occur usually by means of horizontal transfer of plasmids or transposons carrying resistance genes between bacteria.[4] For antibiotics, it is feasible to identify resistance in bacteria only after market release.[4] However, drug resistance in the *Mycobacterium tuberculosis* emerged by chromosomal mutations.[4] The methods for detecting resistance mechanisms include identifying drug-resistant mutants *in-vitro*, *in-vivo* animal models and clinical trials.[4] Mutations in the ATP synthase associated with bedaquiline resistance have been found to emerge in the next-generation sequencing approach. Drug resistance was known 8 years after the mechanism of bedaquiline was well understood.[4] Subunit c of

ATP synthase was encoded by *atp*E gene. Five single nucleotide polymorphisms namely A28V, A28P, G61A, A63P and I66M were associated with bedaquiline-resistance.[3] However, 28% of the bedaquiline-resistant *Mycobacterium tuberculosis* harboured these mutations and the remaining 72% did not have such mutations.[3] The mutational upregulation of an efflux pump was observed to be other mechanism of bedaquiline-resistance and the cross resistance to clofazimine can occur because of this mechanism.[3] As a consequence, regimens including both drugs need to be reconsidered as the combination of these two drugs has significant effects on reduction of treatment success.[3]

A standardised shorter MDR-TB regimen was recommended by World Health Organization for the treatment of MDR/RR-TB patients who are still sensitive to fluoroquinolones or second-line injectable agents such as kanamycin, amikacin or capreomycin.[1] Currently, bedaquiline has been started to be used in 70 countries together with BR. Furthermore, the important information is addition of bedaquiline to BR has advantages of decrease in disability-adjusted life years and reduced total healthcare costs when compared with BR.[5] The significance was observed remarkably in high TB burden countries.[5]

REFERENCES

1. World Health Organization. (2016). Multidrug-Resistant Tuberculosis (MDR-TB) 2016 Update.
2. Goel D. (2014). Bedaquiline: A novel drug to combat multiple drug-resistant tuberculosis. J Pharmacol Pharmacother 5 (1): 76 – 78. DOI: 10.4103/0976-500X.124435.
3. Nguyen TVA, Cao TBT, Akkerman OW, et al. (2016). Bedaquiline as part of combination therapy in adults with pulmonary multi-drug resistant tuberculosis. Expert Rev Clin Pharmacol 9 (8): 1025 – 1037. DOI:10.1080 /17512433.2016.1200462.
4. Köser CU, Javid B, Liddell K, et al. (2015). Drug-resistance mechanisms and tuberculosis drugs. Lancet 385: 305 – 307.
5. Lu X, Smare C, Kambili C, et al. (2017). Health outcomes of bedaquiline in the treatment of multidrug-resistant tuberculosis in selected high burden countries. BMC Health Serv Res 17: 87. DOI: 10.1186/s12913-016-1931-3.

Pesticide Toxicity and Oxidative Stress: A Review

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ABSTRACT

Oxidative stress is an imbalance in redox coupling in the body. Lack of antioxidants to scavenge the reactive oxygen species produces adverse effects on health. The causes for an imbalance in redox coupling are multi-factorial. Though, reactive oxygen species are beneficial in the body, excessive generation and lack of proper scavenging may pose a threat. Both internal and external factors may elevate the level. Environmental pollution is a major contributor. Man-made chemicals such as pesticides, heavy metals, and carbon combustion products are blamed. Chronic exposures lead to disease processes through oxidative stress. They mediate pro-inflammatory cytokines and produce free radicals. Pro-oxidant to antioxidant mismatch leads to the adverse effects. Nrf2 activates a number of genes that encode the antioxidants. Glutamate cysteine ligase is activated in response to Nrf2 and it is a key enzyme for GSH production. Nrf2 functionality protects the cells from environmental pollutants. Nrf2 mediates the antioxidant response due to chemical insults, translocated in the cell nucleus. Oxidative stress is known to induce a number of diseases such as genetic abnormalities, carcinogenesis, cardiovascular and respiratory diseases, neuro-degeneration - Parkinson's and Alzheimer's diseases. Pesticides are the major pollutants. Studies confirm oxidative stress and environmental pollution need to be addressed for public welfare.

Keywords: oxidative stress, pesticide, Nrf2, antioxidant, pollution

INTRODUCTION

Environmental pollution is a global phenomenon and the risks and outcomes on human health are a worrying factor. The present situation of pollution is a man-made calamity though the fact of benefit-risk ratio also needs to be weighed equally before complaining on the issue of pollution. From time immemorial, scientists, researchers and policy makers focused on improving the quality of life of people. New technologies, chemical products and medicines were able to increase the life expectancy over the years. Food and water are the basic needs of life; population explosion in turn potentiated the green revolution to increase the food production. Better yield, protection of farm yields from pesticides and other predators were the basic necessity. As a result, different chemicals and pesticide productions were made their way in view of supporting the green revolution. Pesticides are classified into insecticides, herbicides and fungicides based on their target of predator or pest. Over the years new formulations targeted specifically different breeds of pests with the mission of providing basic food need in the world for growing population. The benefit aspect of pesticides was hunger alleviation which is largely met but on the other hand; the risks of exposure, contamination of environment and short and long-term health issues of world population were a new challenge. Pesticides and its metabolites reach easily to every organ system including endocrine glands, reproductive, nervous, cardiovascular, immune, respiratory and renal systems which are the targets for the pesticides. Genetic anomalies and different types of cancers on exposed population challenged the scientific

world. However, the researchers focus in finding the basic mechanisms of pesticide toxicity at organ and cellular levels could yield future path to find solutions.

Chronic exposure to pesticides is directly linked to chronic diseases and mortality to an extent of 60%. According to WHO (2009)[1] reports, around 36 million people died due to the chronic diseases in the world. Pesticides results in production of reactive oxygen species which in turn brings down the antioxidant levels and their defense against oxidative damage in the cellular system. Lipids, proteins and nucleic acids are targeted due to the imbalance and cellular signalling pathways are affected. Oxidative stress and reactive oxygen species induce the long-term health effects such as carcinogenesis, neuro-degeneration, cardiovascular, respiratory, renal, endocrine and reproductive problems. When pesticides disturb the oxidative balance, they pave way for these diseases and homeostasis. Nrf2 is a leucine zipper protein which plays a role in expression of antioxidant proteins against the oxidative stress induced damage. Nrf2 function is maintaining the cellular homeostasis on exposure to oxidative stress due to chemical exposure. On exposure to different pesticides, Nrf2 expression is increased which protects against the oxidative damage.

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 of this journal.

Oxidative Stress

Way back in year 2011 by Sies, based on Nernst formula, oxidative stress and imbalance in redox coupling are same and synonymous. According to Lushchak, oxidative stress in the cells is as result of transient and chronic elevation in reactive oxygen species that harms the normal cellular metabolism and the regulatory process hence adversely affecting the homeostatic balance[2]. Moses Gomberg has described the reactive oxygen species or free radicals almost a century back which are constantly produced in in all the living systems[3]. Their role in varied pathological conditions and disease states were gradually proved over the years by constant research in this area, confirming the deleterious effects in biological systems of animal kingdom and human beings. Free radical scavenging enzyme superoxide dismutase discovery by McCord and Fridovich further strengthened the constant production of free radicals[4]. Though free radicals were thought as agents that produce only adverse effects in the body were disproved and were proved to be useful in attacking the infection producing organisms through activation of immunity and also in the endothelial derived relaxation in response to the production of nitric oxide by arginine and its good effects in the body. Though oxidative stress is dangerous, its positive effects and role in biological functions are thoroughly updated gradually. It is the imbalance and lack of natural scavenging system in combating the deleterious effects which mostly a subject of constant challenge in understanding the oxidative stress among the scientific discoveries.

Most of the reactive oxygen species (abbreviated as ROS) were produced in the mitochondria in eukaryotic organisms. More than 90% of oxygen in the body is converted into water by cytochrome oxidase by reduction process in the electron transport chain (ETC) through four-electron mechanism, but ROS were not released. Electron transport chain in eukaryotes was generally present at interior membrane of mitochondria but in prokaryotes in plasma membrane. Remaining 10% of oxygen is converted into superoxide anion, then to hydrogen peroxide (H_2O_2) which is further yielding hydroxyl radical and anion by addition of an electron. Hydrogen ion from lipids and proteins initiates a chain reaction by abstraction. In the meantime O_2^- and HO^- are the major free radicals, in addition peroxide of proteins, lipids and nucleic acids also constitute their role as free radicals. Any toxic compounds in the biological system may induce an imbalance in the redox

state. Toxic compounds such as heavy metals, drugs and pesticides basically inhibit free radical scavenging enzymes such as superoxide dismutase and glutathione peroxidases while enhancing the generation of malondialdehyde and release of lactate dehydrogenases. Liver is a common target of such insults and generation of superoxide radicals resulting in oxidative stress. A variety of pesticides, organophosphates, chlorpyripos, carbamates, benomyl and diazinon[5, 6, 7]. Diquat induced an increase in reactive oxygen species and long term oxidative stress in genetically CuZn superoxide dismutase enzyme genetic ablated has upregulated the thiol antioxidants mediated by redox sensitive Nrf2 transcription factor[8]. A soil fumigant, 1, 3 –dichloro-2-propanol (1, 3-DCP) induces hepatic toxicity through oxidative stress, nuclear translocation of Nrf2 with an expression of Nrf2 genes[9]. This clearly indicates that, pesticides induce oxidative stress along with translocation of Nrf2 gene expressions. Pesticides are known to induce disruption of endocrine and reproductive axis and a number of molecular mechanisms are disrupted such as enzymes involved in metabolic pathways, synthetic steps of hormones, membranous receptors and nuclear receptors. Pesticides which are also named as xenobiotics mediate their toxicity via the receptor interaction. These receptors are membrane receptors and nuclear receptors. In the nuclear receptors, xenosensors get activated mainly to initiate the metabolism of pesticide molecule so that it may be excreted. Secondly it also lead to the activation of different hormone receptors and the normal hormonal pathways are disturbed. Most of the pesticide induced toxicity is receptor and nuclear mediated in the organism. Around 127 pesticides are classified under endocrine disrupting chemicals which are in use for at least 55 to 60 years in the globe[10]. Promotion of oxidative stress by generation of reactive oxygen species and there by induction of apoptosis by activation of caspases as well as genetic mutation that have procarcinogenic effects through epigenetic alterations, induction of oncogenes and suppression of tumour genes.

Pesticides

Pesticides are artificially manufactured chemical compounds, developed to contain the pests such as different vectors of diseases, agricultural produces, and harmful plants. It may affect the growth of unwanted plants, helps to protect the yields of plants produce such as grains, fruits and vegetables. Overall, it arrests the pests, insects, and acts as a defoliant or desicant. They are the toxic organic substances. Though pesticides were used very long back in history, 19th century documents the usage of them in human welfare in terms of better food production and health. Poisonous substance arsenic was in used against the insects. Gradually sulphur was also used as a pesticide. In 1873, Ziedler developed a compound dichlorodiphenyltrichloroethane, popularly called DDT and Paul Muller tested insect containing properties of same[11]. As the population of the globe was increasing, demand for food need to be met and around 1950s green revolution was initiated in Mexico to increase the agricultural produces. Since then a number of pesticides compounds were manufactured and marketed widely irrespective of their hidden potential towards adverse health effects. Based on their hazard, WHO classified pesticides into a number of different classes. Secondly based on the pest they control, pesticides are classified into different groups – insecticides, herbicides, rodenticides, nematicide, fungicide, acasicide and bactericide.

Based on their chemical nature, classified into:

- Organochlorines
- Organophosphates
- Carbamates
- Synthetic pyrethroid
- Microbial insecticides
- Insect growth regulators

Functional basis of classification of pesticides was on the target organisms. Accordingly they are classified as shown in Table 1[12].

Table 1 Classification of pesticides based on the pests they target

Pesticide class	Target/Action	Example(s)
Acaricide	Mites	Aldicarb, Bifenazate
Algaecide	Algae	Copper sulphate
Attractant	Attracts wide range of pests	Pheromones
Avicide	Birds	Avitrol (aminopyridine)
Bactericide	Bacteria	Copper complexes, streptomycin
Bait	Wide range of organisms	Anticoagulants
Biopesticide	Wide range of organisms	Bacillus thuringiensis
Defoliant	Removes plant foliage	Tribufos
Desiccant	Removes water	Boric acid
Fumigant	Wide range of organisms	Aluminum phosphide
Fungicide	Fungi	Azoxystrobin, chlorothalonil
Herbicide	Weeds	Atrazine, glyphosate, 2,4-D
Insect growth regulator	Insects	Diflubenzuron
Insecticide	Insects	Aldicarb, Carbaryl, imidacloprid
Molluscicides	Snails, slugs	Metaldehyde
Nematicide	Nematodes	Aldicarb, fenamiphos
Piscicide	Fish	Rotenone
Plant growth regulator	Regulates plant growth	Gibberellic acid, 2,4-D
Predacide	Mammal predators	Strychnine
Repellent	Vertebrates and invertebrates	DEET, methiocarb
Rodenticide	Rodents	Warfarin
Silvicide	Trees	Tebuthiuron
Termiticide	Kills termites	Fipronil

All the different types of pesticide compounds have the potentiality to induce toxicity in normal cells of both animal and human and may pose long term threat to homeostasis in the body, thus disturbing the health. Earlier studies have proved evidences towards organophosphate induced oxidative stress in both in vivo and in vitro models of liver and brain. They increase the production of malondialdehyde by glutathione and superoxide dismutase inhibition and DNA strand break[13]. Endosulphan in low doses induce lipid peroxidation in a dose-dependent manner with simultaneous decrease in antioxidant status in liver and heart tissues[14]. Paraquat is capable of inducing oxidative stress by over expression of SOD enzymes, apoplastic reactive oxygen species, activation of NADPH oxidase complex and apoptosis[15]. Methyl parathion induces oxidative stress, damage of DNA and apoptotic cell death in human gingival fibroblasts which acts as a cytotoxic and genotoxic pesticide through generation of free radicals[16]. Methyl parathion has weak genotoxic and cytotoxic effects induced by a decrease in ascorbic acid in the testicular cell lines in rat model[17].

Exogenous Agents and Oxidative Stress

Oxidative stress is the results of production of reactive oxygen species (ROS) sometimes they are also have nomenclature as reactive oxygen intermediates (ROI). They are a result of metabolism of tissues. ROS act as signalling mediators as most of the time may be beneficial. Most of the exogenous substances may activate the production of these ROS. Cigarette smoke, UV radiation in the atmosphere, alcohol, drugs and cancer chemotherapeutic agents and radiotherapy treatments as well induce oxidative stress. Petroleum combustion products, heavy metals as well as pesticide particles and their metabolic end products too initiate the oxidative stress. Infections, tissue injury, and ischaemia also contribute in the elevated levels of ROS. Cigarette smoke is one of the exogenous agents that induce oxidative damage in cell line. Cigarette smoke induces oxidation

of structural and functional components and also able to decrease the endothelial growth factors[18, 19]. In a recent study, Kau et al. (2016) reported that, exposure to cigarette smoke has elevated the oxidative stress as indicated by the significant elevation in the MDA levels in orbital fibroblasts[20]. Exposure to smoking during gestational period has resulted in oxidative stress and hypoxia in BALB/c mice; antioxidant manganese superoxide dismutase activity was reduced with an increase in nitrotyrosine, a protein damage marker[21]. Pathogenesis of lung parenchymal cell population by smoking has a direct correlation with reactive oxygen species generation. In animal model, exposure to cigarette smoke has confirmed a reduction in superoxide dismutase, catalase and glutathione peroxidase with a simultaneous elevation in thiobarbituric acid levels in the lung tissues of mice[22]. E- Cigarette or vap is gaining more popularity among the adolescents and young generations as the perception of society at large as a reduced risk for higher levels of nicotine compared to the conventional cigarettes. Exposure of lung epithelial cells to e cigarette vapours resulted in higher levels of oxidative stress[23]. Few natural antioxidants such as icaritin was proved to decrease cigarette smoke induced oxidative stress by up-regulation of glutathione through P13K-AKT-Nrf2 dependent pathways. Host defense functions and redox homeostasis and mitochondrial biogenesis are possible by the transcription factor Nrf2 which is also a key element on metabolism and cell cycle processes. Heavy metal such as cadmium is a toxic compound that is present in the environment. Cadmium toxicity results in a decline in antioxidant status and an increased oxidative stress levels. When cadmium intoxicated rats were supplemented with proanthocyanidins, Nrf2 expression were increased in cardiac cells[24]. It is also speculated and proved that, neurodegenerative effects in Parkinson's disease is an outcome of oxidative stress leading into the loss of dopaminergic neurons. Heavy metals were basically are the root cause such as cobalt, iron and copper by generating reactive oxygen species that gradually deteriorate the functional ability of dopamine neurons[25]. Hepcidin is a hepatic antimicrobial peptide which helps in the absorption of iron which is generated by the presence of xenobiotic and heavy metals, but the cells are protected and regulated by Nrf2 which defences against the hepcidin and extending its role as an antioxidant[26]. Another heavy metal, antimony induces oxidative stress in biological system leading to programmed cell death. Nrf2 is expressed in response to this toxicity which tries to nullify the apoptotic mechanism induced by antimony[27]. Heavy metals such as Pb, Cd, Ni, Al, Mn and Zn though are not directly generating reactive oxygen species in the biological system, they contribute indirectly in oxidative stress by NADPH oxidase system and the subsequent ROS produced affect the expression of genes, cell cyles and programmed cell death in both plant and animal cells. Heavy metals in the environment are a constant threat to mankind and along with petroleum combustion products which also enhances the oxidative stress and subsequent disease processes.

