Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal of Medical Research: K

Interdisciplinary



Multidrug Resistance Protein

Effects of Exercise on Doxorubicin

Highlights

Association between Breast Cancer

Assessment of Barriers of Behavioral

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 6 VERSION 1.0

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Volume 16 Issue 38 was originally issued as K6

Global Journal of Medical Research: K
Interdisciplinary

Volume 16 Issue 38
Originally issued as K6

Global Journal of Medical Research: K Interdisciplinary

Volume 16 Issue 6 (Ver. 1.0)

Open Association of Research Society

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Is Medical Research at the Right Track

By Rafal Al-Rawi
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Abstract- *Background:* As it is well known that a disease is any disorder or incorrectly functioning organ, part, structure or system of the body resulting from the effect of genetic or developmental errors, infection (caused by pathogenic microorganisms), poisons, nutritional imbalance or deficiency, toxicity or unfavorable environmental factors [1,2]. Fortunately, during twentieth century, medical investigation and research have been diagnosed, treated, cured and prevented many diseases. The primary treatment is through using vaccines and drugs, which are required and beneficial. Medical research is a vital to the health and wealth of societies, as scientific knowledge can improve health and the quality of life.

The objectives of this article are to discuss many topics (immunity, immunogenetics, molecular genetics, pharmaceutical, histopathology and other relevant subjects), to get what has medical research done to improve health of human, to figure out whether these research are at the right track? and to suggest recommendations for future medical research.

GJMR-K Classification: *NLMC Code: W 20.5*



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Is Medical Research at the Right Track

Rafal Al-Rawi

Abstract- Background: As it is well known that a disease is any disorder or incorrectly functioning organ, part, structure or system of the body resulting from the effect of genetic or developmental errors, infection (caused by pathogenic microorganisms), poisons, nutritional imbalance or deficiency, toxicity or unfavorable environmental factors [1,2]. Fortunately, during twentieth century, medical investigation and research have been diagnosed, treated, cured and prevented many diseases. The primary treatment is through using vaccines and drugs, which are required and beneficial. Medical research is a vital to the health and wealth of societies, as scientific knowledge can improve health and the quality of life.

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Life Span: It is written that life span of ancient people were more than hundred years. A progressive decline over the generations from Adam's 930-year life, to Noah's 500 years, to Abraham's 175 [3]. As the life was so simple and there was no pollution, no food preservative, no drugs abuse, no depression, no hypertension, no pesticides, no insecticides, no competence, no hypocrisy and no hate. Mean life span varies with susceptibility to diseases and other environmental (risk) factors [4, 5].

Immunity: Immunity refers to having a resistance to a disease or illness. It is well documented that natural immunity possessed by individuals at birth prior to exposure to pathogens or antigen and that includes components such as neutrophils and natural killer cells, which provide an initial response against infection [6]. Maternal passive immunity (Innate immunity) is a type of naturally acquired nonspecific passive immunity, and refers to antibody conveyed to a fetus or infant by its mother. It provides resistances through several physical, chemical and cellular approaches. Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies. Subsequent general defenses include secreted chemical signals (cytokines), antimicrobial substances and phagocytic activity associated with the inflammatory responses. Through these approaches, innate immunity can prevent the colonization and spread of microbes. On the other hand, naturally acquired immunity occurs through contact with a disease causing agent. Whereas, artificially acquired immunity develops through vaccination [7, 8 & 9].

Immunogenetics investigations showed the normal immunological pathways and the identification of genetic variations that result in immune defects, which may result in the identification of new therapeutic targets for immune diseases. Studies have indicated that host genetic factors are major determinants of susceptibility to infectious diseases [10, 11]. Also found high heritability estimates (proportion of genetic variation to the total phenotypic variation) for many immune responses to pathogen antigens [12, 13 &14]. Successful research results were obtained, in which candidate gene have implicated several immunogenetic polymorphisms in human infectious diseases. Human Leukocyte Antigen (HLA) variation has been associated with susceptibility or resistance to malaria, tuberculosis, leprosy, AIDS, and hepatitis virus persistence [15]. Variation in the tumor necrosis factor gene promoter has been reported to associate with several infectious diseases. It is likely that susceptibility to most microorganisms is determined by a large number of polymorphic genes, and identification of these should provide insights into protective and pathogenic mechanisms in infectious diseases. Genetic variations among individuals may affect susceptibility (or resistance) to various diseases [16]. It is important to identify genetic variations and its contributions to naturally acquired immune responses. This may lead to better health care of human being. Furthermore, most of the molecular genetic variations in immune responses remained unexplored. Therefore, the genetic variations among individuals within families in antibody response need to be investigated. As such investigation in human may be not easy it is recommended to investigate such issue in mice or Guinea pigs as a model for human. Results revealed that seven antibody isotypes of IgG4 heritability estimates were found to be high (above 0.5) of all isotypes responses to all antigens, indicating that IgG4 responses are most strongly genetically regulated [16]. Furthermore, they indicated that genetic variation in regulation of cytokines may also contribute. On the other hand, there is considerable heterogeneity in immunological parameters between individuals, but its sources are largely unknown. Non-heritable influences explain much of the variation in immune measurements [17].

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Vaccination: Vaccines are suspensions of infectious agents used to artificially induce immunity against specific diseases. A tremendous amount of biological events are triggered which are essential in developing immunity. Results revealed that cytokines are low-molecular weight proteins that control, coordinate, and regulate various immune or inflammatory responses. Recently, gene therapy and DNA vaccination has been used to produce memory against a number of cytokines that promote inflammation. Antibodies to the product of each inserted gene were produced. These antibodies were found to prevent the effects of the cytokines. Recent vaccine research and development has focused on recombinant DNA vaccines as a way of duplicating natural immunity, but the findings demonstrate that they work by suppressing the immune system as well. DNA vaccines consist of a bit of DNA containing a gene for a marker from the pathogen. The idea is that when the DNA is injected into the muscle tissue, it works its way into cells where it is incorporated into cellular DNA. Recent research indicated that instead of being immunized to the protein encoded by a DNA vaccine, it actually learns to tolerate it [18]. However, when later injected with the same protein, no antibodies were developed at all. This finding indicated that the possibility that a DNA vaccine could convert someone who normally would be able to clear a pathogen-albeit they might get sick first, to someone who would be unable to clear it at all. The science of immunology is on a fast track due to recent advances in molecular biology and genetics research. Though there is still much to be done. Thus it will need an increased emphasis on nutrition, exercise, and structural integrity of the human frame, all of which maximize the body's innate healing power. Lately, there have been complaints from individuals about vaccine side effects and the lack of long-term scientific studies and safety data on vaccines especially; nowadays there are more than 200 vaccines in use. Medical research in vaccination technique has successful stories and impact through eradication of many diseases in the past 100 years including polio, smallpox, whooping cough and diphtheria [19].

Diseases and Drugs: A disease is a particular abnormal condition, a disorder of a structure or function, which affects part or all of an organism. Disease is often associated with specific symptoms and signs. It may be caused by external factors such as pathogens, or it may be caused by internal dysfunctions particularly of the immune system such as an immunodeficiency, or hypersensitivity. There are four main types of disease: infectious diseases, deficiency diseases, genetic diseases and physiological diseases [2]. Medicines are commonly used worldwide. Drugs are used to diagnose, cure, treat, or prevent disease [20].

Drug therapy is an important part of the medical field and relies on the science of pharmacology for continual advancement and on pharmacy for appropriate management. Drug discovery and drug development are complex and expensive endeavors undertaken by pharmaceutical companies, academic scientists, and governments. Recently, there are many successful results, implications and advancement in pharmaceutical research and industry, especially when technology of sequencing of human genome where applied. Such technique allowed rapid cloning and synthesis of large quantities of purified proteins, it has become common practice to use high throughput screening of large compounds libraries against isolated biological targets which are hypothesized to be disease modifying in a process known as reverse pharmacology. Hits from these screens are then tested in cells and then in animals for efficacy. Moreover, scientists have been able to understand the shape of biological molecules at the atomic level, and to use that knowledge to design drug candidates. Modern drug discovery involved the identification of screening hits, medicinal chemistry and optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life), and oral bioavailability. Once a compound that fulfills all of these requirements has been identified, it will begin the process of drug development prior to clinical trials. Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy (expensive, difficult and inefficient process) with low rate of new therapeutic discovery [21]. In 2010, the research and development cost of each new molecular entity (NME) was approximately US$1.8 billion [22]. As it included pre-clinical research (microorganisms/animals) and clinical trials (on human) and may include the step of obtaining regulatory approval to market the drug. Nevertheless, drugs can help and keep people safe, but some are dangerous if they are used in the wrong way [23]. The controversial role of pharmaceutical and how diseases can be prevented is a vital issue to be investigated in details. There are many issues to be investigating such as mixing drugs that interact adversely and a drug overdose or poisoning besides that some people are sensitive to various drugs and how they are able to metabolize them. It is important to investigate the effect of drugs used and its consequences. It was reported that drug abuse had side effect on the gastrointestinal tract (esophagus, small intestine, colon, stomach and duodenum and jejunum). Furthermore, it has been showed histopathology consequences of drugs induced lesions, ulcers, hemorrhage, necrosis and gastritis [24]. Moreover, many bioactive natural products have been identified and characterized from cyanobacteria. Some compounds are of medical and/or pharmaceutical importance as they exhibit antimicrobial, antifungal, and anticancer activities [25]. However, some of these metabolites are toxic toward a great variety of

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organisms, including humans. Histopathologist researchers have been investigated the toxicity of such compounds via sectioning, staining and evaluation of cells and tissues. Furthermore, they focus on how a tissue is interacting with other tissues or drugs. Histopathological findings provided a correct diagnosis and assessment of many drug-induced allergies or diagnosis of new drugs toxicity or any adverse effects in patients. It highlights issues in evaluation of carcinogenicity of drugs [24]. Recently, it was reported that scientifically evaluation of biomarker performance in relation to histopathologic changes is essential as guidance for qualification process for drug development [26].

Human Genome Project: In the middle of the twentieth century, Watson and Crick revealed the chemical basis of heredity with their discovery of the double helical structure of DNA. Recently, sequence the entire human genome (Human Genome Project, HGP) has become an international effort, to develop a catalog of human DNA variations. While DNA sequences in peoples are 99.9% identical to each other, the 0.1% of variation is expected to contain many clues about the genetic risk for illnesses [27]. Technology developed to study the expression of many genes at once. The new technique can allow researchers to observe in a single experiment whether as many as 10,000 genes are turned on or off in various cells or under different conditions. Such studies will help reveal how different tissue types differ and what alterations in gene expression accompany the development of diseases. Such analyses have already helped find differences among tumors that otherwise seemed identical. Because large-scale experiments are flooding researchers with information, a field called computational biology is emerging and will become increasingly important for analyzing all these data [27].

Medical Research in the Twenty-First Century:

Having the human genome sequence and knowing the DNA spelling variations among people will help reveal which genes contribute to the risks for common diseases. This will be a challenging task. For diabetes, for example, researchers expect that five to ten - and perhaps more - genes are involved, all of which have forms that increase the risk for disease slightly. Those genes interact with each other and with the environment in complex ways. Finding a gene involved in such diseases is many times harder than in cases where a disease stems from variations in a single gene.

Even so, researchers are optimistic that by precisely diagnosing different forms of diseases like diabetes, heart disease, and cancer and by developing a large catalog of genetic variations, they will begin to find genes for some of the most common illnesses in the near future.

Researchers will need to develop and apply methods that analyze many drugs at a time for their potential affect on disease-related genes and gene products. The pharmaceutical industry has been gearing up for this opportunity, and most pharmaceutical companies now expect that the majority of future drug development will come from the field of genomics. New, efficient ways of analyzing the effects of many drugs should identify those that block or stimulate particular genetic pathways. Genomics is likely to help allow the prediction of individuals' responsiveness to particular drugs, since variations in drug response often stem from genetic differences. For example individuals break down particular drugs at different rates. Researchers are beginning to correlate variations in the spellings of genes with variations in responsiveness to different drugs. This new field is called pharmacogenomics and promises to make prescription of drugs a much more individualized affair in the future [27]. By 2020 the impact of genetics on medicine will be even more widespread. The pharmacogenomics approach for predicting drug responsiveness will be standard practice for many drugs. New gene-based "designer drugs" will be coming on the market for diabetes, hypertension, mental illness, and a long list of other conditions. The diagnosis and treatment of cancer will likely be transformed. By 2020, it is likely that every tumor will undergo precise molecular fingerprinting, to catalog the genes that have gone awry, and therapy will be individually targeted to that fingerprint [28].

Concluding Remarks: Although, it was reported that progress being made in individual's longevity is entirely due to medical and public-health efforts, rising standards of living, exercise, better education and healthier nutrition are also vital issues to be considered. Among medical researchers, progress and success is assessed according to professional and objective criteria such as volume of publication output in scientific journals and associated citations, securing research grants and running a large-scale research by multidisciplinary team. Outside the system, people want to know the impact of medical research on health of human and mortality [29].

God created man in his best form, especially when he lives in his natural environment. That means that his body is designed to be able to defend itself against foreign invaders. Nevertheless, with currently existing high risk environmental conditions, mankind has to cope with such situation. Therefore, many questions should be investigated in details and addressed to medical researchers, among these are the followings:

- What are the contributions of genetic versus non-genetic (environmental) factors to disease incidence?
- Is there any specific age at which active gene become inactive (or vice versa)? Or is there any

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specific age at which gene (allele) under specific environmental factors show its expression or switched off? Are oxygen (fresh air), chemicals, depression and hypertension play important role in incidence of diseases?

- How long can allele tolerate or how fast can allele respond to unfavorable environmental factors?
- Why do many people of more than 70-year old still healthy and enjoy life without serious diseases? Is there any variation in gene stability or sensitivity, and at what age?

Final Conclusion and Suggestions: It is concluded from this review that although huge scientific information and results of much medical research is just few drops from ocean. Moreover, although there were too many successful stories, achievements and progress made in medical scientific research, nobody knows where and how far medical research is going. Even though, people should be optimistic.

It is suggested that integrated multidisciplinary international team (pharmacists, molecular biologist, pathologists, geneticists, toxicologists and statisticians) should work together in all medical research activities to have more efficient and tangible impact on health of human.