Pesticide Induced Oxidative Stress

Agricultural workers those are continuously exposed for a long duration of pesticides had a remarkable decrease in antioxidant enzyme levels such as superoxide dismutase; in addition genetic polymorphism of paraoxonase-1 (PON1), glutathione S-transferases and cholinesterases metabolizing enzymes. It is clear in the literature that, pesticides generate oxidative stress by production of ROS which in turn decreases the antioxidant status. Oxidative stress is the major toxic pathway that affects the cell cycles and death on exposure to xenoestrogens. Exposure to environmental toxins is known to affect the neuro-physiological processes that may develop autistic spectrum of conditions. Pathogenesis of autism was speculated to be based on environmental toxins such as pesticides. Gestational stage exposure of mice to chlorpyrifos has resulted in an oxidative stress which led to the autism features exhibiting delay in functional and somatic growth at the postnatal stages[28]. Organophosphate compounds which are in common usage induce

lipid peroxidation, nitric oxide synthase are activated that synthesizes nitric oxide which also forms a pro-oxidant damaging the normal neurons. Exposure to diuron resulted in DNA damage through oxidative stress in both male and female germinal cell lines of Pacific oyster crassostrea gigas[29].

In aerobic organisms oxidative stress is a continuous process that keep generating reactive oxygen species. In normal conditions of health, ROS scavenging system is able to bring a balance by nullifying the toxic potential of these oxidative stress insults. If there is any imbalance in scavenging the oxidative stress, it may result in toxic effects at molecular, genetic and cellular levels. Mitochondria and endoplasmic reticulum, the two cell organelles along with cytochrome P450 which forms the electron transport chain in animal kingdom are the main molecules. In plants, chloroplasts function as alternative sources. In response to oxidative stress, a series of products are released which actually has a tissue damaging effect. As a marker of oxidative stress, lipid peroxidation products such malonyldialdehyde (MDA) thiobarbutyric acids, and 8-hydroxy-2-deoxyguanosine (8-OHdG) are released and in response to these, different antioxidant enzymes such as superoxide dismutase, GSH, catalase, xanthine oxidase, etc.

Organophosphate, organochlorine and flurorines, herbicides, carbamates and pyrethroids, etc. were known to generate oxidative stress. Organophosphate compounds inhibit the enzyme acetyl cholinesterases which favours the lipid peroxidation. This will follow ATPase activity disturbances. Prolonged exposure also depletes superoxide dismutase, GST, GPX, etc.[30] Depletion of glutathione S- transferase activity with an elevated hydroxyperoxide levels are also common in few vital organs on exposures. In our own previous studies, diazinon has elevated the level of oxidative stress in testis and endosulphan in the vital organs[7, 14]. In addition others study have also confirmed a reduction in Na^+/K^+ - ATPase activities. Methyl parathion increased the MDA and a reduction in GSH and also in SOD. Chlorpyriphos, carbamates and monocrotophos have also increased the lipid peroxidation with a reduction in CAT, SOD and GST levels in experimental models in different organs. Similarly paraquat, cypermethrin, atrzine rotenone and diurons have also depleted the antioxidant status with an increase in lipid peroxidation parameters.

Antioxidants

Natural antioxidants are commonly found in the nature. Phytochemicals, enzymes and few vitamins are few of the antioxidants which generally are the plant sources. Some co-factors such as selenium, copper, zinc magnesium and iron are essential for the activity of antioxidant enzymes in the biological system. Few antioxidant enzymes are superoxide dismutase (SOD), catalases, glutathione peroxidases and reductases which are synthesized in the body. Dietary vitamins which are not synthesized also function as antioxidants such as vitamin A, C, and E, beta carotenes and folates. Some phytochemical molecules play an important role in the antioxidant properties mainly derived from plants. Polyphenols, flavonoids and carotenoids are few examples. Chlorpyrifos exposure is known to decrease SOD activity, catalase, glutathione reductase in experimental rats[31]. Dichlorvos and lindane treatment in brain tissues of the rats have significantly attenuated the concentrations of SOD, catalases and glutathione transferase activities which was reversed by ginger[32]. A mixture of pesticides-molinate, thiobencarb, linuron, phorate, primiphos methyl, fenvelerate and lambda – cyhalothrin treatment to Drosophila and analysis of CAT, GS and Mn-SOD genes by real time PCR exhibited a rise in their expressions with a concomitant increase in heat shock proteins (HSP26) and a decrease in HSP60 transcription[33]. Organophosphate, dichlorvos induced lipid peroxidation in human erythrocytes were attenuated by vitamin C and E, and a simultaneous beneficial effects on a series of antioxidant enzymes in in-vitro studies[34]. In a meta-analysis report, there was a higher

antioxidant levels and lesser concentration of cadmium and pesticides in organic farming compared to that of non-organic practices[35]. This is another fact that, pesticides and other heavy metals impact the antioxidant system in the food chain. Nuclear factor erythroid 2 - Nrf2, a NF- E2 transcription factor plays an important role in the oxidative stress induced by any chemical compounds. Chemical insults lead to exhaustion of glutathione and activate Nrf2 in the cell nucleus. This in turn affects the heme oxygenases. Nrf2 is a leucine zipper protein and activates a number of genes that encode the antioxidants. Glutamate cysteine ligase is activated in reponse to Nrf2 and it is a key enzyme for GSH production. Nrf2 functionality protects the cells from environmental pollutants. Nrf2 mediates the antioxidant response element which is expressed in response to chemical insults and is translocated in the cell nucleus. Nrf2 regulates the antioxidant related genes thereby both normal and adverse effects of oxidative stress will be nullified. Reactive oxygen species and nitrogen species are mainly neutralized by different antioxidants to maintain the redox balance in biological cells. Majority of afore-mentioned antioxidants are low molecular weight molecules which enable $NADP^+$/NADPH and NAD^+/NADH. NADPH further helps in the reduction process. Nrf2-Keap 1 signalling pathway provides the functional support in the antioxidant system. Nrf2 involves suppression and activation processes. A variety of molecules produce ARE gene such as environmental pollutants such as few pesticides, chemicals, therapeutic drugs, photochemical and few endogenous substances like nitric oxide through Nrf2. So Nrf2 is the key factor in homeostasis by regulatory control on antioxidant system on oxidative stress markers.

Pesticides – Oxidative Stress and Diseases

In toxicology studies, pesticide induced oxidative stress is a major area of research as environmental factors that aggravate the disease processes due to the residues of these chemicals. Pesticides have deleterious effects on biological system and are able to generate oxidative stress. Though oxygen is the basic gas that is indispensable for sustaining life, it may be toxic enabling the formation of toxic substances in the body. These chemicals are the reactive oxygen species which is able to transfer oxygen forming free radicals. These are unstable molecules with unpaired electrons. They have hydroxyl, lipid peroxyl, and superoxide and nitric oxide moieties[36]. Since they are the unstable molecules, they attack the neighbouring like carbohydrates, proteins, nucleic acids and lipids to accept an electron and meantime producing a damaging effect. These adverse effects induce genotoxic effects and may lead to carcinogenic effects and also atherosclerosis and neuro-degenerative problems and Parkinsonism[37]. These reactive oxygen species are generated either due to external insults or also due to normal biochemical metabolic functions. Pesticides, heavy metals, cigarette smoke, drugs, etc. are few of the common external insults. Oxidative stress is a common basis for many of the disease processes that may have chronic or permanent health effects. Few of the diseases in which oxidative stress was responsible for the pathophysiological changes are – autoimmune diseases, ophthalmic conditions like retinopathy, cataract, bronchial asthma, neurodegenerative diseases such as Parkinsonism, Alzheimer's, and dementia are the common among them. Various types of malignancies, cardiovascular diseases such as atherosclerosis, stroke, ischaemia, thalassaemia and inflammatory conditions to have their pathophyiological sequel which are directly correlated to the oxidative stress[38].

Pro-oxidant to anti-oxidant imbalance lead to the intracellular damage, DNA, RNA was also targeted and these reactive oxygen species produce 8-hydroxy-2$^{'}$ –deoxyguanosine which may produce gene-mutations and nicks in the DNA. This may increase the carcinogenesis[39]. As genetic factors are contributing to the development of cancer, metabolic activities in cancer cells further enhances the generation of reactive oxygen species. Nrf2 in turn activates a number of genes that promote antioxidant

enzymes and few immune and inflammation inducing genes. Nrf2 and its suppressor protein Keap 1 help to regulate the harmful oxygen species but carcinogenesis and metastasis may also promote due to the ROS[40]. Secondly Ras pathways are also activated by oxidative stress which may induce point mutations and oncogenes. Subsequently oncogenic proteins will be over expressed and silencing of tumour suppressor genes. Oxidative stress is also the prime factors that induce inflammation and atherogenesis in blood vessels. It leads the development of fatty streaks in the vascular system. When mitochondrial respiratory chain is dysfunctional, it paves the way for the atherosclerosis. A number of mediators of oxidative stress are released from the dysfunctional mitochondria such as NADPH oxidases, xanthine, lipogenase, myeloperoxidase and nitric oxide synthases which are the causative factors in the formation of plaques and atherosclerosis. This is mainly affected whenever there is an imbalance in the pro-oxidant to antioxidant ratio[41]. Prolonged exposure to organophosphate compounds has been reported to accelerate the coronary blood vessel atherosclerosis by a decline in paraxonase activity. Cholesterolemia and increase in LDL are the two sources that promote premature atherosclerotic incidences and generally LDL is a major source having atherogenic potential. LDL once oxidized it enhances generation of monocyte colony stimulating factor by a series of steps. It leads to the formation of macrophages which helps in the uptake of LDL. Oxidized LDL promotes a number of biological activities and along with oxidative stress agents it promotes the atherosclerotic potential by the generation of NADPH oxidase, eNOS, myeloperoxidases xanthine oxidizes cyclooxygenase and oxidative phosphorylation in the mitochondria[42].

Parkinson's disease is a neuro-degenerative disorder which keeps progressing over time. There is loss of dopamine secreting neurons in the selected nuclei of basal ganglia. Mitochondrial respiratory chain dysfunction which lead to the generation of ROS and in turn the toxic potential of these oxidative stress products lead to the neuronal cell death in substantia nigra in the corpus striatum. This neuronal loss is regulated by the microglia cells, when stimulated produce ROS like superoxide and nitric oxide. This is the basis for inflammation of neurons and a neurodegenerative process in dopaminergic neurons. Further, dopaminergic neuronal dysfunction lead to the activation of microglia which release neuromelanin and a vicious cycle of loss of dopamine secreting cells are set in for the Parkinson's symptoms[43]. Overall, most of the pesticides which are discussed in this chapter generate the oxidative stress and are the basis for different diseases of cardiovascular, neuronal, respiratory, genetic, reproductive, and hepatic and many other system of the body.

CONCLUSIONS

Pesticides are basically used for the purpose of mitigation of hunger, contain pests and diseases. Use of pesticide has impacted the hunger alleviation of the world. Along with its benefits, untoward health problems have been increased tremendously; genetic toxicity, carcinogenesis, mutations, infertility, respiratory, neurodegenerative and many more diseases are on a rise. It is alarming to confirm that, this health related adverse conditions are directly proportional to the environmental pollution and pesticides are blamed greatly. Scientific evidences prove the mediation of these toxicities is through an imbalance in the redox equilibrium and they constantly generate reactive oxygen species at cellular levels of living beings. Mediators of oxidative stress are released from the dysfunctional mitochondria; NADPH oxidases, xanthine, lipogenase, myeloperoxidase and nitric oxide synthases. Nuclear factor erythroid 2 - Nrf2, a NF- E2 transcription factor plays an important role in the oxidative stress induced by these chemical compounds. Chemical insults lead to exhaustion of glutathione and activate Nrf2 in the cell nucleus. This in turn affects the heme oxygenases. Nrf2 is a leucine zipper protein and activates a number of genes that encode the antioxidants. Glutamate cysteine ligase is activated in

response to Nrf2 and it is a key enzyme for GSH production. Nrf2 functionality protects the cells from environmental pollutants. Nrf2 mediates the antioxidant response element which is expressed in response to chemical insults and is translocated in the cell nucleus. Nrf2 regulates the antioxidant related genes thereby both normal and adverse effects of oxidative stress will be nullified. It is a major concern as many of the incurable health conditions and diseases are making their way in human life. Genetic defects, inborn errors, mutagenecity, infertility are of greater concern. Though benefit-risk ratio outweighs towards the benefit to mankind, the extent of health risk on exposure to pesticides need to be heavily relooked and measures to minimize the adversities need to be addressed in the 21st century for the greater cause of healthy human existence.

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REFERENCES

1. WHO. (2009). The WHO recommended classification of pesticides by hazard and guidelines to classification 2009. Geneva World Health Organization, International Programme on Chemical Safety. Available from http://www.who.int/ipcs/publications/pesticides_hazard_2009.pdf
2. Lushchak VI. (2014). Review. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 224C: 164 – 175.
3. Gomberg M. (1900). An instant of trivalent carbon: triphenylmethyl. J Am Chem Soc 22: 757 –771.
4. McCord JM, Fridovich I. (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem 244: 6049 – 6055.
5. Yamano T, Morita S. (1992). Hepatotoxicity of trichlorfon and dichlorvos in isolated rat hepatocytes. Toxicol 76: 69 – 77.
6. Bagachi D, Bhattacharya, Stohs SJ. (1996). In vitro and in vivo induction of heat shock (stress) protein (hsp) gene expression by selected pesticides. Toxicol 112: 57 – 68.
7. Leong CT, D'Souza Urban J, Iqbal M, Mustapha ZA. (2013). Lipid peroxidation and decline in antioxidant status as one of the toxicity measures of diazinon in the testis. Reodx Rep 18: 155 – 164.
8. Han ES, Muller FL, Perez VI, Qi W, Liang H, Xi L, Fu C, Doyle E, Hickey M, Cornell J, Epstein CJ, Roberts LJ, Van Remmen H, Richardson A. (2008). The in vivo gene expression signature of oxidative stress. Physiol Genomics 34: 112 – 126.
9. In-Chul Lee, Je-Won Ko, Sang Min Lee, Sung HK, In-Sik S, Og Sung M, Won KY, Hyoung CM, Jong CK. (2015). Time-course and molecular mechanism of hepatotoxicity induced by 1,3 –dichloro-2 propanol in rats. Environ Toxicol Pharmacol 40: 191 – 198.
10. Pesticide Action Network (PAN). (2005). List of lists: a catalogue of lists of pesticides identifying those associated with particularly harmful health or environmental impacts.
11. Othmer, K. (1996). Encyclopedia of Chemical Technology, John Wiley and Sons Inc. New York, USA.
12. Fishel FM. (2012). Fungicide Resistance Action Committee's (FRAC) classification scheme of fungicides according to mode of action. Pesticide information office: UF/IFAS Extension, Gainesville.
13. Osman, KA. (1999). Lindane, chlorpyrifos and paraquat induced oxidative stress in female rats. Alex. J. Agric Res 44: 345 – 355.
14. Alva S, Damodar D, D'Souza A, D'Souza UJ. (2012). Endosulfan induced early pathological changes in vital organs of rat: A Biochemical approach. Ind. J. Pharmacol 44: 512 – 515.
15. Lascano L, Munoz N, Robert G, Rodriguez M, Melchiorre M, Trippi V, Quero G. (2012). Herbicides – properties, synthesis and control of weeds. Available from https://cdn.intechopen.com/pdfs/25618.pdf.