Accordingly, below some suggestions to medical researchers:

1- A well defined experimental design should be followed by medical researchers to investigate sequencing the entire human genome to develop a catalog of human DNA variations, The experimental design should contain many experimental groups to identify risk for diseases incidence via screening people who are healthy and highly resistant to diseases, versus people who developed the disease across various age groups. Age groups may be: more than 80, 70 - 80, 60 – 70, 50 - 60, 40 – 50 and less than 40 years old. Moreover, each age group can be breakdown to whether person is smoker or nonsmoker and whether he is living in polluted or rural area. Such experimental design allow to investigate and identify the highest gene frequency (represent best disease resistant gene) in smoker, living in polluted area of healthy advanced aged person, which may suggest the possibility of that gene(s) to be responsible for diseases resistance and/or influence life span of peoples.

2- It needs well defined research and investigation for increase the level of natural immune (antibodies) to achieve natural disease resistance. As the human body is designed to be able to defend itself against foreign invaders. Therefore, a chance to natural immunity to defend body should be considered (given) in many diseases.

3- Nutrition plays an essential role in human health. Poor diet quality influences weight status and cardio-vascular health. Thus medical research should examine nutrition (nutrients balance and interaction among essential amino acids, essential fatty acids, minerals and vitamins) at all stage of life and investigate related issues including immunity and disease resistance. This will contribute to the understanding the role of diet in many diseases and also to undertake whether improve diet can improve resistance to disease risk. In addition, changing life style, exercise and using herb and vegetables producing natural antimicrobials are vital issues to be encouraged and investigated in medical research. Last but not least it is vital issue to investigate variations in molecular DNA and relate such variations to metabolic rate, effectiveness of sebaceous and sweat glands secretion as well as variations in their contents.

4- Focus on immunogenetic polymorphisms as well as molecular genetics variations at DNA level for the cytokine, antimicrobial substances and phagocytic activity associated with healthy individuals and that of inflammatory responses.

5- Encourage pharmacogenomics research to correlate variations in molecular biochemical genetics in responsiveness to different drugs, as this issue promises to make prescription of drugs a much more individualized affair in the future.

6- It is important to assess the relative contribution of heritable versus non-heritable factors in disease resistance/susceptibility.

7- Human tissue culture technique should be focused on in the future of medical research. By such technique one can investigate resistance and/or susceptibility to chemicals and disease infection. Impact and applicable results can be secured through well design experiments using virus, bacteria and/or chemicals on tissues inoculated (growing human cells in vitro) of various healthy people to follow up controlling growing condition and observing changes occurred and any abnormalities during experimental period.

8- It is recommended that multidisciplinary team (pharmacists, molecular biologist and pathologists) should work together in drug industry with a focus on biopharmaceutical products manufacturing and evaluation.

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Type 2 Muscle Fiber Predominance: A Case Report

By Kirill Alekseyev, Shruti Amin, Malcolm Lakdawala, Nada Farooqui & Marc K. Ross

Introduction- Skeletal muscles are a heterogeneous group of tissues categorized into different fiber types.[1] These fibers are divided into slow-twitch type 1 and fast-twitch type 2 fibers, which are identified by their expression of specific myosin heavy chain isoforms.[5,7,9] The predominance of type 2 muscle fibers has been observed in various diseases involving skeletal muscle such as Duchenne muscular dystrophy, hyperthyro-idism, and type 2 muscle fiber predominance.[2,4] Type 2 muscle fiber predominance is a rare muscular disease whose primary manifestations include proximal muscle weakness, exertional myalgia, fasciculations, and episodes of prolonged painful muscle cramping.[3,4] These symptoms eventually led our patient to be wheel-chair bound with subsequent disuse muscle atrophy. In this case we describe a patient with type 2 muscle fiber predominance, the progression of his disease, and his experience in our inpatient rehabilitation facility.

Keywords: proximal muscle weakness, exertional myalgia, type 2 muscle fiber predominance, rehabilitation, dysphagia.

GJMR-K Classification: NLMC Code: WE 500

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Type 2 Muscle Fiber Predominance: A Case Report

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I. Introduction

Skeletal muscles are a heterogeneous group of tissues categorized into different fiber types.[1] These fibers are divided into slow-twitch type 1 and fast-twitch type 2 fibers, which are identified by their expression of specific myosin heavy chain isoforms.[5,7,9] The predominance of type 2 muscle fibers has been observed in various diseases involving skeletal muscle such as Duchenne muscular dystrophy, hyperthyroidism, and type 2 muscle fiber predominance.[2,4] Type 2 muscle fiber predominance is a rare muscular disease whose primary manifestations include proximal muscle weakness, exertional myalgia, fasciculations, and episodes of prolonged painful muscle cramping.[3,4] These symptoms eventually led our patient to be wheelchair bound with subsequent disuse muscle atrophy. In this case we describe a patient with type 2 muscle fiber predominance, the progression of his disease, and his experience in our inpatient rehabilitation facility.

II. Case

A 68-year old Caucasian male with a past medical history of congenital type 2 muscle fiber predominance formally diagnosed in 1980 via multiple muscle biopsies presented to the inpatient rehabilitation facility (IRF). The patient was evaluated and treated for proximal muscle weakness in his thighs and calves, as well as less pronounced weakness in the upper extremities, exertional myalgia, and chronic dysphagia. The patient also reported episodes of severe spastic muscle cramping that involved all extremities, sparing his face, with exacerbations occurring 2 to 12 times per year for the past 48 years.

The disease first affected the patient with exertional myalgia at age five, which led to frequent falls. The patient has since then avoided running and exercise. The patient's muscular weakness progressively worsened, and he eventually became wheelchair bound at age 38. At age 30, he was admitted to the hospital for substernal chest pain and diaphoresis that the patient states "felt like having a heart attack". Work-up was negative for a myocardial infarction, and multiple subsequent episodes of substernal chest pain have also have also been negative for myocardial infarct.

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Prior to his admission, the patient was unable to transfer from his bed to wheelchair. At the time of admission, his musculoskeletal exam demonstrated bilateral calf fasciculations at rest. Sensation and proprioception were intact in all four limbs. Muscle strength was 4/5 in shoulder girdle secondary to pain. In his lower extremities he had 1/5 strength proximally and 4+/5 strength distally. The patient was noted to hold a cup in either hand for approximately 10-15 seconds without dropping it. The patient's deep tendon reflexes were diminished to 1/4 in biceps brachii, triceps, and brachioradialis reflexes, and 0/4 in patellar and Achilles reflexes bilaterally.

After rehabilitation, the patient was able to perform bed to chair transfers independently with a transfer score of 6. The patient was also able to perform bicep curls and shoulder press-ups with four pounds of weight for 10 repetitions and the use of a thera-band. With balance, endurance, and neuromuscular facilitation training the patient advanced from 0 feet of ambulation at the beginning of his stay to 16 feet with parallel bar assistance. During the hospital course, the patient's diet was advanced from full liquids to solid food. Moist heat packs and bio freeze gel were applied to his shoulder and neck area to decrease pain and stiffness.

Nerve conduction studies were conducted as shown in Table 1. The patient had no response in the right peroneal motor nerve at the ankle with reduced amplitude (B Fib 1.7 mV and Popliteal 1.6 mV) and decreased nerve velocity (Pop-fib 36 m/s). The left tibial motor showed reduced amplitude (Ankle 3.5 mV) and reduced amplitude (Popliteal 1.7 mV). The right tibial motor nerve showed reduced amplitude (Popliteal, 0.8 mV). The left and right sural sensory nerves showed no response. F-wave studies indicated that the right tibia response had prolonged latency (59.11 ms).

On repeat nerve conduction studies, evaluation of the left tibial motor nerve showed reduced amplitude (Ankle 3.5 mV) and reduced amplitude (Popliteal 1.7 mV). On electromyography studies, the patient was found to have delayed recruitment and the poor activation in right and left vastus medialis, right medial gastrocnemius, and left anterior tibialis. Increased duration was seen in bilateral vastus medialis and left anterior tibialis muscles. The overall objective impression of the study showed primarily axonal and demyelinating sensorimotor peripheral polyneuropathy affecting the bilateral lower extremities.

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III. Discussion

Type 2 muscle fiber predominance is a rare congenital muscular disorder that primarily affects the strength of the proximal muscles. The main manifestations as seen in this patient include proximal muscle weakness, exertional myalgia, fasciculations, episodes of severe painful muscle cramping requiring muscle relaxants, and dysphagia. Normally, slow-twitch type 1 fibers are the first to contract during aerobic exercises such as running.[6] Patients with this disease have shown to have a decrease in type 1/type 2 fiber ratio in proximal muscles[3]; thus, have difficulty with aerobic exercises. Most cases reported of this disease include patients ranging in age from the teenage years to our patient's age of 68.

In 1985, the first documented report of "type 2 fiber predominance" was studied.[3] In this study, all 13 male patients demonstrated muscle cramping and exertional myalgia, but no abnormalities were found on physical neurologic or laboratory examinations.[3] However, pathologic abnormalities of muscle biopsies of proximal muscles, such as the vastus medialis identified, a decrease in type 1/type 2 fiber ratio with 72.91% of type 2 fibers increased versus 59.9% in the control group.[3]

Similar findings were reported in a study in 2002, in which three cases of otherwise healthy men aged 18, 19 and 22 were identified with this disease despite being otherwise healthy.[4] In all three cases, muscles of facial expression were spared and pseudohypertrophy or true hypertrophy of the calf muscle was absent. Concluded from these studies concluded that type 2 muscle fiber predominance is a diagnosis of exclusion. It is important to note that laboratory findings were normal in both studies. Normal findings in all patients of the Kim et al. study included serum creatinine kinase, aldolase, lactate dehydrogenase, electrolytes, and thyroid function tests. Findings of increased lactate levels during ischemic exercise rules out glycogen storage diseases. Thyroid function tests are important, as patients with hyperthyroidism have been shown to have an increased expression of type 2 muscle fibers.[4]

Pathologic studies in all three cases reported no significant abnormality observed on physical findings and normal expression of dystrophin along the sarcolemmal membrane on immunohistochemical studies. To note, all three cases demonstrated an increased percentage of type 2 fibers on enzyme histochemistry. To elaborate, in case 1, a 73% increase in type 2 fiber in the vastus medialis, 80.2% in vastus lateralis in case 2, and 75% in vastus lateralis in case 3. Furthermore, a marked decrease in type 1 fibers was shown in myofibrillar ATPase preincubated at pH 9.4.[4]

Hayat et al., reports one case of a 28-year-old male with proximal muscle weakness and motor neuron dysfunction on electrophysiological studies. This patient had a 97% type 2 fiber predominance on muscle biopsy of vastus lateralis with type 2A being the largest compared to type 2B and 2C. The pathogenesis of type 2 fiber predominance remains unknown however, this study proposes a functional abnormality of motor neuron inutero leading to abnormal muscle fiber differentiation and growth.[10] Another theory suggested in the literature is that the diminished type 1 fibers are directly affected from a depletion of an oxidative enzyme along with fiber switching of type 1 to type 2 fibers.[4]

Limited studies exist on the effect of rehabilitation and electrical stimulation on the transformation of type 2 to type 1 muscle fibers. One study states that long duration and high volume endurance type events promote the conversion of type 2 to type 1 fibers.[8] High volume resistance training may also result in similar fiber changes as high volume endurance training.[9] Another study suggests that at least two months of slow pattern stimulation is needed for transformation of original type 2B or 2X fibers into type 1 fibers.[11] Further investigation is needed to determine if endurance events or stimulation conversion of type 2 to type 1 fibers will therapeutically benefit patients with type 2 muscle fiber predominance.

IV. Conclusion

Patients affected by type 2 muscle fiber predominance exhibit proximal muscle weakness, exertional myalgia, and an additional finding unique to this case, chronic progressive dysphagia. As a result, patients with type 2 muscle fiber predominance have difficulty running by approximately age 5 and are often wheelchair bound in their late 20s to 30s. In this disease lower extremities seem to be affected more than upper extremities. However, our patient showed more improvement in the lower extremities than the upper extremities in his overall outcome. He improved in activities of daily living from moderate assistance to modified independent with wheelchair. The integration of physical and occupational therapy as well as the use of muscle relaxants are essential for patients with this disease. Few cases have been reported on type 2 muscle fiber predo-minance, but further studies are vital in order to improve the quality of life of current and future patients.

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Table 1: Electromyography Study Results

Site	Onset (ms)	Norm Onset (ms)	O-P Amp (mV)	Norm O-P Amp	NegDur (ms)	Site 1	Site 2	Delta-0 (ms)	Dist (cm)	Vel (m/s)	Norm Vel (m/s)
Left Tibial Motor (Abd Hall Brev) Ankle Poplit	4.5 16.3	<6.0	3.5 1.7	>4.0 >4.0	4.69 5.0	Poplit	Ankle	11.8	48.0	41	>40
Right Peroneal Motor (Ext Dig Brev) B Fib Poplt	14.5 17.3	- -	1.7 1.6	>5.0 >5.0	6.09 5.78	Poplit	B Fib	2.8	10.0	36	>40
Right Tibial Motor (Abd Hall Brev) Ankle Poplt	5.5 16.4	<6.0	4.3 0.8	>4.0 >4.0	5.78 4.38	Poplit	Ankle	10.9			>40

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Effects of Exercise on Doxorubicin Accumulation and Multidrug Resistance Protein Expression in Striated Muscle

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Abstract- The chemotherapy drug doxorubicin (DOX) is well known to induce cardiac and skeletal muscle dysfunction. Previous studies demonstrate that exercise can mitigate dysfunction, reduce myocardial DOX accumulation, and depress markers of oxidative stress, but a putative mechanism is unknown. The aim of this study was to determine whether multidrug resistance protein (MRP) expression contributes to the protective effects of exercise against DOX-induced muscular dysfunction. Lower left ventricle (LV) and soleus DOX concentrations were observed in exercised animals, and MRP- 1, MRP-2, and MRP-7 expression was significantly increased in the LV with exercise. No MRP variations were apparent in skeletal muscles following the exercise protocol. As a marker of oxidative stress, malondialdehyde+4 hydroxyalkenal levels were analyzed, and exercise reduced both cardiac and skeletal muscle levels from exercised trained animals treated with DOX had significantly lower levels than SED-DOX. This study suggests increased MRP expression with exercise may contribute to exercise-induced protection in cardiac muscle but not skeletal muscle.