16. Argentin G, Divizia M, Cicchetti R. (2015). Oxidative stress, cytotoxicity and genotoxicity induced by methyl parathion in human gingival fibroblasts: Protective role of epigallocatechin -3-gallate. J. Toxicol Environ Health A 78: 1227 – 1240.
17. Narayana K, Prashanthi N, Nayanathara A, Kumar HH, Abhilash K Bairy KL. (2005). Effects of methyl parathion (O,O- dimethyl-o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility are associated with decreased ascorbic acid levels in the testis. Mutat Res 588: 28 – 24.
18. Repine JE, Bast A Lankhorst. (1997). Oxidative stress in chronic obstructive pulmonary disease. Oxidative stress study group. Am J Respir Crit Care Med 156: 341 – 357.
19. Kasahara Y, Tuder RM, Cool CD, Lynch DA, Flores SC, Voelkel NF. (2001). Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. Am J Respir Crit Care Med 163: 737 – 744.
20. Kau HC, Wu SB, Tsai CC, Liu CJL, Wei YH. (2016). Cigarette smoke extracts-induced oxidative stress and fibrosis-related genes expression in orbital fibroblasts from patients with Graves' Opthalmology. Oxidative Med & Cellular Longevity 4676289: 1 – 10. doi: 10: 1155/2016/4676289.
21. Chan YL, Saad S, Pollock C, Oliver B, Al-Odat, Zaky AA, Jones N, Chen H. (2016). Impact of maternal cigarette smoke exposure on brain inflammation and oxidative stress in male mice offspring. Sci Rep 12: 6: 25881.
22. Nesi RT, de Souza PS, Dos Santos GP, Thirupathi A, Menegali BT, Silveira PC, da Silva LA, Valenca SS, Pinho RA. (2016). Physical exercise is effective in preventing cigarette smoke-induced pulmonary oxidative response in mice. Int J Chron Obstruct Pulmon Dis. 11: 603 – 610.
23. Scheffler S, Dieken H, Krischenowski O, Forster C, Branscheid D, Aufderheide M. (2015). Evaluation of E-ciga-ette liquid vapour and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells. Int J Environ Res Public Health 12 (4): 3915 – 3925. doi: 10.3390/ijerph120403915 PMID:25856554.
24. Nazimabashir, Manoharan V, Miltonprabu S. (2015). Cadmium induced cardiac oxidative stress in rats and its attenuation by GSP through the activation of Nrf2 signaling pathway. Chem Biol Interact 242: 179 – 193.
25. Lan AP, Chen J, Chai ZF, Hu Y. (2016). The neurotoxicity of iron, copper and cobalt in Parkinson's disease through ROS-mediated mechanisms. Biometals 29 (4): 665 – 678. Doi: 10: 1007/s 10534-016-9942-4.
26. Bayele HK, Balesaria S, Srai SK. (2015). Phytoestrogen modulate hepcidin expression by Nrf2: Implications for dietary control of iron absorption. Free Radic Biol Med 89: 1192 – 1202.
27. Jiang X, An Z, Lu C, Chen Y, Du E, Qi S, Yang K, Zhang Z, Xu Y. (2016). The protective role of Nrf2-Gadd45b against antimony-induced oxidative stress and apoptosis in KEK293 cells. Toxicol Lett 256:11 – 18.
28. De Felice A, Greco A, Calamandrei G, Minghetti L. (2016). Prenatal exposure to the organophosphate insecticide chlorpyrifos enhances brain oxidative stress and prostaglandin E2 synthesis in a mouse model of idiopathic autism. J neuroinflammation 13: 149.
29. Barranger A, Heude-Berthelin C, Rouxel J, Adeline B, Benabdelmona A. Burgeot T, Akcha F. (2016). Prenatal exposure to the herbicide diuron results in oxidative DNA damage to germinal cells of the Pacific oyster Crassostrea gigas. Comp Biochem Physiol C Toxicol Pharmacol 180: 23 – 30.
30. Gupta J, Datta CH, Sarkar A, Senugupta D. (1992). Effect of malathion on antioxidant defense system in human fetus an in vitro study. Ind J Exp Biol 30: 352 – 354.
31. Agarwal K, Singh D, Singla SK. (2014). Studies on the effect of oxidative stress induced by chlorpyripos on antioxidant hepatic enzyme in rat. World J Phar & Pharmaceutical Sci 3: 523 – 533.
32. Poonam S, Singh R. (2012). Dichlorvos and lindane induced oxidative stress in rat brain: Protective effects of ginger. Pharmacognosy Res 4: 27 – 32.
33. Oguzhan D, Zeynep BD. (2015). Responses of antioxidant enzymes and heat shock proteins in Drosophila to treatment with a pesticide mixture. Arch Biol Sci, Belgrade 67: 869 – 876.

34. Sema E, Dilek P, Fatma GU, Hatice B. (2013). Protective role of vitamins C and E in dichlorvos – induced oxidative stress in human erythrocytes in vitro. Biol Res 46: 33 – 38.
35. Barański M, Srednicka-Tober D, Volakakis N, Seal C, Sanderson R, Stewart GB, Benbrook C, Biavati B, Markellou E, Giotis C, Gromadzka-Ostrowska J, Rembiałkowska E, Skwarło-Sońta K, Tahvonen R, Janovská D, Niggli U, Nicot P, Leifert C. (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-anlysis. Br J Nutr 112: 794 – 811.
36. Cochranc CG. (1991). Cellular injury by oxidants. Am J Med 92: 235 – 305.
37. Banerjee BD, Seth V, Ahmed RS. (2001). Pesticide-induced oxidative stress perspectives and trends. Rev Environ Health 16: 1 – 40.
38. Goodyear-Bruch C, Pierce JD. (2002). Oxidative stress in critically ill patients. Am J Crit Care 11: 543 – 351.
39. Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, Watanabe M, Hibi T, Kitajima M. (2000). Increased formation of oxidative DNA damage, 8-hydroxy-2' – deoxyguanosine in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. Cancer Letters 151: 87 – 95.
40. Hybertson BM, Gao B, Bose SK, McCord JM. (2011). Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. Mol Aspects of Med 32: 234 – 246.
41. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. (2008). Atherosclerosis and oxidative stress 23: 381 – 390.
42. Zamzila AN, Aminu I, Niza S, Razman MR, Hadi MA. (2011). Chronic organophosphate pesticide exposure and coronary artery disease: Finding a bridge. IIUM Research, Invention and Innovation exhibition (IRIIE).
43. Onyou H. (2013). Role of oxidative stress in Parkinson's disease. Exp Neurobiol 22: 11 – 17.

*Kat*G 315 Mutation as a Molecular Determinant for Isoniazid Resistance in *Mycobacterium tuberculosis*

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ABSTRACT

Emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) is one of the reasons why tuberculosis (TB) continues to cause great mortality and morbidity in less-developed countries. The development of rapid diagnostic methods targeting genetic mutations associated with resistance to the anti-tuberculous drugs is essential to fight this deadly pathogen. Isoniazid (INH) has been included in the multidrug regimens for the treatment of drug-susceptible TB for the decades. In the worldwide setting, isoniazid resistance was highly prevalent and was observed in one of every seven TB cases. Since *kat*G315 mutation is highly prevalent, the common mutation in the enzyme essential for the activation of the INH concerned with the mechanism of drug resistance and associated with high level resistance to INH, *kat*G315 mutation was necessary to be identified by molecular method as a molecular determinant of INH resistant *Mycobacterium tuberculosis*. The prevalence of *kat*G315 mutation in various countries was discussed in this report and a new molecular method for the detection of the mutation was proposed.

Keywords: *Kat*G 315 mutation, molecular determinant, isoniazid resistance, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis (TB) continues to cause great mortality and morbidity in less-developed countries although an effective drug regimen has been available for decades. The reason for high mortality is prevalence of TB in HIV/AIDS pandemic population and emergence of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB). These two factors made the control of the TB complicated worldwide.[1, 2] About 85% of new cases of TB were in Asia and Sub-Saharan Africa with 8.8 million new cases of TB were reported in 2010 and 1.4 million deaths occurred due to TB worldwide.[3]

Conventional methods for detection of drug resistance were usually undertaken in the reference laboratory in case of poor resource country with the delay of 4 – 8 weeks to get the results. Within that period, the patient was not properly treated with consequence of acquiring more serious drug resistance and having problem of unnecessary toxic effects of drugs, especially in HIV patients superimposed by TB. Rapid detection of drug resistance and starting of the correct treatment could overcome spread of multidrug resistant pathogens in the community and these measures are the top priorities in TB control. The development of rapid diagnostic methods targeting genetic mutations associated with resistance to the anti-tuberculous drugs in the tubercle bacilli is beneficial to fight this deadly pathogen.[4, 5] Studies of mutations and Single Nucleotide Polymorphisms (SNPs) within the genes associated with drug resistance were the research area which comes to the frontier in the control of TB.

History of Drug Resistance in *Mycobacterium tuberculosis*

Two anti-TB drugs, para-aminosalicylic acid and isoniazid (INH) were discovered in 1946 and 1952 respectively.[6, 7, 8] Although both of

the drugs were active against *Mycobacterium tuberculosis* (*M. tuberculosis*), drug resistance emerged within short period during the clinical usage of single drug.[9, 10, 11] Drug resistance has emerged to these two drugs in a short time because the drugs were used singly instead of combination with other anti-TB drugs. These two drugs, together with streptomycin (SM), which was discovered earlier, were combined in a first successful multidrug regimen for TB.[12] This three drug regimen was very effective that it was thought a foe of man has been already fought. The limitation of this regimen was long and expensive so that dropped out cases among patients made the therapy incomplete and problem of drug resistance emerged. In 1984, a new short-course treatment was started and this regimen had advantages of improved efficacy and better compliance of patients. The short course includes four drugs for 2 months followed by two drugs for 4 months. Four drugs were INH, rifampicin (RIF), pyrazinamide (PZA), and ethambutol and two drugs included INH and RIF. Strains resistant to INH and RIF started to emerge in 1985. To date, 20% of previously treated TB cases are caused by MDR-TB whereas nearly 4% of new cases were infected by these strains.[13] The *M. tuberculosis* strains which are resistant to INH, RIF, fluoroquinolones and one second-line drug that has to be given by injection are called XDR-TB. XDR-TB isolates are observed in at least 84 countries and prevalent up to 9% of MDR-TB. In addition, totally drug-resistant (TDR-TB) *M. tuberculosis* strains have already emerged in India, Iran, Africa and Europe and these strains are characterized by resistance formation to 10 TB drugs.[14] The reason for the starting and spreading of drug resistance is the delay in diagnosis because of unavailability of rapid diagnostic facilities.[15] Drug resistance mechanisms are essential for the diagnosis of these XDR-TB and TDR-TB. For MDR-TB, drug resistance mutations are well known.

INH Resistance in *M. tuberculosis*

INH is the prodrug and catalase peroxidase (*kat*G) activates INH which reacts with NAD+ resulting in INH-NAD. This compound inhibits InhA with the consequence of stoppage of mycolic acid biosynthesis which ends in mycobacterial cell death. SNPs in *kat*G gene result in inactive *kat*G with loss of activation of INH. S315T mutation of *kat*G was observed in 94 – 95% of INH-resistant clinical isolates.[15]

INH was synthesized in 1912 and its anti-tuberculous activity was known after 40 years. It has been included in the multidrug regimens for the treatment of drug-susceptible TB. In addition, INH monotherapy has been recommended for the management of latent TB. INH preventive therapy was well effective for HIV-infected individuals. In the worldwide setting, INH resistance was highly prevalent and was observed in one of seven TB cases.[16] In this review prevalence of *kat*G 315 mutations and other *kat*G mutations for INH resistance in different countries will be described.

*Kat*G Mutation in Multiple Drug-resistant Strains and Isoniazid Mono-resistant Strains

Fifty multiple drug-resistant, 50 INH mono-resistant and 50 susceptible strains of *M. tuberculosis* from the National Tuberculosis and Lung Diseases Research Institute in Warsaw, Poland were investigated for the prevalence of isoniazid resistance-associated mutations. Mutation distribution patterns between INH-monoresistant and MDR strains were compared in this study.

Of 109 INH resistant isolates, *kat*G 315 was observed in 46 isolates of the MDR strains, 31 of the INH-monoresistant strains whereas it was present in 2 pan-susceptible strains. G944C mutations at nucleotide level resulting in Ser315Thr (S315T) amino acid change was found in 33 MDR and 21 INH-monoresistant strains. G944C and C945T substitutions were coexistent in three of the isolates resulting in

same S315T mutation. G383A (Arg128Gln) and C701G (Ala234Gly), G1388T (Arg463Leu) mutations were observed in more than one isolate in MDR strains whereas A1197G (Glu399Glu) was present in three mono-resistant strains.[16]

*Kat*G Arg463Leu more Common than *kat*G Ser315Thr in Taiwan

In the study in Taiwan, the results were different with the observations in the other studies. Seventy *M. tuberculosis* isolates collected during the period from 1999 to 2011 were included in the study with the observation of 41 INH resistant isolates among 46 drug resistant isolates. Mutations were *kat*G Arg463Leu (R463L) (51%), S315T (29%), Ser315Asn (S315N) (9.8%), and other loci (22%). The canonical mutation S315T was relatively uncommon when compared with the other studies. This result makes the GenoType MTBDR plus molecular technique unreliable as this molecular diagnostic method used the *kat*G S315T as the probe (see Table 2).[17] However, the most prevalent mutation observed in the study, *kat*G R463L did not correlate with INH resistance as shown by the study in Netherlands where the mutation was nearly equalled in proportion among INH-susceptible isolates and INH resistant isolates.[18] In addition, the activity of the catalase-peroxidase in *M. tuberculosis* was not significantly changed by R463L which is induced in site-directed mutagenesis.[17]

*Kat*G315 Mutations in 49 Countries: A Literature Review

Although more than 95% of RIF resistance is associated with mutations in Rifampicin Resistance determining region (RRDR) which is 81bp region of the *rpo*B single gene, INH resistance is associated with mutations in multiple genes.[19, 20, 21, 22, 23, 24, 25] The existing molecular diagnostic methods for rapid detection of INH resistance have focused on the identification of the "canonical" mutations which are *kat*G codon 315 and *inh*A promoter region -15 nucleotide. However, the common one of the two canonical mutations was the point mutations in *kat*G codon 315. *Kat*G 315 mutations were prevalent up to 95% in the previous studies.[26, 27, 28] *Kat*G 315 mutation was averagely common up to 64.2 % of 8416 INH resistant isolates and 0.1% of 2462 INH sensitive isolates. S315T mutation was the most common mutation among *kat* gene of INH resistant isolates with second most common mutation has been S315N in the *kat* gene.[29] These two mutations can be used as molecular determinants of INH resistance in most of the studies although their frequency did not reach 100% in most of the studies.

*Inh*A promoter region -15 mutation was the most common mutations in the *inh*A gene and it was common up to 19% together with other mutations in the promoter region of the gene.[29]

Of 4505 isolates which had change of nucleotide with consequent change of amino acid information in *kat*G 315 mutation, *kat*G S315T (AGC-ACC) was present in 93.4% of isolates, whereas Ser315Asp (S315D) was 3.6% and *kat*G S315T (AGC-ACA) was 1.6%. Other mutations occurred among less than 1% of the isolates. As a common sense, those mutations with one nucleotide change were more common than those with two nucleotide change. However, S315T (AGC-ACA) was more common than Ser315Ile (S315I) (AGC- ATC).[29]

Different *kat*G Mutation at other Codons in Malaysia

The study in HUSM, Kelantan for mutations in *kat*G gene for INH resistant genes indicated that mutations in *kat*G 315 codon were not observed. Of 9 drug resistant isolates, only four isolates were observed to have INH resistant phenotypes. One isolate of these four isolates has Gln247His mutation at codon 247, another one has Val61Gly mutation at codon 61 and one also has Ala62Thr mutation at codon 62. The other INH resistant isolate has no mutation in the amplified region of *kat*G gene or no mutation in the *inh*A gene or its promoter region or in the other genes in which mutation leads to

INH resistance by *M. tuberculosis*.[30] However, *kat*G Leu238Arg, Ser238Ala mutations were collectively observed in 7 isolates with no phenotypic resistance to INH.[30]

Whole Genome Sequencing (WGS) Approach for Detection of Drug Susceptibility in Myanmar

Myanmar is highly prevalent for MDR-TB and was included in high-burden tuberculosis (TB) countries. It is of no doubt that earlier detection of MDR-TB is important for the control of tuberculosis.[31] Well-resourced, low-TB burden countries have facilities for WGS and it was considered for the diagnosis of drug-resistant TB. However, in resource-limited, high-TB burden country like Myanmar, routine implementation was not yet planned. As the improvement of TB control should be adopted earlier in the countries in which the facilities are needed most, evaluation of the usage of WGS in the diagnosis of MDR-TB and XDR-TB was conducted.[32]

Moreover, drug susceptibility testing (DST) with conventional method is time-consuming and taking weeks due to the prolonged culture necessary in *M. tuberculosis* with subsequent phenotypic testing. For these reasons, molecular methods such as GenoType MTBDRplus v.2.0 and GeneXpert MTB/RIF have been established in Myanmar. However, these methods can detect drug resistant mutations for the limited number of anti-tuberculous drugs. Whole-genome sequencing (WGS) is the possible way to supersede these methods.[33, 34, 35] Fourteen MDRTB isolates were sequenced by WGS and the results were consistent with phenotypic drug susceptibility testing (DST). Of 14 MDRTB isolates, all the isolates were resistant to INH with the mutations observed were *kat*G315 in 10 isolates and *inh*A promoter mutation in 2 isolates. The rest of the two isolates had G299C mutation in *kat*G gene in one isolate and frame shift mutation in *kat*G gene in the other isolate. *Kat*G315 mutations in 10 isolates were the same with change from Serine to Threonine whereas *inh*A mutations were at −15 nucleotide in the promoter region (Table 1).[32]

INH Resistant Mutations in Brazil

MDR-TB isolates were randomly chosen and collected from Central Public Health Laboratory, State of Bahia, Brazil.[36] These strains were isolated from sputum samples collected from local patients. Molecular determinants for MDR-TB isolates commonly used in the previous studies and observed to be prevalent were S315T in *katG*, −15C/T in the promoter region of *inh*A, and H526D and S531L mutations in *rpoB* genes. Regarding *kat*G S315Tpolymorphism, it was observed to be 100% of INH resistant strains in some countries (Table 2)[37, 38, 39] and variable number of percentage in other countries whereas the study in Brazil indicated 41.9%. The −15C/T (*inhA*) polymorphism, the other mutation for INH resistance was observed to have frequency of 25.6% in the study. From the previous studies, there was an information that type of mutations and frequency of these mutations associated with drug resistance in *M. tuberculosis* varied according to the geographical regions.[40, 41]