Keywords: *ABC transporters, adriamycin, anthracyclines, cardiotoxicity, chemotherapy, oxidative stress, myotoxicity.*

GJMR-K Classification: *NLMC Code: WE 500*



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Effects of Exercise on Doxorubicin Accumulation and Multidrug Resistance Protein Expression in Striated Muscle

Colin J Quinn [α], Noah M. Gibson [σ], Keith B. Pfannenstiel [ρ], Alex C. Bashore [ω], Reid Hayward [¥] & David S. Hydock [§]

Abstract- **The chemotherapy drug doxorubicin (DOX) is well known to induce cardiac and skeletal muscle dysfunction. Previous studies demonstrate that exercise can mitigate dysfunction, reduce myocardial DOX accumulation, and depress markers of oxidative stress, but a putative mechanism is unknown. The aim of this study was to determine whether multidrug resistance protein (MRP) expression contributes to the protective effects of exercise against DOX-induced muscular dysfunction. Lower left ventricle (LV) and soleus DOX concentrations were observed in exercised animals, and MRP-1, MRP-2, and MRP-7 expression was significantly increased in the LV with exercise. No MRP variations were apparent in skeletal muscles following the exercise protocol. As a marker of oxidative stress, malondialdehyde+4 hydroxyalkenal levels were analyzed, and exercise reduced both cardiac and skeletal muscle levels from exercised trained animals treated with DOX had significantly lower levels than SED-DOX. This study suggests increased MRP expression with exercise may contribute to exercise-induced protection in cardiac muscle but not skeletal muscle.**

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I. Introduction

Anthracycline antibiotics are commonly used chemotherapeutic agents that have a wide range of applications to treat many cancer types including solid tumors. Doxorubicin (DOX, trade name: Adriamycin) is one of the most effective anthracyclines; however, it is associated with cardiotoxicity. This cardiotoxicity is dose-dependent and can eventually lead to congestive heart failure. Mechanisms of the cardiotoxic milieu associated with anthracyclines are mainly attributed to oxidative stress. DOX molecules undergo redox cycling at complex I of the electron transport chain[1]. Additionally, DOX impairs mitochondrial respiration and elevates the formation of reactive oxygen species (ROS). An overproduction of ROS can damage cellular components and induce signaling pathways that lead to apoptosis[2, 3], necrosis[4], and autophagy [5, 6].

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Endurance exercise interventions have been used as an effective strategy to mitigate the severity of anthracycline cardiotoxicity[7, 8]. With both voluntary wheel running and treadmill training, our laboratory has observed attenuated DOX-induced cardiac dysfunction analyzed both in vivo and ex vivo[9-11]. With echocardiographic functional analyses, exercise preconditioned animals receiving DOX show increased fractional shortening, aortic and mitral valve maximal blood flow velocity, and mean blood flow velocity when compared to sedentary animals receiving DOX[12, 13]. Furthermore, isolated working heart experiments have shown preservation in left ventricular developed pressures, maximal rate of pressure development, and maximal rate of pressure decline with exercise[11, 14].

In addition to the cardiotoxicity of anthracyclines, myotoxic effects are also evident and can manifest as increased fatigue and reduced force production[16, 17]. This has been reported as being due to oxidative stress as well, which can affect calcium handling and actin-myosin cross bridge cycling[18, 19]. While there is relatively little research examining these mechanisms, it has been reported that exercise plays a beneficial role by mitigating skeletal muscle dysfunction[20], autophagic signaling[5, 21], and proteolysis [22].

The multidrug resistance protein (MRP) family, which is part of the super family of ATP binding cassette (ABC) transporters, includes a group of drug efflux pumps and transporters known to extrude cellular anthracyclines[23-26]. Gene transfected upregulation of MRPs has been shown to confer multidrug resistance on cultured cells by decreasing intracellular drug concentrations[27]. Additionally, MRP-1 knockout cardiomyocytes show increased 4-hydroxynonenal production, a marker of oxidative stress, when exposed to DOX[28]. Parry and Hayward[29] indicated cardiomyocyte expression of MRP-1 and MRP-2 was increased in previously-exercised rats receiving DOX versus paired sedentary animals.

Our laboratory has previously reported that exercise preconditioning is associated with a decrease in cardiac DOX accumulation[9] and reduced lipid peroxidation[30]. Additionally, Marques-Aleixo et al.[31]

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demonstrated treadmill and free-wheel running in rats preserved oxidative phosphorylation proteins, mitochondrial complexes' I and V content and activity, and mitochondrial biogenesis while decreasing oxidative stress associated with DOX treatment. Taken together, prior exercise may influence MRP content and consequent uncontrolled ROS generation. The purpose of this study was to determine if MRP expression in striated muscle is up regulated with exercise and if its expression is linked with decreases in DOX accumulation and oxidative stress.

II. Methods

a) Animal Care and use

All procedures were approved by the University of Northern Colorado Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act guidelines. Ten week old male Sprague-Dawley rats (n=32) were purchased from Harlan Laboratories (Indianapolis, IN) and housed in an environmentally controlled facility on a 12:12 hour light: dark cycle. Rat chow (Teklad 2016: Harlan) and distilled water were provided *ad libitum*. Rats were randomly assigned to sedentary (SED, n=16) or wheel run (WR, n=16) groups. WR rats were housed in cages equipped with voluntary running wheels and allowed 24-hour access to wheels for 10 weeks. SED animals were restricted to normal cage activity for the duration of the study. Following completion of the ten weeks of exercise, WR animals were removed from wheel cages.

Twenty-four hours following removal from wheel access, both groups (SED & WR) were randomly assigned to receive a single bolus injection of DOX or saline as a placebo (SED-DOX, n=8; SED-SAL, n=8; WR-DOX, n=8; WR-SAL, n=8). DOX animals received 15 mg DOX/kg body mass i.p. and SAL animals received an equivalent volume of 0.9% SAL i.p. injection. All animals were euthanized 24 hours following DOX or SAL injection with an i.p injection of heparini zed (500 U) sodium pentobarbital (50 mg/kg). Immediately following the absence of a tail-pinch reflex, the heart was rapidly excised, and the aorta cannulated and flushed of blood with Krebs-Hansleit buffer (in mM, 120 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 17 glucose, and 0.5 EDTA, pH 7.4) through retrograde perfusion. Upon clearance of blood, the left ventricle (LV) was isolated and flash frozen in liquid nitrogen for subsequent biochemical analyses. Skeletal muscles were isolated quickly following heart excision. The soleus (SOL) and extensor digit orum longus (EDL) were obtained from the right hind limb to represent type I and type II dominant muscle, respectively and flash frozen in liquid nitrogen.

b) Quantification of doxorubicin

High performance liquid chromatography (HPLC) was used to quantify DOX accumulation as previously described[17]. Approximately 50 mg of LV or skeletal muscle tissue was diluted with a 0.067 M phosphate buffer (pH 7.4) and homogenized using a Virtishear homogenizer (Virtis: Gardner, NJ). Homogenates were then subjected to protein precipitation by adding 200 μL of a 50:50 (v/v) mixture of HPLC-grade methanol and 40% ZnSO4 to 150 μL of homogenized tissue. Fifty μL of daunorubicin (DAUN) (Sigma: St. Louis, MO) at an initial concentration of 500 ng/mL was added to the sample as an internal standard. The sample was vortexed for 1 min before centrifugation at 1,500 *g* for 10 minutes. The supernatant was filtered through a 0.2 μM syringe filter and injected directly onto the column to initiate the analytical method. Quantification of DOX and DAUN was performed using a Shimadzu HPLC system (Shimadzu: Kyoto, Japan) equipped with the following components: DGU-14A degasser, dual pump LC-10A LC chromatograph, SPD-M10A diode array detector, SLC-10A system controller, and RF-10Axl fluorescence detector. The stationary phase consisted of a reverse phase Zorbax C8 column (Agilent Technologies: Santa Clara, CA). The fluorescence detector used to quantify DOX and DAUN was operated at an excitation of 470 nm and emission of 550 nm. A volume of 20 μL of sample was injected directly onto the column.

c) MRP expression

LV and skeletal muscle homogenates were analyzed for MRP-1, MRP-2, and MRP-7 by Western blotting. Approximately 100 mg of tissue was homogenized in a dilution of 1:10 w:v in RIPA buffer (Sigma-Aldrich) using a Virtishear homogenizer. Homogenates were centrifuged at 10,000 *g* for 10 minutes, and supernatant was removed for analysis. Total protein was determined by a Bradford assay [31]. Protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane in a Novex Sure Lock blotting unit (Invitrogen: Carlsbad, CA). Membranes were incubated overnight with primary antibodies (Santa Cruz Biotechnology: Dallas, TX) for MRP-1 (anti-goat), MRP-2 and MRP-7 (anti-rabbit) in a 1:500 dilution and developed using Novex Western Breeze Immuno-detection kits (Invitrogen). Developed membranes were scanned and proteins bands were analyzed using densitometry (ImageJ: NIH, Bethesda, MD).

d) Lipid peroxidation

A commercially available assay kit (Bioxytech LPO-586, Oxis Research: Foster City, CA) was used to measure malondialdehyde + 4-hydroxyalkenals (MDA + 4-HAE) as an indicator of cellular lipid peroxidation. Tissue homogenates in RIPA were measured for the assay. A 200 μL aliquot of each sample was added to a micro centrifuge tube followed by 650 μL of N-methyl-2-

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phenylindole in acetonitrile and briefly vortexed. Next, 150 μL of methanesulfonic acid was added, vortexed, and incubated at 45°C for 60 minutes. Samples were then centrifuged at 10,000 *g* for 10 minutes. The resulting supernatant was transferred to a cuvette, and absorbency was measured using a spectro photometer at 586 nm. MDA + 4-HAE was estimated from a standard curve. All samples were assayed in duplicate, and any samples varying more than 5% were reassayed.

e) Statistical analysis

All data are expressed as mean ± standard deviation (mean±SD). A Student's *t*-test was performed to compare tissue DOX accumulation in DOX-treated groups (SED-DOX vs. WR-DOX). A two-way analysis of variance (ANOVA, drug x exercise) was performed to determine significant main effects and interactions for MRP expression and MDA + 4-HAE in LV, SOL, and EDL. If a significant difference was observed, a Tukey's *post hoc* test was performed to identify where differences existed. For all procedures, significance was set at the $\alpha = 0.05$ level.

III. Results

a) Animal characteristics

Heart and skeletal muscle masses are presented in *Table 1*. No main effects or interactions ($p>0.05$) were observed between groups in skeletal muscle mass. A drug effect was observed for heart mass with animals receiving DOX having a lower heart mass ($p=0.025$), *post hoc* testing revealed that WR-DOX had significantly lower heart mass than SED-SAL ($p<0.05$).

b) Doxorubicin accumulation

Figure 1 illustrates DOX accumulation in cardiac and skeletal muscle. DOX accumulation varied between tissue types, which is consistent with previous reports from our laboratory [17]. As expected, the LV accumulated the greatest quantity of DOX with a significantly lower level observed in WR animals when compared to SED ($p<0.05$, WR: 1055±188 ng/g versus SED: 1284±150 ng/g, Figure 1A). Similarly, exercise groups had a lower level of DOX in the SOL ($p<0.05$, WR: 505±99 ng/g versus SED: 626±86 ng/g, Figure 1B). In contrast to the oxidative muscle fibers found in the heart and SOL, no DOX level differences were observed in the EDL between exercised and sedentary animals ($p>0.05$, WR: 219±89 ng/g versus SED: 287±85 ng/g, Figure 1C).

c) MRP expression

Analysis of MRP expression in the LV by Western blotting revealed a significant DOX effect with an up regulation of MRP-1, MRP-2, and MRP-7 (+79%, +107%, and +193%, respectively; $p<0.05$) as well as a significant exercise effect (+56%, +99%, and +179%, respectively; $p<0.05$, Figure 2). In addition, MRP-1 and MRP-7 appear to have significant ($p<0.05$) additive increases in WR-DOX when compared to WR-SAL (MRP-1: +80%; MRP-7: +150%). In contrast to LV MRP expression, no significant main effects were observed in SOL MRP-1, MRP-2, or MRP-7 or EDL MRP-2 or MRP-7 expression (Figures 3 & 4). An interaction was observed in SOL MRP-7 ($p=0.0240$). No main effects or interactions were observed for MRP-2 or MRP-7 in the EDL ($p>0.05$). It should be noted that MRP-1 expression was not detected in the EDL.

d) Lipid peroxidation

MDA + 4-HAE was quantified as a marker of oxidative stress-induced cell damage (Fig. 5). With all tissues analyzed, WR reduced lipid peroxidation in DOX-treated tissues (LV, -23%; SOL, -44%; EDL, -34%), suggesting exercise protects against DOX induced lipid peroxidation. Significant drug and activity effects were observed in the LV ($p<0.05$) while significant drug effects and interactions were observed in SOL and EDL ($p<0.05$). SED-DOX MDA + 4-HAE was significantly greater than all other groups across in the three tissues examined ($p<0.05$).

IV. Discussion

Exposure to DOX provokes damage to the structure and function of both cardiac and skeletal muscles[16, 17, 33, 34]. DOX-induced structural alteration was evident by decreases in heart mass. Exercise as a preventative and complementary treatment, however, has demonstrated increased tolerance to DOX in cardiac and skeletal muscle tissues[7, 22, 35, 36]. Specifically, reduction of tissue DOX accumulation with exercise preconditioning has previously been linked to the maintenance of cardiac function following DOX treatment[9]. The current experiments provide further evidence in support of a mechanism that may contribute to exercise-induced cardio protection in this setting. It is suggested that an up regulation of MRP expression in the myocardium is associated with a decrease in intracellular DOX accumulation. In addition, these data also suggest that MRP expression contributes to the reduced LV oxidative stress observed in exercised groups. This study observed drug and activity main effects with significantly elevated of MRP-1, -2 and -7 expression in the LV with DOX and prior treadmill training, respectively. Additionally, an interaction was observed in LV expression of MRP-2. No significant changes in skeletal muscle MRP expression with exercise or drug treatments; however, WR groups exhibited less DOX accumulation in the SOL and lower levels of oxidative stress in both the SOL and EDL.