***Kat*G 315 Mutations in African Countries**

A total of 63 drug resistant *M. tuberculosis* clinical isolates were screened for genetic mutations associated with INH, RIF, SM and Ethambutol resistance among the positive pulmonary tuberculosis patients enrolled from April 2010 and March 2011. Thirty two of 44 isoniazid resistant isolates were observed to have *kat*G315 and/or the −15 *inh*A promoter mutations. All the *kat*G315 mutations, three (−15C/T) *inh*A promoter mutation and 6 wild types exhibit high level drug resistance. The details were shown in Table 1 and described under the heading of Minimum Inhibitory concentration for INH resistant isolates.[42]

Between 2008 and 2011, two drug resistance surveys were conducted in Uganda by using WGS method. Of these two surveys, 90 *M. tuberculosis* isolates which are phenotypically resistant to RIF and/or INH were selected and sequenced for whole genome. Mutations observed were *kat*G S315T in 44 cases, S315N in 2 isolates, S315R in 2 isolates, S315T with

*inh*A promoter mutation −15 C/T in 1 isolate, S315T with *ahp*C 48 G/A mutation in 1 isolate. High-level INH resistance was observed in *kat*G codon 315 mutations whereas low-level resistance was associated with *inh*A promoter mutations. However, very high MIC to INH was found in isolates carrying both *kat*G315 and *inh*A promoter mutation. Furthermore, *kat*G mutations were associated with high incidence of tuberculosis with higher transmission rates and unfavourable outcome worldwide.[43]

Methodology Applied in Studying INH Resistant Mutations

In most of the studies, resistance to INH was detected on Lowenstein-Jensen medium by using agar proportion method with INH concentration of 0.2 mg/L. The MIC of INH was measured and determined by 2-fold incremental concentrations of INH starting from 0.05 mg/L, ending at 60 mg/L.[16]

Methodology for studying mutations includes genomic DNA isolation, PCR of DNA fragments flanking *kat*G 315 mutation and *inh*A mutations and sequencing of the PCR product using same PCR primers using ABI Big dye terminator sequencing kit.[16, 17, 30, 42] In the study in Taiwan, four overlapping pairs of forward and reverse primer pairs were applied for PCR reactions and 1710 bp length DNA sequence was studied to get the mutations of the *kat*G gene. Similarly, two overlapping sets of primers were used for 810 bp PCR product in the PCR for *inh*A mutations both in promoter region and open reading frame. Comparison was undertaken with the *Mycobacterium tuberculosis* reference strain H37RV to find out SNPs[17]. For the *kat*G 315 mutation, 210 bp was amplified and sequenced whereas in case of *inh*A promoter mutations, 248 bp PCR product was amplified and sequenced in the study of INH resistant mutations in Cameroon.[42]

WGS approach was used to study the resistant mutations to all anti-tuberculous drugs in Myanmar and Uganda after DNA extraction and purification as described in Aung et al.[32] and Ssengooba et al.[43].

Reasons of *kat*G Mutation is Common in some Countries and Rare in Other Countries

A conclusion was drawn in the study on isoniazid resistant *M. tuberculosis* in Brazil that type and frequency of the SNPs in *kat*G gene varied according to the geographical regions without the reasons to explain it. However, the following explanation can be undertaken. There were many genotypic lineages in *M. tuberculosis* infecting human worldwide such as Beijing, T- families, LAM, Haarlem, etc.[44]. In several countries, studies indicated *M. tuberculosis* isolates carrying the Beijing genotype had the *katG315* mutation associated with high-level resistance to INH when compared with other mutations. The Beijing genotype appears to develop this *kat*G mutation in comparison with other genotypes.[45] The genotypic lineage varies with the various regions of the world. Therefore taken together, the mutations associated with INH resistance varies with the spreading of *M. tuberculosis* genotypes in different countries. Another example is *kat*G R463L mutation is common in Netherlands and Taiwan although it is not associated with INH resistance as shown by experimental finding.[17, 18]

Table 1 INH resistant mutations in different countries with frequency of *kat*G 315 mutations and *inh*A mutations

Countries of study and no. of INH resistant isolates studied	***kat*G mutations and frequency of *kat*G S315T mutations**	**Mutations in *inh*A promoter region and *inh*A gene**
Poland (109 INH resistant isolates)[16]	*kat*G Ser315Thr, Arg128Gln, Ala234Gly, Arg463Leu, Glu399Glu 57 of 109 isolates	Mutations in the *inh*A promoter region were detected in eight MDR strains (−15C/T in seven strains and −8T/C in one strain). Mutations in the *inh*A gene were of four types.
Taiwan (41 INH resistant isolates)[17]	*kat*G Arg463Leu, Ser315Thr, Ser315Asn, and other loci 12 of 41 isolates	Only one isolate with *inh*A promoter mutation (−15C/T) was observed.
*Myanmar (14 INH resistant isolates)[32]	*kat*G Ser315Thr, Gly299Cys, frameshift. 10 of 14 isolates	2 isolates with *inh*A promoter mutation (−15C/T).
Malaysia (4 INH resistant isolates)[30]	*kat*G Gln247His, Val61Gly, Ala62Thr,	-
Cameroon (44 INH resistant isolates)[42]	*kat*G315 32 of 44 isolates	13 isolates with *inh*A promoter mutation (−15C/T).
*Uganda (50 INH resistant isolates)[43]	*kat*G315 Ser315Thr, Ser315Asn, Ser315Arg 50 of 50 isolates	9 isolates with *inh*A promoter mutation (−15 C/T) 1 isolate with *inh*A promoter mutation (−8T/C)

*Method of study is WGS.

Minimum Inhibitory Concentration for INH Resistant Isolates Carrying *kat*G Mutation

MIC was expressed in mg/L in some literatures and μg/ml in other literatures. In this report, mg/L will be used for simplicity purpose. In the study in Cameroon, the researchers divided the MIC into high level, 1 mg/L and low level, 0.2 mg/L. Twenty-four of 44 INH resistant isolates had 1 mg/L MIC showing high level drug resistance. Of these, 17 *kat*G S315T mutant isolates had high level drug resistance whereas 2 isolates showed low level resistance. Three isolates carrying (−15C/T) *inh*A promoter point

mutation displayed high level resistance and ten of these isolates showed low level resistance. Five (–47G/C) *inh*A promoter mutant isolates showed high level resistance with no low level resistance.[42] In the literature review in which INH resistance isolates in 49 countries were studied, it was observed that *kat*G codon 315 was associated with high level resistance to INH with 4 mg/L or more MIC value.[29] The finding in Warsaw, Poland indicated that *M. tuberculosis* isolates with *kat*G 315 mutants had the MIC values between 1 to 10 mg/L (Table 2) with the average MIC of 2.5 mg/L in the MDR and isoniazid mono-resistant phenotypic strains.[16]

Advantages and Limitations of Currently using Molecular Methods

Although there was variation of the sensitivity and specificity of the results in different regions of the world where these were applied, GeneXpert and GenoType MTBDRplus were relatively simple and extremely rapid in detection of drug-resistant TB.[4, 46] However, the main disadvantage of these molecular methods is resistance detection can be available for fewer drugs and limited number of mutation. GeneXpert cannot detect INH-monoresistant cases (Table 2) that might become MDR-TB in the future[47]. In most TB endemic countries, Mono-INHR tends to rise as a result of inability to capture these isolates by molecular methods.[48] Moreover, as observed in the previous studies S315T, a canonical mutation was lower in prevalence in INH mono-resistant isolates whereas it was highly prevalent in MDR-TB isolates.[49] Research on development of rapid diagnostics which can encompass novel mutations conferring drug resistant TB are essential.[49]

Table 2 Significance of *kat*G 315 mutations in INH resistant *M. tuberculosis*

In some countries, *kat*G 315 mutations were prevalent up to 100%.[37, 38, 39]
M. tuberculosis isolates with *kat*G 315 mutants were commonly associated with the MIC values between 1 to 10 mg/L showing high level INH resistance.[16]
*Kat*G 315 mutations were more common in MDR-TB isolates than INH monoresistant isolates.[16]
Of two canonical mutations, *kat*G 315 was the more common one than (−15C/T) *inh*A promoter mutation. Other mutations associated with INH resistance were not included in canonical mutations.[29]
Ser315Thr (AGC-ACC) was present in more than 90% in INH resistant isolates with *kat*G 315 mutations.[29]
Ser315Asp was the second most common mutation among *kat*G 315 mutations.[29]
*Kat*G 315 mutation was included in the GenoType MTBDRplus molecular diagnostic method.[17] The rapid molecular diagnostic methods come to the front line in the diagnosis of drug resistant TB because conventional methods for drug sensitivity test usually take 8 weeks in most regions and treatment of the patients will be delayed.[32]
*Kat*G 315 mutation was not detected in the GeneXpert MTB/RIF molecular diagnostic method.[49]

Discussion on Molecular Determinants in MDR-TB and XDR-TB

Although WGS is a perfect platform to detect MDR-TB as well as XDR-TB, implementation was still earlier for the routine purpose in the low resource-high TB burden countries. Findings of other molecular diagnostic methods are the main areas of research. Researchers in the drug resistant TB now focus on identification of mutations associated with drug resistance in the genome of *M. tuberculosis* and innovation of molecular diagnostics to detect these mutants. Accuracy up to 100% was not obtained in these methods because the association between the molecular determinants and the phenotypes of the isolates with these mutations is not strong enough.[50]

WGS approaches in Uganda and Myanmar indicated that *rpo*B S531L and *kat*G S315T were the highly prevalent molecular markers for MDR-TB isolates. In Myanmar, *rpo*B S531L was observed in 11 of 14 isolates with H526Y as the second common mutation. Regarding INH resistant mutation, *kat*G S315T was also found in 11 isolates whereas *inh*A C-15T promoter mutation was present in two isolates as the second common mutation. In Uganda, S531L and H526D were the most two common mutations for RIF resistance whereas *kat*G S315T and *inh*A C-15T promoter mutation were the most two common ones for INH resistance. Although these results were observed in these two studies, it was clear that *rpo*B S531L and *kat*G S315T are the pre-dominant molecular determinants for RIF and INH respectively. The outstanding characteristic of *kat*G S315T SNP in INH resistance is relatively more common than *rpo*B S531L in RIF resistance. This fact indicated that *kat*G S315T was a stronger marker for INH resistance than *rpo*B S531L in RIF resistance.[32, 43] The observations in other studies of various countries were consistent with these two studies. In conclusion, drug resistant molecular markers for MDR-TB were well established.

Besides mutations associated with RIF and INH, it is necessary to study drug resistant mutations commonly associated with resistance to fluoroquinolones and other second line drugs (SLD) to identify XDR-TB. Drug resistant mutations for SLD were less studied and less understood. Although detection of *gyr*A and *gyr*B mutations additively gave rise to sensitivity of 93% for the fluoroquinolone resistance, *gyr*B mutations were widespread and molecular determinants were inconsistently associated with drug resistant phenotypes. Taken together, these mutations were not reliable to prepare the rapid molecular diagnostics. In the study in France, *gyr*A mutants Ala90Val, Asp94Gly, Asp94Ala were common molecular determinants for fluoroquinolone resistance whereas 13 SNPs in *gyr*B were observed. In addition, these SNPs were observed to be present in fluoroquinolone sensitive *M. tuberculosis* isolates[51]. SNP *rrs* A1401G was highly prevalent in isolates resistant to these SLD which have to be given by injection like capreomycin (CAP), amikacin (AMK) and kanamycin (KAN). However, the *rrs* A1401G mutation was present only in 70 – 80% of *M. tuberculosis* strains resistant to CAP and AMK whereas this mutation was observed in 60% of strains resistant to KAN. MTBDRsl line probe assay (LPA) is based on the principle of hybridization and mutations in clinical strains were detected by the probes that are complementary to the mutated DNA. MTBDRsl LPA is the only available rapid molecular diagnostic method widely used for identification of XDR-TB. However, the method has variable sensitivity with the range from 40 – 100%.[50]

CONCLUSIONS

*Kat*G gene encodes catalase peroxidase which activates anti-TB drug INH and is an important enzyme for the survival of the bacteria in the macrophages and it is regarded as virulence factor of *M. tuberculosis.*[52]

Although *kat*G315 mutations has shown to be highly common up to 95% in the previous studies, other *kat*G mutations are found in the literatures and observed to be associated with INH resistance. In addition, *kat*G315 mutation was uncommon as low as 25% in INH monoresistant cases. It will be necessary for the researchers to develop the molecular methods which can include probes possible to detect all novel mutations.

Dissemination of MDR-TB and XDR-TB will be dangerous and cause high mortality in the TB endemic countries. In addition, second line TB drugs have higher toxicity and are expensive so that there may be many dropped out during the regimen which leads to increasing mortality. Therefore finding of hotspot areas and screening of hotspots within the localities in the poor resource countries with earlier diagnosis and rapid implementation of appropriate anti-tuberculous drug regime will be the essential

measure in the control of TB. Hotspot areas are defined as TB endemic localities with the prevalence rate of more than 0.5 – 1%.

In spite of the fact that *kat*G315 mutation is not the only mutation for INH resistance, it is highly prevalent and it is the common mutation in the main enzyme essential for the activation of the INH regarding the mechanism of drug resistance. Moreover, *kat*G S315T mutation has been shown to be associated with high level resistance to INH with average MIC of 1 ug/ml in the previous studies. As a consequence, rapid method of identification of this point mutation with mismatch amplification mutation assay (MAMA) is recommended in this report.

SNPs that are not detectable by other polymerase chain reaction or PCR-RFLP can be detected by MAMA PCR. One nucleotide change in quinolone resistance determining region of *gyr*A gene responsible for fluoroquinolone resistant bacteria such as *Klebsiella pneumoniae*, *Campylobacter jejuni* and *Neisseria gonorrhoeae* can be identified by PCR assays using MAMA method.[53] Discrimination of the *ctxB* alleles in classical, El Tor, and Haitian type *Vibrio cholerae* can be undertaken by Double-mismatch-amplification mutation assay (DMAMA) PCR.[54] The canonical mutations for INH resistance, *kat*G S315T mutation and (−15C/T) *inh*A promoter mutation, the two SNPs can be proposed to be detected by DMAMA.

In Malaysia, the study in Kelantan has shown totally different mutations in four cases with INH resistance. The common mutations were not observed in the study because the total number of samples studied was only nine. It will be interesting if a large survey of drug resistant *M. tuberculosis* isolates is undertaken in the near future within Malaysia.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Cauthen GM, Dooley SW, Onorato IM, Ihle WW, Burr JM, Bigler WJ, Witte J, Castro KG. (1996). Transmission of Mycobacterium tuberculosis from tuberculosis patients with HIV infection or AIDS. Am J Epidemiol 144 (1): 69 – 77.
2. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G. (2006). Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368 (9547): 1575 – 1580.
3. WHO: Global Tuberculosis Control: WHO Report. (2011). Geneva, Switzerland: WHO/ HTM/TB/2011. 16; 2011.
4. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. (2010). Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363 (11): 1005 – 1015.
5. Garcia De Viedma D. (2003). Rapid detection of resistance in Mycobacterium tuberculosis: a review discussing molecular approaches. Clin Microbiol Infect 9(5):349–59.
6. Lehmann J. (1946). Para-Aminosalicylic acid in the treatment of tuberculosis. Lancet 247: 15.
7. Bernstein JW, Lott A, Steinberg BA, Yale HL. (1952). Chemotherapy of experimental tuberculosis. Am Rev Tuberc 65: 357 – 374.
8. Fox HH. (1952). The chemical approach to the control of tuberculosis. Science 116: 129 – 134.
9. Medical Research Council Investigation. (1950). Treatment of pulmonary tuberculosis with streptomycin and para-amino-salicylic acid. Br Med J 2: 1073 – 1085.
10. Medical Research Council Investigation. (1952). The treatment of pulmonary tuberculosis with isoniazid. Br Med J 2: 735 – 746.
11. Crofton J, Mitchison DA. (1948). Streptomycin resistance in pulmonary tuberculosis. Br Med J 2: 1009 – 1015.
12. Crofton J. (1959). Chemotherapy of pulmonary tuberculosis. Br Med J 1: 1610 – 1614.
13. WHO. (2012). Global Tuberculosis Report 2012. Geneva, Switzerland.