Myocardial alterations of MRP expression and oxidative stress with exercise predicate the examination for a relationship with cell health. Glutathione (GSH) is an antioxidant present in a variety of cells, and DOX has been shown to deplete populations, specifically in the heart[37]. Kwok and Richardson[38] showed DOX-treated

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myocardial cells to increase levels of oxidative stress. Lipid peroxidation products (alkenals) react with GSH, reducing levels and have been correlated with apoptosis [39]. Highly oxygen-dependent tissues may be particularly susceptible to reactive oxygen species damaging proteins and lipids, leading to cell dysfunction and death. MRP-1, MRP-2 and MRP-7 are known to extrude anthracyclines, such as DOX, as well as GSH conjugates[40, 41]. Krause et al.[42] reported that 60 minutes of exercise per day for only 1 week induced an up regulation of MRP-1 in rat myocardium (2.4-fold). This resulted in an increase in MRP-1 function and reduced redox imbalance associated with DOX exposure. In the current study, DOX treatment induced elevated levels of MRP-1, -2, and -7 in the LV. Levels of MRP-1 and -7 were highest in WR-DOX groups suggesting an additive effect. Accordingly, levels of DOX accumulation in LV were significantly higher in the DOX-treated SED than WR groups.

The aforementioned study by Krause et al.[42] reported that MRP-1 expression in whole gastrocnemius was absent. This is consistent with our results in the EDL, a muscle of similar fiber type; however, we did detect MRP-1 expression in the SOL, a muscle with greater oxidative capacity. It may be the case that the greater the oxidative muscle fiber content, the more apt the cells are to handling oxidative stress with the expression of MRP-1. Another possible explanation for MRP-1 expression variability is the localization of the protein in the mitochondrial membrane following DOX exposure. It was reported that following DOX treatment, a 2-fold MRP-1 expression aggregated at the mitochondrial membrane of cardiomyocytes versus observations in whole heart homogenate samples[28]. This evidence suggests that MRP-1 may be expressed specifically where greatest redox cycling occurs. Additionally, DOX preferentially accumulating at the mitochondria, due to cardiolipin binding, may drive higher localized MRP expression[17]. Furthermore, the mitochondrial content between muscle fiber types varies greatly (20-30% in cardiomyocytes, 6% in oxidative skeletal muscle, and 2-3% in glycolytic skeletal muscle) which may contribute to the differential expression between muscle types. However, no studies have examined the effect of exercise on MRP-1 expression in the mitochondrial membrane, and thus, this warrants future investigation.

Our lab has observed a significant decline in skeletal muscle function 24 hours following DOX treatment with increasing loss of function 5 days following treatment[17]. While skeletal muscle did not show significant changes in MRP expression, there was a significant decrease in SOL DOX accumulation and a trend toward a decrease in EDL DOX accumulation. One possible explanation for this may be the increase in mitochondrial function with chronic endurance training regardless of mitochondrial quantity. Exercise induces an improvement in skeletal muscle metabolic capacity, which enhances the function and efficiency of the mitochondria. This could be achieved through a process whereby increased mitochondrial biogenesis replaces mitochondria damaged by DOX exposure resulting in an overall healthier mitochondrial population within the cell without an increase in mitochondrial density per se. DOX has been shown to acutely (2 hours-post) reduce mitochondrial function by inhibiting complex-I and II and increasing H_2O_2 production as well as disrupting membrane potential and compromising respiratory capacity 72 hours following exposure[43]. Prior exercise may contribute to reducing this dysfunction by improved mitochondrial function at the onset of treatment there by better maintaining function and possibly improving mitochondrial turnover following DOX-induced damage. Further studies are necessary to elucidate these mechanisms.

It should be noted that the xenobiotic function of MRP-1 in rodent species exhibits lesser DOX export capacity than that of human cells[44, 45]; however, MRP-1 maintains its ability to transport oxidized glutathione, which may contribute to the protection against DOX-induced oxidative stress with exercise preconditioning. Additionally, Hopper-Borge et al.[27] characterized MRP-7's DOX resistance as significantly lower activity levels than MRP-1 and -2. In spite of lesser transport activity, greater expression of MRP-1 and -7 seen in this study may have contributed to lesser DOX accumulation in the LV in WR animals. Of additional interest, elevated MRP expression has been linked to vincristine resistance and increased release of GSH disulfides thereby reducing oxidative stress[46, 47]. To our knowledge, this is the first study examining striated muscle expression of MRP-2 and MRP-7 with exercise.

V. Conclusion

The current report examined the role of MRP expression as a possible mechanism of protection against DOX-induced striated muscle toxicities. Exercise promoted an up regulation of MRPs, decreased DOX accumulation and decreased lipid peroxidation in the LV. However, no modulation of MRP expression was observed in either the SOL or EDL, but a decrease in DOX accumulation was observed in the SOL. Additionally, a reduction in lipid peroxidation was observed with WR in both skeletal muscles analyzed. These findings suggest that cardioprotection may be induced by an up regulation of MRPs, which may reduce DOX accumulation and increase the ability of the heart to with stand oxidative stress, but MRP changes do not explain the observed exercise-induced reductions in lipid peroxidation in skeletal muscle with DOX treatment. These findings also further contribute to the notion that chronic exercise training plays an important and applicable role in reducing DOX-induced

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side-effects which may improve quality of life, but more work needs to be done exploring tissue-specific mechanisms.

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Figure Captions

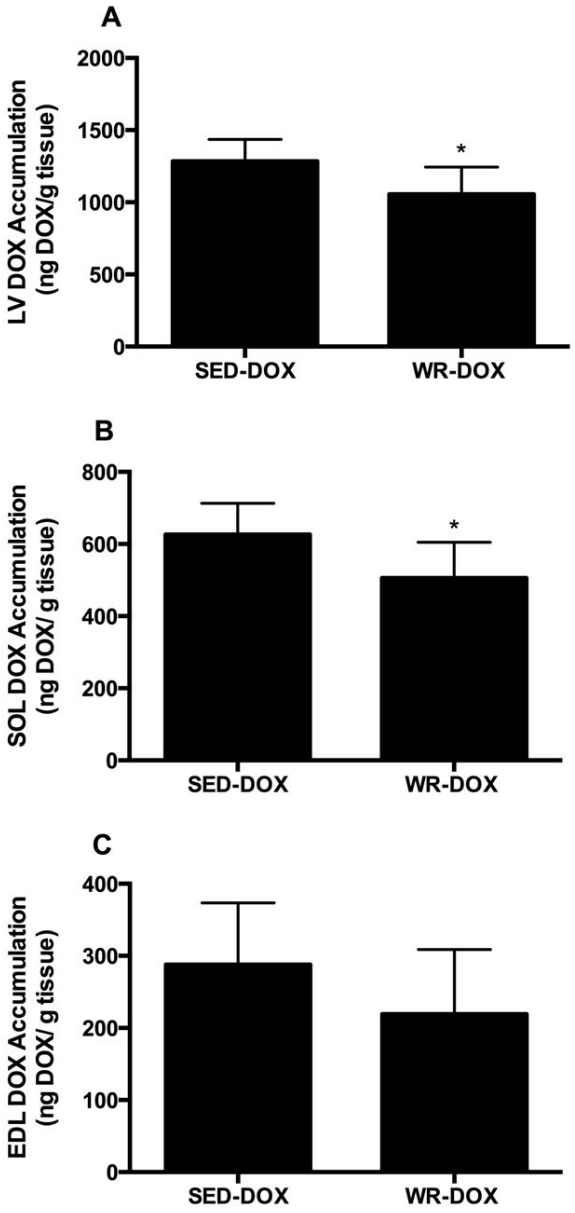


Figure 1: DOX accumulation in LV (A), SOL (B), & EDL (C)

Data are mean ± SD. LV, left ventricle; SOL, soleus; EDL, extensor digitorum longus; SED, sedentary; WR, wheel-run; DOX, doxorubicin-treated.