14. Cegielski P, Nunn P, Kurbatova EV, Weyer K, Dalton TL, Wares DF, Iademarco MF, Castro KG, Raviglione M. (2012). Challenges and controversies in defining totally drug-resistant tuberculosis. Emerg Infect Dis 18: e2.
15. Vilchèze C, Jacobs WR. (2014). Resistance to isoniazid and ethionamide in Mycobacterium tuberculosis: genes, mutations, and causalities. Microbiol Spectrum 2 (4): MGM2-0014-2013. doi:10.1128/microbiolspec.MGM2-0014-2013.
16. Jagielski T, Bakuła Z, Roeske K, Kamiński M, Napiórkowska A, Augustynowicz-Kopeć E, Zwolska Z, Bielecki J. (2015). Mutation profiling for detection of isoniazid resistance in Mycobacterium tuberculosis clinical isolates. J Antimicrob Chemother 70: 3214–21.
17. Tseng ST, Tai CH, Li CR, et al. (2014). The mutations of katG and inhA genes of isoniazid-resistant Mycobacterium tuberculosis isolates in Taiwan. J Microbiol Immunol Infect 48: 249 – 255.
18. van Doorn HR, Kuijper EJ, van der Ende A, Welten AG, van Soolingen D, de Haas PE, Dankert J. (2001). The susceptibility of Mycobacterium tuberculosis to isoniazid and the Arg–>Leu mutation at codon 463 of katG are not associated. J Clin Microbiol 39: 1591 – 1594.
19. Laurenzo D, Mousa SA. (2011). Mechanisms of drug resistance in Mycobacterium tuberculosis and current status of rapid molecular diagnostic testing. Acta Trop 119: 5 – 10. doi:10.1016/j.actatropica.2011.04.008 PMID: 21515239
20. Rossetti ML, Valim AR, Silva MS, Rodrigues VS. (2002). Resistant tuberculosis: a molecular review. Rev Saude Publica 36: 525 – 532. PMID: 12364929
21. Zhang Y, Heym B, Allen B, Young D, Cole S. (1992). The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 358: 591 – 593. PMID: 1501713
22. Vilchèze C, Wang F, Arai M, Hazbón MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs WR Jr. (2006). Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. Nat Med 12: 1027 – 1029. PMID: 16906155
23. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs WR Jr. (1994). InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science 263: 227 – 230. PMID: 8284673
24. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. (1993). Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. Lancet 341: 647 – 650. PMID: 8095569
25. Ramaswamy S, Musser JM. (1998). Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuber Lung Dis 79: 3 – 29. PMID: 10645439
26. Rattan A, Kalia A, Ahmad N. (1998). Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerg Infect Dis 4: 195 – 209. PMID: 9621190
27. Ahmad S, Mokaddas E. (2009). Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis. Respir Med 103: 1777 – 1790. doi:10.1016/j.rmed.2009.07.010 PMID: 19660927
28. Sreevatsan S, Pan X, Zhang Y, Deretic V, Musser JM. (1997). Analysis of the oxyR-ahpC region in isoniazid-resistant and-susceptible Mycobacterium tuberculosis complex organisms recovered from diseased humans and animals in diverse localities. Antimicrob Agents Chemother 41: 600 – 606. PMID: 9056000
29. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. (2015). Genetic Mutations Associated with Isoniazid Resistance in Mycobacterium tuberculosis: A Systematic Review. PLoS ONE 10 (3): e0119628. doi:10.1371/journal.pone.0119628
30. Ismail NA, Ismail MF, Noor MDSS, Camalxaman SN. (2016). Gene Mutations Associated with Rifampicin and Isoniazid Resistance in Mycobacterium Tuberculosis Isolates: A Local Scenario (Kelantan). Malays J Med Sci 23 (1): 22 – 26.
31. World Health Organization. (2014). Global tuberculosis report 2014. Geneva, Switzerland.
32. Aung HL, Tun T, Moradigaravand D, Köser CU, Nyunt WW, Aung ST, Lwin T, Thinn KK, Crump JA, Parkhill J, Peacock SJ, Cook GM, Hill PC. (2016). Whole-genome sequencing of multidrug resistant Mycobacterium tuberculosis isolates from Myanmar. Journal of Global Antimicrobial Resistance 6: 113 – 117.
33. Köser CU, Bryant JM, Becq J, Török ME, Ellington MJ, Marti-Renom MA, Carmichael AJ, Parkhill J, Smith GP, Peacock SJ. (2013). Whole-genome sequencing for rapid susceptibility testing of M. tuberculosis. N Engl J Med 369: 290 – 292.

34. Köser CU1, Ellington MJ, Cartwright EJ, Gillespie SH, Brown NM, Farrington M, Holden MT, Dougan G, Bentley SD, Parkhill J, Peacock SJ. (2012). Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog 8: e1002824.
35. Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elias C, Bradley P, Iqbal Z, Feuerriegel S, Niehaus KE, Wilson DJ, Clifton DA, Kapatai G, Ip CL, Bowden R, Drobniewski FA, Allix-Béguec C, Gaudin C, Parkhill J, Diel R, Supply P, Crook DW, Smith EG, Walker AS, Ismail N, Niemann S, Peto TE. (2015). Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis 15: 1193 – 1202.
36. Oliveira LNC, Muniz-Sobrinhoa JS, Viana-Magnob LA, Melo SCO, Macho A, Santos FR. (2016). Detection of multidrug-resistant Mycobacterium tuberculosis strains isolated in Brazil using a multimarker genetic assay for katG and rpoB genes. Braz J Infect Dis 20 (2): 166 – 172.
37. Blackwood KS, He C, Gunton J, Turenne CY, Wolfe J, Kabani AM. (2000). Evaluation of recA sequences for identification of Mycobacterium species. J Clin Microbiol 38: 2846 – 2852.
38. Durmaz R, Gunal S, Yang Z, Ozerol H, Cave MD. (2003). Molecular epidemiology of tuberculosis in Turkey. Clin Microbiol Infect 9: 873 – 877.
39. Elia-Pasquet S., Dabis F., Texier-Maugien J., DessusBabus S., Meynard J., Bouiges M., Portel L., Salamon M, Tessier JF, Courty G (2000). Transmission of tuberculosis in Gironde: epidemiologic investigation by genomic analysis of Mycobacterium tuberculosis. Rev Epidemiol Sante Publique 48: 127 – 136 [in French].
40. Filliol I, Motiwala AS, Cavatore M, Qi W, Hazbón MH, Bobadilla del Valle M, Fyfe J, García-García L, Rastogi N, Sola C, Zozio T, Guerrero MI, León CI, Crabtree J, Angiuoli S, Eisenach KD, Durmaz R, Joloba ML, Rendón A, Sifuentes-Osornio J, Ponce de León A, Cave MD, Fleischmann R, Whittam TS, Alland D. (2006). Global phylogeny of Mycobacterium tuberculosis based on single nucleotide polymorphism (SNP) analysis: insights into tuberculosis evolution, phylogenetic accuracy of other DNA fingerprinting systems, and recommendations for a minimal standard SNP set. J Bacteriol 188: 759 – 772.
41. Brudey K, Driscoll JR, Rigouts L, et al. (2006). Mycobacterium tuberculosis complex geneticdiversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol 6: 23.
42. Tekwu EM, Sidze LK, Assam JPA, Tedom JC, Tchatchouang S, Makafe GG, Wetewale ALT, Kuaban C, Eyangoh S, Ntoumi F, Beng VNP, Frank M. (2014). Sequence analysis for detection of drug resistance in Mycobacterium tuberculosis complex isolates from the Central Region of Cameroon. BMC Microbiol 14: 113. http://www.biomedcentral.com/1471-2180/14/113
43. Ssengooba W, Meehan CJ, Lukoye D, Kasule GW, Musisi K, Joloba ML, Cobelens FG, de Jong BC6. (2016). Whole genome sequencing to complement tuberculosis drug resistance surveys in Uganda. Infect Genet Evol 40: 8 – 16.
44. Nguyen VAT, Bañuls A, Tran THT, Pham KLT, Nguyen TS, Nguyen HV, Nguyen NLT, Nguyen NLT, Dang DA, Marks GB, Choisyet M. (2016). Mycobacterium tuberculosis lineages and anti-tuberculosis drug resistance in reference hospitals across Viet Nam. BMC Microbiol 16: 167.
45. Duong DA, Nguyen TH, Nguyen TN, Dai VH, Dang TM, Vo SK, Do DA, Nguyen VV, Nguyen HD, Dinh NS, Farrar J, Caws M. (2009). Beijing genotype of Mycobacterium tuberculosis is significantly associated with high-level fluoroquinolone resistance in Vietnam. Antimicrob Agents Chemother 53: 4835 – 4839.
46. Ferro BE, Garcı´a PK, Nieto LM, Soolingen DV. (2013). Predictive value of molecular drug resistance testing of Mycobacterium tuberculosis isolates in Valle del Cauca, Colombia. J Clin Microbiol 51: 2220 – 2224.
47. Jacobson KR, Theron D, Victor TC, Streicher EM, Warren RM, Murray MB. (2011). Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape Province, South Africa. Clin Infect Dis 53: 369 – 372.
48. Varahram M, Nasiri MJ, Farnia P, Mozafari M., Velayati A. A. (2014). A retrospective analysis of isoniazid-monoresistant tuberculosis: among Iranian pulmonary tuberculosis patients. Open Microbiol J 8: 1 – 5.
49. Torres JN, Paul LV, Rodwell TC, Victor TC, Amallraja AM, Elghraoui A, Goodmanson AP, Ramirez-Busby SM, Chawla A, Zadorozhny V, Streicher EM, Sirgel FA, Catanzaro D, Rodrigues C, Gler MT, Crudu V, Catanzaro

A, Valafar F. (2015). Novel katG mutations causing isoniazid resistance in clinical M. tuberculosis isolates. Emerg Microbes Infect 4: e42. doi:10.1038/emi.2015.42

50. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. (2012). Evaluation of Genetic Mutations Associated with Mycobacterium tuberculosis Resistance to Amikacin, Kanamycin and Capreomycin: A Systematic Review. PLoS ONE 7(3): e33275
51. Bernard C, Veziris N, Brossier F, Sougakoff W, Jarlier V, Robert J, Aubry A. (2015). Molecular Diagnosis of Fluoroquinolone Resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 59 (3): 1519 – 1524.
52. Yu S, Girotto S, Lee C, Magliozzo RS. (2003). Reduced Affinity for Isoniazid in the S315T Mutant of Mycobacterium tuberculosis KatG Is a Key Factor in Antibiotic Resistance. J Bio Chem 278 (17): 14769 – 14775.
53. Marzooq FA, Yusof MYM, Tay ST. (2015). Molecular Analysis of Antibiotic Resistance Determinants and Plasmids in Malaysian Isolates of Multidrug Resistant Klebsiella pneumoniae. PLoS ONE 10 (7): e0133654. doi:10.1371/journal.pone.0133654.
54. Naha A, Pazhani GP, Ganguly M, Ghosh S, Ramamurthy T, Nandy RK, Nair GB, Takeda Y, Mukhopadhyay AK. (2012). Development and Evaluation of a PCR Assay for Tracking the Emergence and Dissemination of Haitian Variant ctxB in Vibrio cholerae O1 Strains Isolated from Kolkata, India. J Clin Microbiol 1733 – 1736. doi:10.1128/JCM.00387-12

Case Report: Challenges in Diagnosis and Management of Myasthenic Crisis in Resource-Limited Health Care Setting

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ABSTRACT

Myasthenia gravis, the most common autoimmune neuromuscular disorder, is characterised by muscle weakness and fatigability. A 23-year-old-lady with background history of breathing and swallowing difficulty for six months was presented with respiratory distress to the hospital which is without an in-house neurology service. Her diagnosis remained a challenge as patient presented as an emergency without detailed medical history. She was subjected to bedside ice pack testing and subsequently managed along the diagnosis of myasthenia crisis. She responded to the treatment and survived the critical period. So, diagnosis of myasthenia gravis by ice pack test and managing a case of myasthenic crisis would be possible in limited heath care setting.

Keywords: myasthenia gravis, ice pack test, myasthenic crisis

INTRODUCTION

Myasthenia gravis (MG), the most common autoimmune neuromuscular disorder, is characterised by muscle weakness and fatigability. There are two patterns described; ocular myasthenia gravis and generalised myasthenia gravis.[1] MG is diagnosed based on appropriate clinical context supported by diagnostic tools like serology testing, edrophonium testing, repetitive nerve stimulation testing and single fibre electromyography.[2] Myasthenia gravis is easily diagnosed in tertiary centre with neurology service and adequate resources. However, it is a diagnostic challenge to doctors in smaller hospitals who are mostly the novices in medical hierarchy due to limited resources. Ice Pack Test has long been described as an alternative method in aiding myasthenia gravis diagnosis. Unfortunately, this simple and safe bedside diagnostic tool has been under-utilised and remains unknown to majority of the health care providers.

CASE PRESENTATION

A 23-year-old-lady without any known medical illness was brought in by her family, complaining of difficulty in breathing associated with high-grade fever and cough for two days. She has background of breathing and swallowing difficulties for six months with worsening a day prior to admission. She has been seen and treated by different primary health care providers without any improvement of her symptoms. Her medical problem remains unresolved. As she presented acutely, a detailed history and examination were not possible.

Upon admission she was cyanosed with low Glasgow Coma Scale (Eye: 2, Verbal: 1, Motor: 1). Her blood pressure was 128/67 mmHg, with heart rate of 118 beats/min and SpO2 88% under room air temperature of 38.5°C. Her blood investigations revealed that Hb concentration was 12.7 gm/dl, total white cell count 20.6×10^9 /L, platelet count 399×10^9 /L, ESR > 140 mm/H. Her biochemical profile for urea 2.0 mmol/L, sodium 142 mmol/L, potassium 2.84 mmol/L and creatinine 61 µmol/L. Her total bilirubin was 14.3 µmol/L, albumin 40.6 g/L, ALT 8.4 U/L, AST 17.8 U/L and ALP 35 U/L. C-reactive protein (CRP) was 11.62 mg/L. Her arterial blood gases (ABG)

analysis were pH 7.32, pO2 173 mmHg, pCO2 38.4 mmHg, HCO_3 19.7 mmol/L. Chest x-ray showed heterogeneous opacity over both lungs with lower lung field more prominent over the right side. Computer tomography (CT) of brain was unremarkable without any features suggestive of stroke. Blood and sputum specimen sent for culture and sensitivity but no organism was isolated.

She was intubated for respiratory failure, and treated for aspiration pneumonia with intravenous Ceftriaxone in ICU setting.

During her stay in ICU, two attempts of extubation were tried by anaesthetist but in vain. Upon weaning her off from sedation, there were copious amount of oral secretion needing regular suctioning and obvious bilateral ptosis noted. There was bilateral facial paresis with pupillary sparing ptosis. Neurology assessment of limbs revealed tetraparesis with medical research council power grading 4 out of 5 for all 4 limbs accompanied by intact tendon reflexes and flexor plantar responses.

She was initially planned for a transfer to tertiary centre for further management by neurology team. However this plan appeared unwise given that the land transport (the only mode available in our setting) takes approximately four hours and she was deemed unstable for transfer.

Hence she was subjected to ice pack test based on the clinical findings of bulbar weakness and bilateral ptosis . Figure 1 shows her eyelids position before ice pack test. The ice pack result is as in Table 1. Figure 2 shows her eyelids position after application of ice pack test. There was a drastic improvement in term of her ptosis after two minutes of ice pack application.

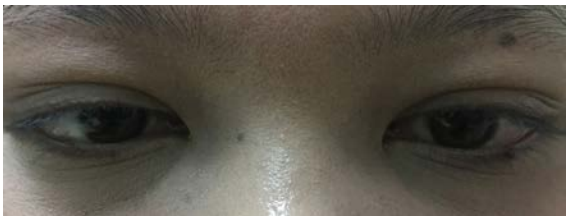

Figure 1 Eyelids position before ice pack test

Figure 2 shows her eyelids position after application of ice pack test. There was a drastic improvement in term of her ptosis after two minutes of ice pack application.

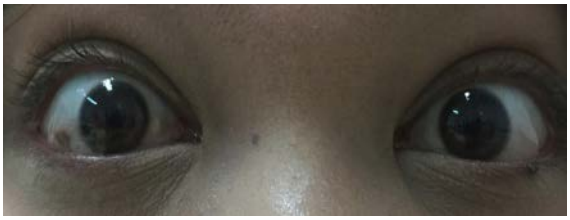

Figure 2 Eyelids position after ice pack test

Table 1 Ice pack test result

Interpalpebral distance	Right	Left
Before ice pack	10 mm	10 mm
After ice pack	12 mm	13 mm

As her ice pack test was positive, blood specimen was taken and sent to tertiary hospital for acetylcholine receptor antibody testing.

There are few differential diagnoses to be considered in a young lady with bulbar weakness and bilateral ptosis, namely myasthenia gravis, Guillain Barre Syndrome (GBS), botulism, and acute stroke with brain stem involvement.

She was treated as myasthenic crisis. This is because she presented with respiratory distress requiring intubation and ventilatory support. Tracheostomy was done for her in view of repeated failed extubation. She was treated with antibiotic (Ceftriaxone for one week duration) for aspiration pneumonia, intravenous immunoglobulin (IVIG), high-dose Prednisolone at 1 mg/kg/day (initiated at day four of IVIG), subcutaneous Enoxaparin for thromboembolic prophylaxis and tablet

Pyridostigmine 30 mg 5 times/day (initiated prior to discharge). Clinically, she progressively showed improvement and was discharged well after 2 weeks of inpatient treatment.

Six weeks after discharge, she achieved full resolution of her weakness and was independent in carrying out activity of daily livings. Tracheostomy tube had been removed. Her Acetylcholine receptor antibody came back as positive during clinic follow-up (> 8.4 nmol/L; normal < 0.4 nmol/L) which confirmed the diagnosis of myasthenia gravis.

DISCUSSION

This is a challenging case seen in a resource-limited health setting. Patient was presented acutely requiring ventilatory support and ICU care without much clinical history. Thus doctors were to treat her based on best clinical judgement and using all the slightest clues available. The unavailability of neurology service added onto this challenge. Escalation of care to tertiary centre was impractical given patient's instability for transfer. Neurological consult with neurologist of neighbouring tertiary hospital was available via phone consultation; yet this is not of much help given that the neurologist did not have the opportunity to assess the patient and had to rely on assessment of the referring doctor. Serological investigations such as acetylcholine receptor antibody take approximately three to four weeks to be ready as the specimen has to be sent to a tertiary centre laboratory for processing. Neurophysiological study and edrophonium test were also not available given the similar reason as above.

According to the clinical judgement, she had myasthenia gravis based on the presence of bulbar weakness, bilateral ptosis and positive ice pack testing. As she presented acutely with respiratory distress requiring intubation and ventilatory support. So, her case was considered as myasthenic crisis. This case was managed based on the latest recommendation.[3]

The initiation of high-dose steroid may worsen myasthenia gravis in 50% of patients and even precipitate a crisis in another 10%.[3] The quick action onset of IVIG helps to prevent the transient worsening of MG associated with steroid initiation.[3] In this case, IVIG was used as acute therapy for myasthenia crisis. Total IVIG given to her was 2 g/kg over the course of 5 days (0.4 g/kg/day). Steroid was introduced at day 4 of IVIG when infection was well controlled with antibiotic treatment based on clinical and microbiological parameter.

Ice pack testing in myasthenia gravis is well described in many literatures.[4, 5, 6, 7, 8] This test is performed by objective measurement of interpalpebral distance before and after the application of ice packs. The ice used should be packed and placed on closed eyes for 2 minutes as to minimise the risk of cold-induced injury. Two independent observers should be available to measure the interpalpebral distance as to prevent bias in result interpretation. Ice pack test is considered positive if there is objective improvement of the ptosis by at least 2 mm of the interpalpebral distance. The principles governing Ice Pack Testing are that acetylcholinesterase activity of skeletal muscle reduces with lower temperature. The risk of this procedure is minimal compare to edrophonium where the later has the risk of precipitating heart block. Edrophonium test can only be conducted by an experienced neurologist with standby resuscitation trolley in case of acute cardiac event.

In limited health care setting, this was the only alternative available. Ice pack test can be conducted at the bedside by non-neurologist, much safer compare to edrophonium test and cost effective. Moreover, a study comparing ice pack test and edrophonium test showed that ice pack test had sensitivity and specificity of 100% in myasthenia gravis patient.[4] However, ice pack test may not be reliable in cases of isolated diplopia without ptosis.[4] In this patient, ice pack test is reliable as she had ptosis.

CONCLUSION

Myasthenic crisis need to be suspected in a patient presented with weakness, difficulty in breathing and swallowing. Ice Pack Test is a simple, reliable, safer bedside test that can aid in the diagnosis of myasthenia gravis especially in resource-limited health setting.

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The author thanks the Director General of Health, Ministry of Health Malaysia for his permission to publish this case report.

CONFLICT OF INTEREST

The author declares that he has no competing interests.

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REFERENCES

1. Bird SJ. (2017). Clinical manifestations of myasthenia gravis. UpToDate.
2. Philllips LH, Melnick PA. (1990). Diagnosis of myasthenia gravis in the 1990s. Semin Neurol 10: 62 – 69.
3. Bird SJ, Levine JM. (2017). Myasthenic crisis. UpToDate.
4. Tabassi A, Dehghani A, Saberi B. (2005). The ice test for diagnosing myasthenia gravis. Acta Medica Iranica 43 (1): 60 – 62.
5. Lertchavanakul A, Gamnerisiri P, Hirunwiwatkul P. (2001). Ice test for ocular myasthenia gravis. J Med Assoc Thai 84 (Suppl 1): S131 – S136.
6. Sethi KD, Rivner MH, Swift TR. (1987). Ice pack test for myasthenia gravis. Neurology 37 (8): 1383 – 1385.
7. Czaplinski A, Steck AJ, Fuhr P. (2003). Ice pack test for myasthenia gravis; a simple, noninvasive and safe diagnostic method. J Neurol 250: 883 – 884.
8. Liu WW, Chen A. (2016). Diagnosing Myasthenia Gravis with an Ice Pack. N Engl J Med 375: e39. DOI: 10.1056/NEJMicm1509523
9. Larner AJ, Thomas DJ. (2000). Can myasthenia gravis be diagnosed with the ‘ice pack test’? A cautionary note. Postgrad Med J 76: 162 – 163.

Case Report: A Rare Yet Life-threatening Mimicker of Chronic Conjunctivitis

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ABSTRACT

Wegener's granulomatosis (WG) is a necrotizing granulomatous inflammation. A 40-year-old lady of Malay descent presented with unresolving bilateral painful red eyes for three weeks. Clinical examination revealed that best corrected vision acuity of 6/9 for both eyes. Slit lamp examination revealed diffuse scleritis. Other Investigations result like UFEME blood cell: 2+, C-reactive protein 70.06 mg/L and ESR of 125 mm/h suggestive on acute inflammations. Connective tissue screening revealed cytoplasmic ANCA was positive and was supported by Anti-Serine Protease3 (PR3) 68. All the investigation results revealed that she had Wegener's granulomatosis with ophthalmology manifestation in the form scleritis. Patient was treated with guttae Maxidex QID to reduce cells that present in anterior chamber and oral ibuprofen 400 mg thrice daily. Subsequently, oral prednisolone and oral cyclophosphamide with oral Bactrim were commenced. Patient responded well and redness resolved. There are many differential diagnoses for chronic conjunctivitis but to rule out connective tissue disease should be one of the primary differential diagnoses in young female. Oral immunosuppressive and Trimethoprim/ Sufmethoxazole (Bactrim) were been found beneficial and symptoms were resolved. Wegener's granulomatosis is a great mimicker as exemplified in this case. This disease can be misdiagnosed and maltreated as conjunctivitis. Thus, the authors wish to emphasize that WG is one the differential diagnoses that need to be considered in a person with bilateral scleritis.

Keywords: Wegener's granulomatosis, scleritis, chronic conjunctivitis

INTRODUCTION

Wegener's granulomatosis (WG) is a necrotizing granulomatous inflammation involving small to medium vessels in many organs.[1] Various tests are needed for diagnosis of Wegener's granulomatosis, based on the clinically, radiological and serological findings.[2] WG is a great mimicker. Chronic conjunctivitis is one of the rare manifestations of WG. Here, the authors report a case of Wegener granulomatosis with ocular involvement.

CASE PRESENTATION

A 40 year-old-Malay-female, presented with episodic bilateral eye redness which did not resolve for the past three weeks. Patient however denied any ocular trauma or contact with foreign body. It was associated with dull aching pain upon eye movement only. Further examination revealed that her best corrected vision acuity for both eyes was 6/9. There was no relative afferent pupillary defect. Slit lamp examination revealed non-necrotizing anterior diffuse scleritis bilaterally. There were cells presented in anterior chambers of both eyes. Slit lamp examination diffuse scleritis bilaterally. Indirect ophthalmoscopy with mydriatics drops revealed a normal fundus. Figure 1 shows the initial appearance of the eyes. Cardiovascular, respiratory and abdominal examinations were unremarkable. The intraocular pressure for both eyes was at 14 mmHg. Blood and urine investigations were done (see Table 1).

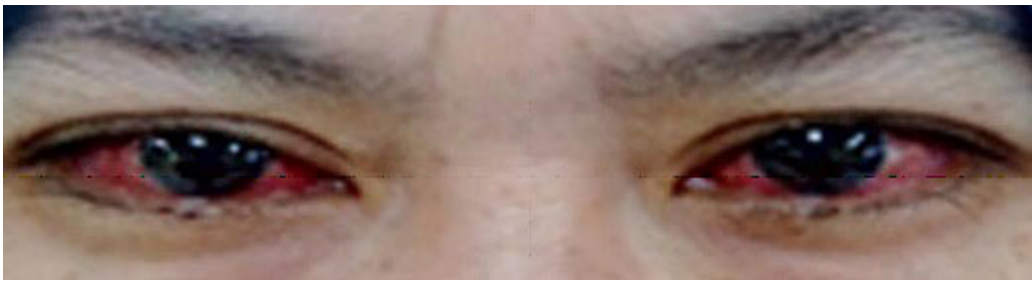

Figure 1 Initial appearance of the eye

Table 1 Investigations

Full blood count	
White Cell Count	12,200/ml
Haemoglobin	11.6 g/dL
Platelet	495,000/ml
Erythrocyte Sedimentation Rate	125
MCV	75.5 fL
MCH	23.3 pg
MCHC	30.9 g/dL
Biochemical parameters	
C-Reactive Protein	70.06 mg/L (<5)
Creatinine	59 mmol/L
Urine Analysis	
pH	6.5
Blood	2+
Protein	Negative
Red Blood Cell Cast	Negative

Immunological parameter	
Antinuclear antibodies	Negative
C-ANCA	Positive
P-ANCA	Negative
RA	Positive
MPO	< 0.0(CU)
PR3	68 (CU)

On investigations, she had hypochromic microcytic anaemia which was confirmed via full blood count accompanied by raised inflammatory markers (Table 1). TB workup for her was negative. She was screened for Staphylococcal Aureus nasal carriage which turned out to be negative. Her urine analysis showed the presence of red blood cells which was later resolved upon treatment commencement. Routine chest x-ray revealed a suspicious right upper lobe with cavitation. So, CT scan of thorax was done. Her CT scan of thorax showed dense consolidation foci at other lobes with ground glass opacity as shown in Figure 2. Immunological parameter revealed cytoplasmic ANCA was positive and was supported by Anti-Serine Protease3 (PR3) 68 (normal < 3.0 CU). Based on these evidences, diagnosis of WG was made.

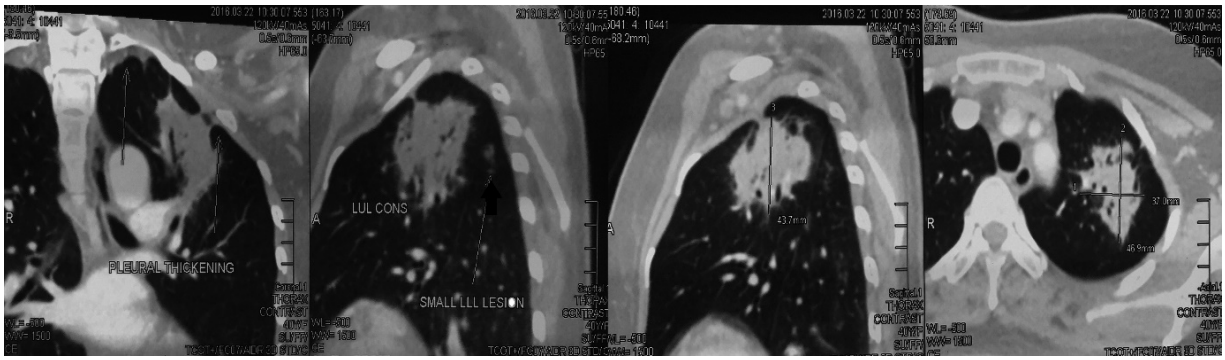

Figure 2 Left upper lobe focal dense consolidation (dark-coloured arrow) with speculation and adjacent fibrosis and pleural thickening on a background of ground glass opacity

Patient was treated with 6-hourly guttae Dexamethasone to reduce cells that present in anterior chamber. In additional to that, the authors added oral ibuprofen 400 mg thrice daily to reduce inflammation. However, redness of both eyes did not resolve. Immunosuppressive therapy in the form of oral prednisolone and oral Azathioprine was initiated with prophylaxis Trimethoprim-Sulfamethoxazole (Bactrim). Patient responded well and eye redness resolved (see Figure 3). Currently she is doing well and on regular follow-up.

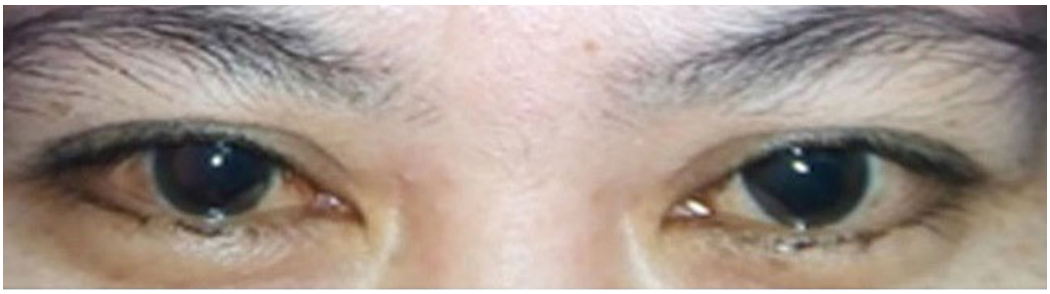

Figure 3 After commencement of treatment

DISCUSSION

Chronic conjunctivitis is one of the rare manifestations of WG. The non-responding nature of conjunctivitis coupled with the lung radiological findings lead to suspect vasculitis. High resolution computer tomography of thorax later confirmed a left upper lobe dense consolidation with speculation with adjacent fibrosis and pleural thickening on a background of ground glass appearance. These radiological features are in consistent with Wegener's granulomatosis.[3] A set of investigations are needed for supportive diagnosis tools. Antineutrophil cystoplasmic antibody test (ANCA) is an important diagnostic criterion for WG. More specific for WG is c-ANCA is an autoantibody directed against the neutrophil serine protease.[2] The c-ANCA test has a high sensitivity (96%) for WG.[4] Tissue biopsy from the suspected region of lesion is essential as to give a confirmatory histology diagnosis. This patient refused to do tissue biopsy. On investigation, raised inflammatory markers supported WG. The aetiology of WG is unknown and postulated causes might be autoimmune origin, genetic predisposition, connective tissue disease, viral or hypersentivity interaction.[5] Clinical presentations are heterogeneous and can be either insidious or acute. The patient was started with oral prednisolone of 1 mg/kg per day and oral Azathioprine. Both her eyes and constitutional symptoms resolved with treatment (Figure 3). Staphylococcus

Aureus is one of the aetiological factors for WG in some patient and serve as trigger factor.[6] Trimethoprim /Sulfamethoxazole (Bactrim) has been found beneficial and prevent S. Aureus infections.[7] As such she was on Trimethoprim/ Sulfamethoxazole (Bactrim) apart from her immunosuppressant therapy.

CONCLUSION

Connective tissue diseases may be one of the differential diagnoses for female in reproductive age. There are many differential diagnoses for chronic conjunctivitis but connective tissue disease especially WG should be kept in mind as one of the primary differential diagnoses in female. High index of suspicion can prevent mistreatment and eventually prognosis of the disease which may be fatal if untreated.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, et al. (2013). 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis & Rheumatology 65 (1): 1 – 11.
2. Ramsey MK, Owens D. (2006). Wegener's Granulomatosis: A review of the clinical implications, diagnosis, and treatment. Laboratory Medicine 37 (2): 114 – 116.
3. Halshtok O, Eshet Y. (2010). Computed tomography scan in necrotizing granulomatosis. IMAJ 12 (3).
4. Csernok E, Holle J, Hellmich B, et al. (2004). Evaluation of capture ELISA for detection of antineutrophil cytoplasmic antibodies directed against proteinase 3 in Wegener's granulomatosis: first results from a multicentre study. Rheumatology 43 (2): 174 – 180.
5. Lapraik C, Watts R, Bacon P, et al. (2003). BSR and BHPR guidelines for the management of adults with ANCA associated vasculitis. Rheumatology. 2007 Oct 1; 46 (10): 1615 – 1616.
6. Popa ER, Tervaert JC. The relation between Staphylococcus aureus and Wegener's granulomatosis: Current knowledge and future directions. Internal medicine 42 (9): 771 – 780.
7. Stegeman CA, Tervaert JWC, de Jong PE, Cees GM, Kallenberg MD. (1996). Trimethoprim–sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. New England Journal of Medicine 335 (1): 16 – 20.

Case Report: A Rare Life-threatening Side Effect of Trimethoprim-Sulfamethoxazole

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ABSTRACT

Trimethoprim-Sulfamethoxazole (TMP-SMX) or Co-Trimazole is the treatment of choice for meliodosis. A 52-year-old man presented with generalized body weakness with reduced appetite. He had bradycardia on examination. After investigations, he was diagnosed as hyperkalaemia. He had life-threatening hyperkalaemia treated with Trimethoprim-Sulfamethoxazole (TMP-SMX) as part of the eradication therapy for Meliodosis. Urgent haemodialysis was done. There were changes done for his meliodosis treatment. This case wished to highlight the importance of considering hyperkalemia in patient treated with Trimethoprim-Sulfamethoxazole especially when risk factor for hyperkalaemia is present.

Keywords:Trimethoprim-Sulfamethoxazole, hyperkalaemia, meliodosis

INTRODUCTION

Trimethoprim-Sulfamethoxazole (TMP-SMX) or Co-Trimazole has been used as an antibiotic since 1974[1]. TMP-SMX is effective against a wide variety of aerobic gram positive and gram negative bacteria, P.jirovecii and some protozoa[2, 3]. In Meliodosis infection, TMP-SMX forms the 'back bone' of its treatment[4]. The efficacy of TMP-SMX in Meliodosis treatment has been well documented in literature[4,5, 6,7]. However, like other antibiotics, TMP-SMX is not free from side effects. One of the often overlook side effects of TMP-SMX is hyperkalemia that may be life threatening if not detected and treated promptly. The association between Trimethoprim-Sulfamethoxazole and hyperkalaemia has been well described in literature[8, 9].

CASE PRESENTATION

A 52-year-old man known case of diabetes mellitus, hypertension, chronic kidney disease and chronic liver disease due to Hepatitis B was seen in the medical outpatient clinic a month after his discharge. He had a recent long stay in the ward and treated for meliodosis with chronic osteomyelitis of left tibia and abdominal prostate abscess. He was discharged with oral Trimethoprim-Sulfamethoxazole as part of Meliodosis eradication therapy. The antibiotic supply was given until his clinic appointment date.

Two weeks after discharged, he was reviewed in the clinic. During clinic review, he complained of generalized body weakness with reduced appetite, other systemic review was unremarkable. He was compliance with his medication. He was neither on nephrotoxic drugs like ACEI or other diuretics nor traditional medication. In view of his significant past medical history and the possibility of meliodosis relapse, he was admitted to the ward for further clinical evaluation.

On examination, he was alert with intact cognition. He had stigmata of chronic liver disease but no asterixis or jaundice to suggest decompensation. There was a discharging sinus over the left anterior shin with minimal clear secretion. Vital sign revealed blood pressure of 130/79 mmHg; heart rate 37 beats/min, temperature 37°C, SpO2 97%. Cardiovascular system: S1 and S2 without murmur; Respiratory system: equal breath sound without added sound; Abdomen system: soft, non-tender without palpable liver/spleen.

In view of the unexplained bradycardia and background chronic kidney disease, we performed urgent serum potassium, venous blood gases and ECG (see Figure 1).

Venous Blood Gases (VBG): pH7.168, pCO2 19 mmHg, HCO3 6.7 mmol/L, urea 25.7 mmol/L, sodium 128 mmol/L, potassium 8.91 mmol/L, creatinine 487 µmol/L, creatinine clearance 12 ml/min, haemoglobin 8.8g/dl, total white cell 9.8x109/L, platelet 257 × 109/L. A month ago, his renal parameters were urea 17.6 mmol/L, sodium 132 mmol/L, potassium 3.73 mmol/L, creatinine 374 µmol/L; creatinine clearance 15.92 ml/min.

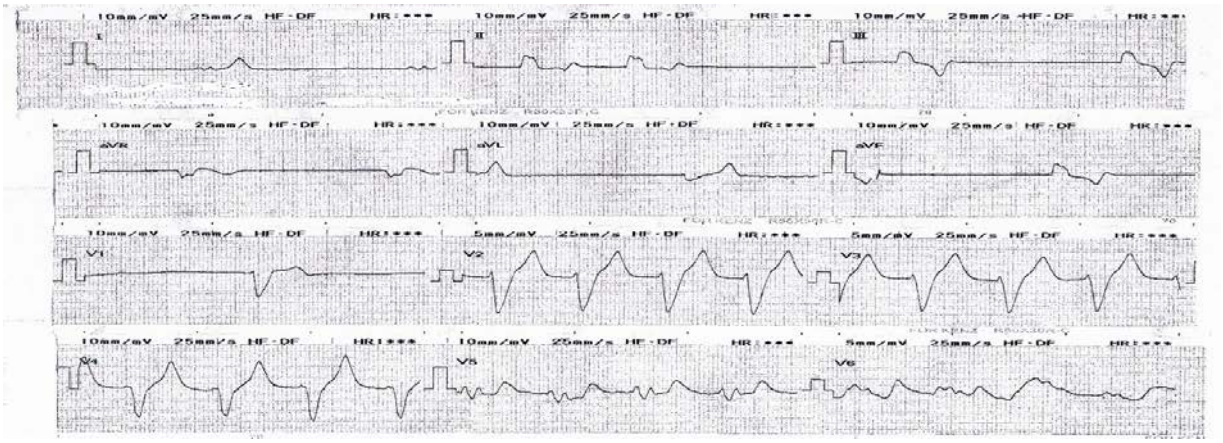

Figure 1 Sinus bradycardia with broad QRS complex and tall tented T-wave consistent with hyperkalaemia changes before haemodialysis

He was treated as life threatening hyperkalaemia secondary to Trimethoprim-Sulfamethoxazole. He was dialyzed urgently via a temporary femoral catheter. While waiting for haemodialysis, he was given two cycles of IV Calcium Gluconate 10% with IV Dextrose 50% and IV Insulin, twice Salbutamol 5 mg nebulization and immediate dose of powder calcium polystylene sulphonate (Kalimate) and Syrup Lactulose. During admission, he underwent twice haemodialysis session to lower down his potassium. Trimethoprim-Sulfamethoxazole was withheld and adverse drug reaction to this agent was notified to the pharmacy unit. Overtime, his serum potassium level reduced to normal range and the initial ECG changes had resolved as shown in Figure 2. His initial bradycardia had resolved once haemodialysis commenced and hyperkalaemia resolved. Upon discharge, his heart rate was in the range of 70 – 85 beats/min, regular with good volume.

He was discharged with oral amoxicillin-clavulanic acid and Doxcycline as an alternative eradication therapy for his meliodosis treatment. Both antibiotics were given for two weeks where he was seen again in clinic after that. His serum potassium has remained in the normal range ever since not on Trimethoprim-Sulfamethoxazole. Two months after discharge, he was admitted again in septic shock with relapse of meliodosis. Unfortunately he did not survive the second admission.

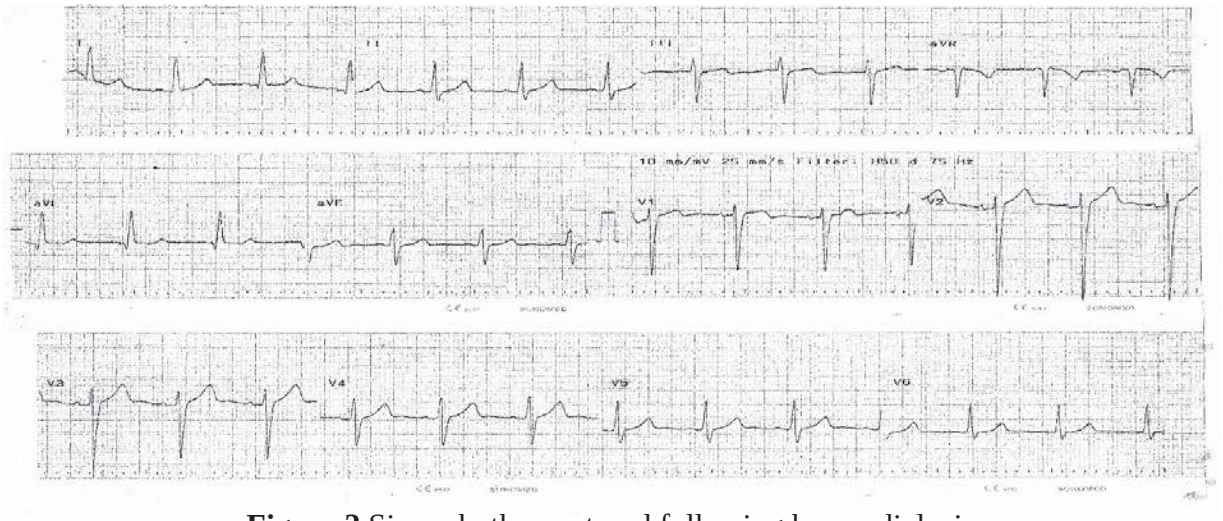

Figure 2 Sinus rhythm restored following haemodialysis

DISCUSSION

This case illustrates a rare yet important side effect of trimethoprim-sulfamethoxazole in causing life threatening hyperkalaemia especially in a chronic kidney disease patient. Often this side effect is overlooked in general practice, exposing patient to unnecessary risk that may threaten life.

The association between Trimethoprim-Sulfamethoxazole and hyperkalaemia has been well described in literature 8, 9. The risk factors for hyperkalaemia predisposition in patient taking Trimethoprim-Sulfamethoxazole include chronic kidney impairment, concurrent usage of angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), potassium sparing diuretic (i.e. spironolactone), underlying immune-compromised state 9, 10. Trimethoprim acts like Amiloride, a potassium sparing diuretics by inhibiting the apical membrane sodium channel of distal nephron. As a result, the transepithelial voltage is reduced causing potassium retention11. In our patient, he had baseline kidney impairment but he was not prescribed with any nephrotoxic medication.

Trimethoprim-Sulfamethoxazole forms the critical component meliodosis treatment4. This is more essential with the presence of deep-seated abscess and osteomyelitis like in our case. Literature review showed that other alternative for meliodosis therapy like Amoxicillin-Clavulanic Acid is less effective, associated with higher relapse rate as compare to regimen containing Trimethoprim-Sulfamethoxazole 7. Based on this finding, hence this patient was prescribed with Trimethoprim-Sulfamethoxazole despite having renal impairment as the benefit outweighs the harm. Duration of Trimethoprim-Sulfamethoxazole treatment is at least 5 months depending on clinical and radiological response.

We wished to emphasize that close monitoring of serum potassium and kidney function warranted once Trimethoprim-Sulfamethoxazole commenced for patient especially for those with high-risk factors 8, 9, 10. A deterioration of kidney function may need adjustment of dosage or even stopping the agent directly. The most serious manifestation of hyperkalaemia are muscle weakness, paralysis, cardiac arrhythmia and conduction abnormalities. Hyperkalaemia has many effects on the heart; the cardiac manifestation can varies from bradyarrhythmia at one end of spectrum to tachyarrhythmia at the other end. In our case, if the hyperkalaemia was not detected earlier and treated promptly, he might went into cardiac arrest due to cardio-toxic effect of hyperkalaemia. The association between sudden death and hyperkalaemia has been strongly supported by various literatures 8, 9. Yet some patient may be apparently well apart from some vague symptoms as shown in this case. Hence a strong clinical suspicion is necessary.

CONCLUSION

Life-threatening hyperkalaemia and sudden death are a known rare complication of Trimethoprim - Sulfamethoxazole treatment. High-risk patient for hyperkalaemia due to Trimethoprim - Sulfamethoxazole include chronic kidney impairment, concurrent usage of angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), potassium sparing diuretic (i.e. spironolactone), underlying immune-compromised state like AIDS. Thus, close monitoring of serum potassium and kidney function warranted especially among the high-risk group patients.

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REFERENCES

1. Oxford handbook of infectious diseases and microbiology. (2009). OUP Oxford. p.56.
2. Kalkut G. (1998). Sulfonamides and Trimethoprim. Cancer Invest 16 (8): 612.
3. Gleckman R, Gantz NM, Joubert DW. (1981). Intravenous Sulfamethoxazole-Trimethoprim: pharmacokinetics, therapeutic indications and adverse reactions. Pharmacotherapy 1 (3): 206.
4. Currie BJ, Fisher DA, Howard DM, Burrow JN, Lo D, Selva-Nayagam S, Anstey NM, Huffam SE, Snelling PL, Marks PJ, Stephens DP, Lum GD, Jacups SP, Krause VL. (2000). Endemic meliodosis in tropical Northern Australia: A 10-year Prospective Study and Review of The Literature. Clin Infect Dis 31 (4): 981 – 986.
5. Chusri S, Hortiwakul T, Charoenmak b, Silpapojakul K. (2012). Outcomes of patients with meliodosis treated with cotrimazole alone for eradication therapy. Am J Trop Med Hyg 87: 927 – 932.
6. Chetchotisakd P, Chierakul W, Chaowagul W, Anunnatsiri S, Phimda K, Mootsikapun P, Chaisuksant S, Pilaikul J, Thinkhamrop B, Phiphitaporn S, Susaengrat W, Toondee C, Wongrattanacheewin S, Wuthiekanun V, Chantratita N, Thaipadungpanit J, Day NP, Limmathurotsakul D, Peacock SJ. (2014). Trimethoprim-sulfamethoxazole versus trimethoprim-sulfamethoxazole plus doxycycline as oral eradicative treatment for meliodosis (MERTH); a multicenter, double-blind, non-inferiority, randomized controlled trial. Lancet 383 (9919): 807 – 814. Epub2013 Nov 25.
7. Rajchanuvong A, Chaowagul W, Suputtamongkol Y, Smith MD, Dance DA, White NJ. (1995). A prospective comparison of co-amoxiclav and the combination of chromphenicol, doxycycline, and co-trimoxazole for the oral maintenance treatment of meliodosis. Trans R Soc Trop Med Hyg 89 (5): 546.
8. PerazellaMA, Mahnensmith RL. (1996). Trimethoprim-sulfamethoxazole: hyperkalemia is an important complication regardless of dose. Clin Nephrol 46 (3): 187 – 192.
9. AlappanR, Perazella M, Buller G. (1996). Hyperkalemia in hospitalized patients treated with trimethoprim-sulfamethoxazole. Ann Intern Med 124 (3): 316 – 320.
10. Antoniou T, Gomes T, Juurlink DN, Loutfy MR, Glazier RH, Mamdani MM. (2010). Trimethoprim-sulfamethoxazole-induced hyperkalemia in patients receiving inhibitors of the renin-angiotensin system: a population-based study. Arch Intern Med 170 (12): 1045 – 1049. doi:10.1001/archinternmed.2010.142
11. Velázquez H, Perazella MA, Wright FS, Ellison DH. (1993). Renal mechanism of trimethoprim-induced hyperkalemia. Ann Intern Med 119 (4): 296 – 301.

Primary Malignant Melanoma of the Pleura: A Rare Case

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ABSTRACT

Primary pleural melanoma is a very rare condition and highly aggressive tumour. A patient presented with productive cough, haemoptysis, pluritic chest pain and breathlessness. On investigation, she was diagnosed as left-sided lung mass with pleural effusion. Pleural biopsy confirmed malignant melanoma of pleura and she was subsequently referred to the oncology team for palliative chemotherapy. In conclusion, primary melanoma of pleura remains an uncommon disease and for this no proven effective treatment regime is available.

Keywords: primary, malignant melanoma, pleura

INTRODUCTION

Malignant melanoma (MM) commonly arises from the skin or the eyes and is the leading cause of death.[1] Primary malignant melanoma is a rare condition. Malignant melanoma can involve any mucosal regions like oral mucosa, oesophagus, larynx and the ano-genital mucosa.[1] It is commonly metastasize from skin cancers.

Few cases have been reported in medical field with the criteria to diagnose primary pleural melanoma.[2] The proposed criteria to diagnose pleural melanoma is difficult to fulfil as all extra pulmonary origin of the tumour should be excluded first.[3] Unfortunately, many patients are very anxious when this diagnosis is being informed to them and hence further invasive investigation to rule out other primary sources are hard to be performed just like in this case that we encountered.

CASE PRESENTATION

A 41-year-old non-smoker Malay lady presented with two weeks history of productive cough associated with haemoptysis and pleuritic chest pain. She also reported weight loss and poor appetite during this period.

Clinically, she was breathless. Respiratory system examination was consistent with a left-sided pleural effusion. There was no palpable lymphadenopathy. Skin examination of the total skin surface revealed no melanoma. She was normotensive and non-diabetic.

Chest radiograph showed homogenous opacity in the left lung (Figure 1). Bedside ultrasound scan of her left lung showed a lung mass with pleural effusion. We proceeded with left pleural biopsy and a thoracostomy tube was inserted and pleural fluid drained was sent for analysis. The results came back as exudative pleural effusion based on Light's criteria. The immunohistochemistry and histomorphological report confirmed the diagnosis of malignant melanoma of the lung. The tumour cells were positive for the expression of intracellular melan-A, human melanoma-45 (HMB-45), vimentin and S-100 in immunohistochemistry. It was negative for calretinin and pancytokeratin. Her positron emission tomography/computed tomography showed a left pleural mass with large pleural effusion in the left hemithorax with raised metabolic activity seen in left pleura, right lung and ribs (Figure 2). There was possible right lung and skeletal metastasis. Retinal examination under slit lamp did not reveal any evidence of melanoma. We had counselled her for an endoscopic examination to rule out any gastrointestinal tract source of her melanoma

but she refused. We did not investigate for leptomeninges melanoma metastasis in view that she was asymptomatic with no headache or signs of raised intracranial pressure. She was referred to the oncology team who counselled her for palliative chemotherapy. She received a cycle of chemotherapy with dacarbazine (200mg/m2, days 1 – 3) and cisplatin (30mg/m2, days 5 – 7) during her inpatient stay but subsequently took self-discharged against medical advice to seek alternative medicine opinion searching for a cure to her illness. She eventually presented after three months with severe dyspnoea and succumbed to the disease.

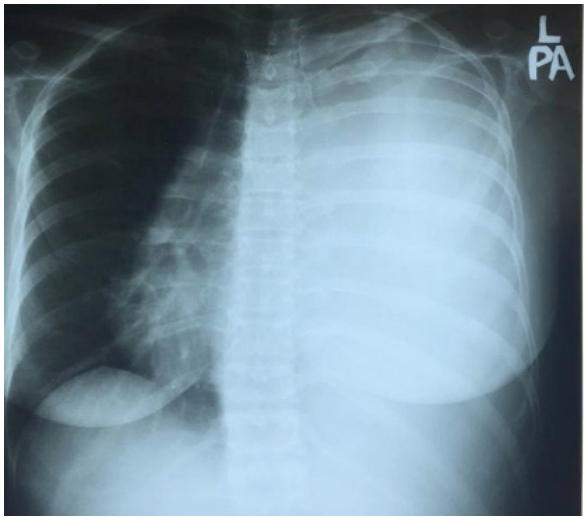

Figure 1 Chest X-ray showed homogenous opacity over the left hemithorax

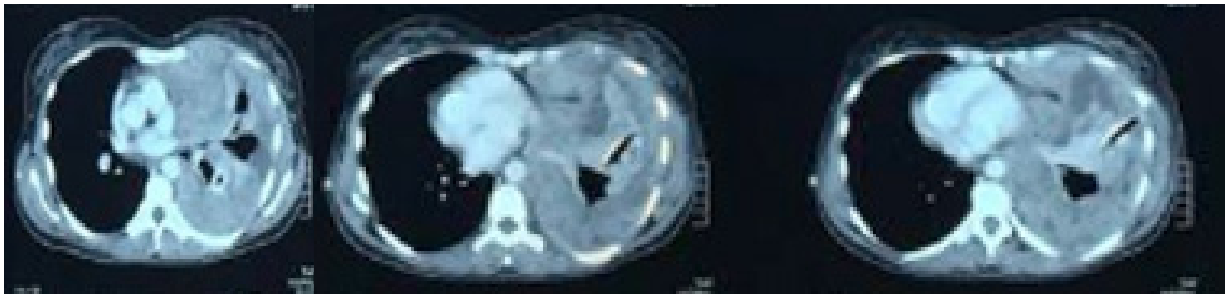

Figure 2 Computed tomography of thorax showed left pleural mass with effusion

DISCUSSION

Primary malignant melanoma (MM) of the pleura is an uncommon condition accounting in about 0.01% of all lung malignancy.[1] It can present endobronchially with respiratory symptoms such as cough, haemoptysis, lung collapsed or atelectasis.[3]

This condition can mimic other lung malignancy and hence a histopathological study would be beneficial. The final diagnosis of primary MM of the lung is established based on clinical, radiological and pathological findings.[3]

The proposed criteria for the diagnosis of primary MM includes the following[3]:

1. Junctional changes like 'dropping off' or nesting of the melanoma cells just beneath the bronchial epithelium.
2. Invasion of the bronchial epithelium by melanoma cells.
3. Malignant melanoma associated with these epithelial changes.
4. A solitary lung tumour.
5. No history of cutaneous, mucous membrane or ocular melanoma.
6. Absence of other detectable tumour at the time of diagnosis.

The pathogenesis of this melanoma of the lung is still poorly understood.[3] One hypothesis is that melanocytes are the cells of neuroendocrine system in the body.[1] Melanocytes migrate to the layers of skin like epidermis and the dermoepidermal junction. Sometimes these cells can also migrate to the viscera during embryogenesis.[3] This has been suggested for the oesophagus, larynx and might be the cause for the lung. The residual primitive melanoblasts that share a common origin with other melanoblasts located in the trachea, oesophagus, and pharynx, giving rise to MM of the lung. Their origin from the neuroectoderm is also the cause of their low incidence in the endodermal epithelium. There is another theory that believes that melanoma cells may be the derivative of pluripotent stem cells.[3]

Treatment of choice would be surgical resection.[3] The role of post-operative adjuvant chemotherapy is not clear. For the mucosal melanoma of the head and neck, radiotherapy had been tried but results were not good.[1] As in this case, chemotherapy is used mainly for palliative only. The prognosis of this disease is poor but available data is inadequate to conclude with conviction.[3]

CONCLUSION

Primary malignant melanoma of the lung is an uncommon pathological entity. It can be diagnosed with careful assessment of both clinical and histopathological studies to establish the diagnosis.

ACKNOWLEDGEMENTS

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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 with its related pictures. A copy of the written consent is available for review by the Chief Editor of this journal.

REFERENCES

1. Smith S, Opipari MI. (1978). Primary pleural melanoma. A first reported case and literature review. J Thorac Cardiovasc Surg 75: 827 – 831.
2. Wang Q, Chen J, Dassarath M. (2015). Primary malignant melanoma of the pleura with rapid progression: A case report and literature review. Oncol Lett 9 (6): 2713 – 2715. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4473647/
3. Agarwal P, Nambiyar K, Manju Kaushal, Bhardwaj M. (2016). Primary malignant melanoma of pleura: A case report and literature review. Diagn Cytopathol 44 (7): 648 – 652. doi: 10.1002/dc.23497.

Case Report: Trigeminal Schwannoma in a Patient with Left-sided Facial Numbness

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ABSTRACT

Trigeminal schwannoma is a rare cause of cerebellopontine angle tumour. This case report of a 65-year-old lady presented with ipsilateral facial numbness and instability. She was finally diagnosed to have trigeminal schwannoma after seeking multiple medical consultations with her doctors. This case report highlights a rare cause of cerebellopontine angle tumour.

Keywords: cerebellopontine angle tumour, trigeminal schwannoma, schwannoma

INTRODUCTION

Trigeminal schwannoma is a rare cause of cerebellopontine angle tumour as it only constitutes 0.2 – 0.4% of all intracranial tumours.[1] This tumour is slightly more common in female than male and usually occurs in the age group of 30 to 40s.[2, 3] The tumour grows slow and may cause compression to the surrounding structure. Due to the slow growing nature, patient with trigeminal schwannoma may experience symptoms for a long time before seeking medical attention and rarely presented with features of raised intracranial pressure as there is sufficient time to compensate for the increase pressure. With the advancement of imaging techniques and refinement of neurosurgical methods, trigeminal schwannoma is highly treatable with a good prognosis if detected and acted upon timely.[2, 3, 4]

CASE PRESENTATION

A 65-year-old lady with dyslipidaemia on diet control under health clinic follow-up. She was referred to the medical outpatient clinic with the complaint of left-sided facial numbness for a year. She initially noticed the symptom of numbness describing as 'pins and needles' over the left side of her face occurring intermittently. However, for the past 3 months, the facial numbness was consistently associated with instability when walking. She felt that her body swayed to the left side when she walks and there was fullness felt in her left ear. She had sought multiple medical attentions at various private general practitioners without any symptomatic relief. There was no associated headache, blurring of vision or vomiting to suggest raised intracranial pressure. Apart from instability, her four limbs were of normal strength without any numbness or pain sensation. Prior to the facial numbness, there was no preceding fall or injury to her face. The numbness was not associated with lancinating facial pain suggestive of trigeminal neuralgia. She had no difficulty in chewing and swallowing food or drink and no drooling of saliva. She has no history of hypertension or diabetes. Her family history was otherwise unremarkable for any brain tumour or neurocutaneous disorder like neurofibromatosis.

Assessment revealed a full Glasgow Coma Scale with normal speech. Her face appeared symmetrical without any involuntary twitching of the facial musculature. Both eyelids were symmetrical without any obvious ptosis or proptosis; pupils were equal and reactive. There was no obvious scar or skin lesions like neurofibroma appreciated on her face and any other part of body. Local palpation of the temporomandibular joints was non-tender, no crepitus felt on passive closure of her mouth. Oro-buccal cavity assessment revealed good dental hygiene without any dental carries. There

was reduced pin prick and light touch sensation on the left side of her face in the V_1 to V_3 distribution of the trigeminal nerve. The corneal reflex was impaired on both sides. Muscle bulk and power of mastication muscle was intact both sides. Auditory assessment revealed Rinne's test of air conduction better than bone condition for both ears; Weber's test was centralised. Formal audiometry assessments were done (Table 1).Otherwise other cranial nerves assessments were unremarkable.

All four limbs showed normal tone, power and sensation with intact normal tendon reflexes. Coordination was intact with negative Romberg sign.

The differentials for ipsilateral facial numbness includes trauma to the face and its underlying nerve branches, dental pathology with referred pain to the ipsilateral face, lesions involving the trigeminal nerve along its route from the brainstem to the cerebello-pontine space, cavernous sinus and lastly skull exit foramina. There was neither associated crossed long tract sign nor opthalmoplegia to suggest brainstem and cavernous sinus involvement respectively.

As she had ipsilateral reduced sensation of V_1 – V_3 distribution of trigeminal nerve, with absence of corneal reflex on both sides and subjective ipsilateral instability over a year duration; cerebello-pontine angle tumour need to be considered.

We proceeded with Computed Tomography of the Brain for her case which revealed a suspicious cerebro-pontine angle mass lesion. A Magnetic Resonance Imaging of the Brain was performed to further delineate the nature and extent of the mass in relation to the surrounding structures (Figure 1).

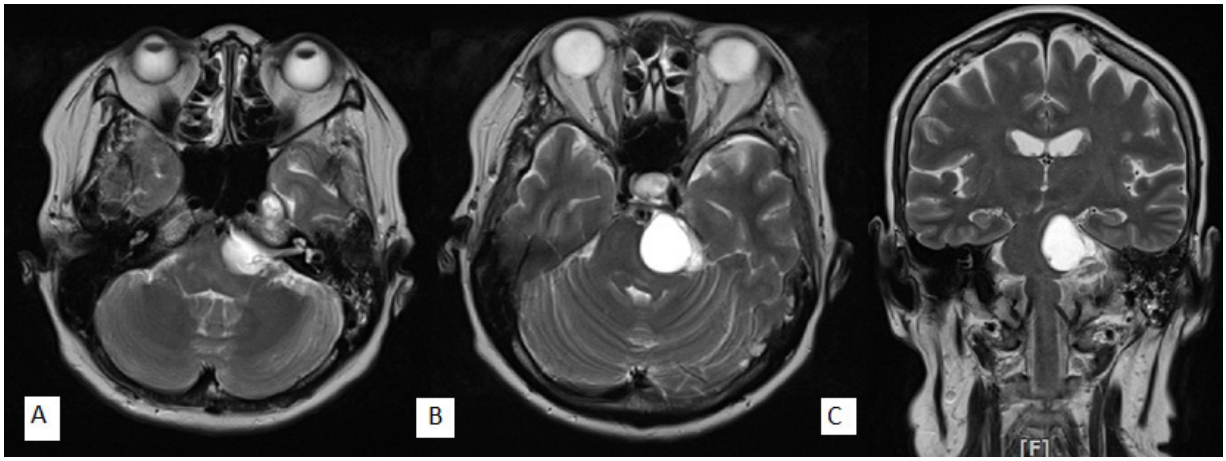

Figure 1 (A) MRI axial view showing tumour occupying the left cerebellopontine angle; (B) A more rostral axial view showing the same tumour compressing the adjacent pons; (C) MRI coronal view showing tumour compressive effect on the brainstem. The tumour measured at 2.8 cm (W) × 2.7 cm (H) × 3.5 cm (AP). The tumour lies superior and medial to the left internal auditory canal without any extension into the canal. The pons, left middle cerebellar peduncle and the 4th ventricle are compressed by the tumour without any hydrocephalus or significant midline shift.

Table 1 Formal Audiometry Assessment

	Left Ear	Right Ear
Tympanometry	Type As bilateral suggestive of middle ear stiffness	
Pure Tone Audiometry	Normal bilateral	

Once diagnosed with cerebello-pontine angle tumour, she was referred promptly to neurosurgical team for evaluation. She underwent neurosurgical operation for excision of the tumour, followed by Gamma Knife Surgical Intervention to remove the residual tumour. The intraoperative specimen was sent for histopathological examination which was later reported as trigeminal schwanoma. Six months post-operation, she still had left-sided reduced pin prick and light touch sensation over $V_1 - V_2$ distribution with intact corneal reflex. Other aspects of neurological assessment were unremarkable. She was able to ambulate without feeling unstable unlike prior to neurosurgical intervention. Figure 2 shows residual tumour after neurosurgical intervention.

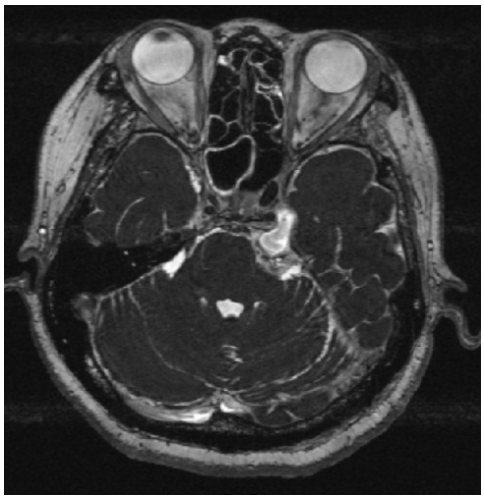

Figure 2 MRI axial view illustrates the residual tumour after resection. The adjacent brainstem compression is significantly improved compared to Figure 1B. The residual tumour measured at 1.4 cm (W) × 1.7 cm (H) × 2.5 cm (AP).

DISCUSSION

Cerebellopontine (CP) angle is a shallow triangle space bounded by the undersurface of cerebellar hemisphere, lateral aspect of pons and the superior surface of the inner third petrous ridge. This space spans cranially from the cranial nerve V^{th} to the cranial nerve IX^{th} rostrally.[5] In view of the close proximity of the structures, a lesion in CP angle (mostly tumours) causes a constellation of signs and symptoms. The extent of involvement depends on the location and size of the lesion. CP angle tumours consist of 5 – 10% of all intracranial tumours. Of all these CP angle tumours, 80 – 90% comprise of vestibular schwannoma or better known as acoustic neuroma. The remainder 10 – 20% consists of trigeminal schwannoma, epidermoid cysts, dermoid cysts, meningioma, arachnoid cysts, lipomas and secondary tumours.[1, 2] Trigeminal schwannoma as in our case constitutes only 0.2 to 0.4% of all intracranial tumours. Hence it is a very rare cause of CP angle tumours.[1]

Trigeminal schwannoma is a benign tumour of the Schwann cells which surrounds the trigeminal nerve. This tumour can arise from the trigeminal nerve root entry zone, the Gasserian ganglion, or any of the three branches of trigeminal nerves, namely ophthalmic, maxillary and mandibular branches.[6] Patients with trigeminal schwannoma commonly present with facial numbness, pain, hypoesthesia like this case. Other clinical features include diplopia, gaze abnormalities, nystagmus, wasting of mastication muscles. Less frequently, they may have hearing loss, ataxia, dysarthria or pathological crying due to brainstem compression.[2] The symptomatology varies largely depending on the size and location of the lesion. In view of the benign nature, most trigeminal schwannoma grows slowly and are treatable with neurosurgical intervention. Magnetic Resonance Imaging of the brain is the best diagnostic imaging modality to guide neurosurgical intervention given its high soft tissue resolution compare to computer tomography of the brain.[6] The outlook and prognosis have gotten better given the improvement in diagnostic imaging and operative techniques.[7]

We highlight the challenges in the diagnosis of cerebellopontine (CP) angle tumour. The patient's symptom had been there for over a year and the only reason that prompted her to seek medical consultation was her facial numbness that had been more frequent

of late. Her symptom was overlooked as CP angle tumour, a rare diagnosis especially more so in our setting. The authors had only seen two cases of CP angle tumour in a year including the present case. During her initial visit to the doctors, there was no prompting to perform brain imaging given the rather subjective sign and symptom. Her presentation of ipsilateral facial numbness with subjective instability was typical for CP angle tumour. The finding of large tumour upon diagnosis was expected given that she had been experiencing the symptoms for a year before diagnosis. However, the absence of other neurological deficits was a surprise given that there was significant compression of the surrounding brain structures in particularly the brainstem. The absence of corneal reflex on the contralateral side when she initially presented could well represent a false localising sign yet there was no other features like abducens nerve palsy to support this postulation. Reduced facial sensation to light touch and pin prick persisted postoperatively as there was still residual tumour. Complete resection was not possible given the close proximity of tumour to the adjacent cranial nerves and brainstem.

CONCLUSION

Cerebellopontine angle tumour, although rare, need to be considered for any individual who presented with facial symptoms and subjective ipsilateral instability due to the good prognosis if detected early and treated. We have highlighted the challenges in diagnosing cerebellopontine angle tumour in our healthcare setting which could be due to unfamiliarity of this clinical entity.

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The authors thank the Director General of Health, Ministry of Health Malaysia for his permission to publish this case report.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

CONSENTS

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REFERENCES

1. Day JD, Fukushima T. (1998). The surgical management of trigeminal neuromas. Neurosurgery 42: 233 – 241.
2. Fukaya R, Yoshida K. (2010). Trigeminal schwannomas: Experience with 57 cases and a review of the literature. Neurosurgical Review 34 (2): 159 – 171.
3. Goel A, Muzumdar D, Raman C. (2003). Trigeminal neuroma: Analysis of surgical experience with 73 cases. Neurosurgery 52: 783 – 790.
4. Renowden S. (2014). Imaging of the cerebello-pontine angle. Pract Neurol 14 (5): e2. doi:10.1136/practneurol-2014-000949.
5. Bone I, Hadley DM. (2005). Syndromes of the orbital fissure, cavernous sinus, cerebellopontine angle and skull base. J Neurol Neurosurg Psychiatry 76 (III): 29 – 38.
6. Agarwal A. (2015). Intracranial trigeminal schwannoma. Neuroradiol J 28 (1): 36 – 41. doi: 10.15274/NRJ-2014-10117.
7. Alam S, Khair A, Hassan R, Munir SF, Wakil. (2009). The surgical management of trigeminal schwannomas. The ORION Medical Journal 32 (2): 659 – 662.

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