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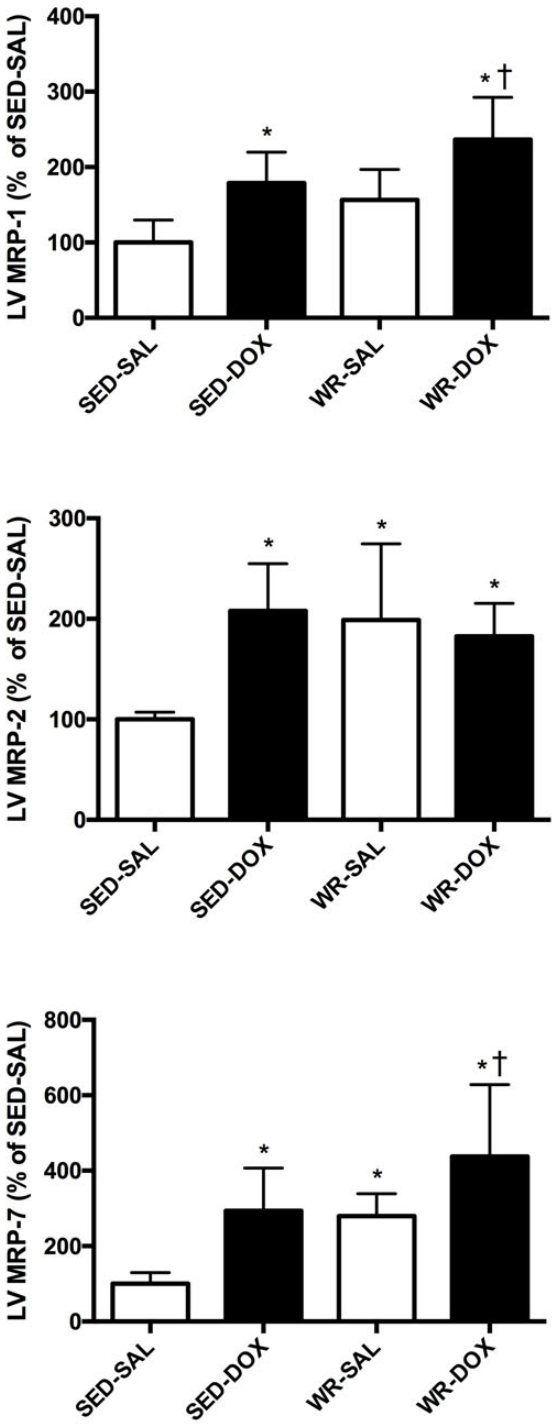


Figure 2: MRP expression as a percentage of SED-SAL in LV.

Data are mean ± SD. LV, left ventricle; SOL, soleus; EDL, extensor digitorum longus; SED, sedentary; WR, wheel-run; SAL, saline-treated; DOX, doxorubicin-treated.

Main activity effect in MRP-1, -2, and -7 ($p<0.05$).

Main drug effect in MRP-1, -2, and -7 ($p<0.05$).

Interaction in MRP-2 ($p<0.05$).

* Significantly different as compared to SED-SAL group ($p<0.05$).

† Significantly different as compared to WR-SAL group ($p<0.05$).

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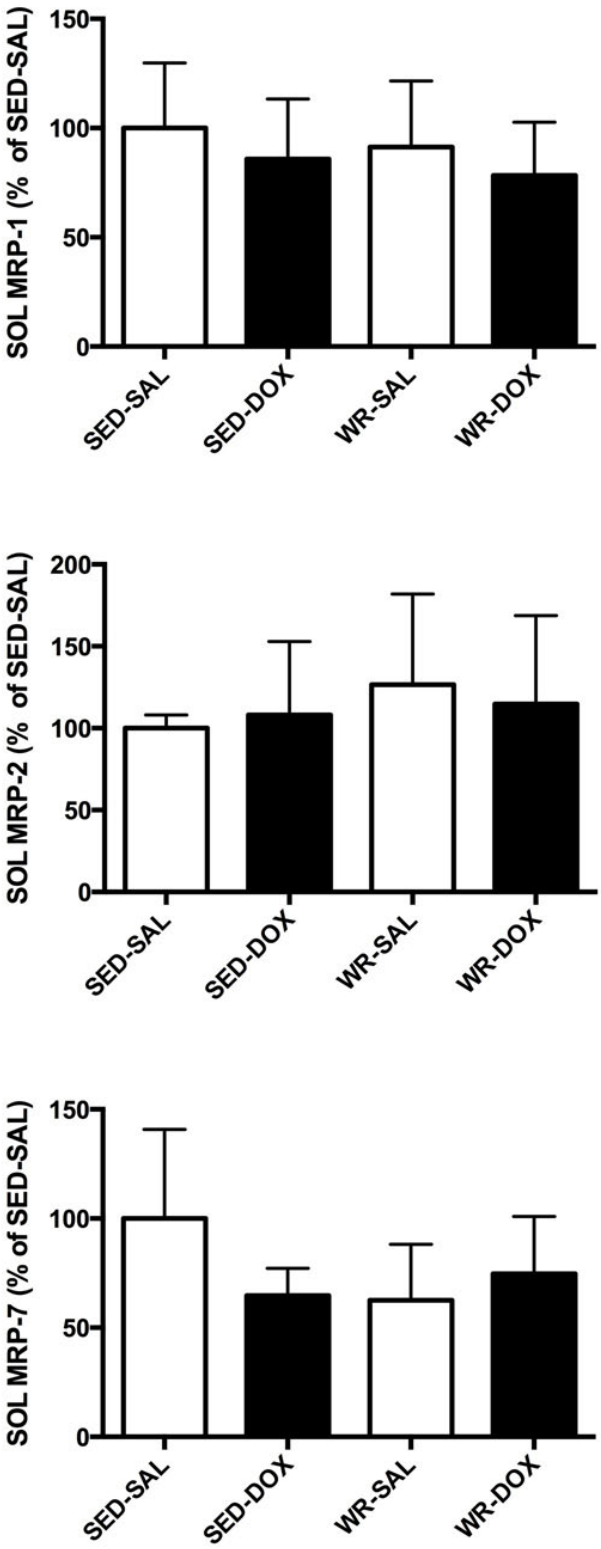


Figure 3: MRP expression as a percentage of SED-SAL in SOL.

Data are mean ± SD. SOL, soleus; SED, sedentary; WR, wheel-run; SAL, saline-treated; DOX, doxorubicin-treated. Interaction in MRP-7 ($p<0.05$).

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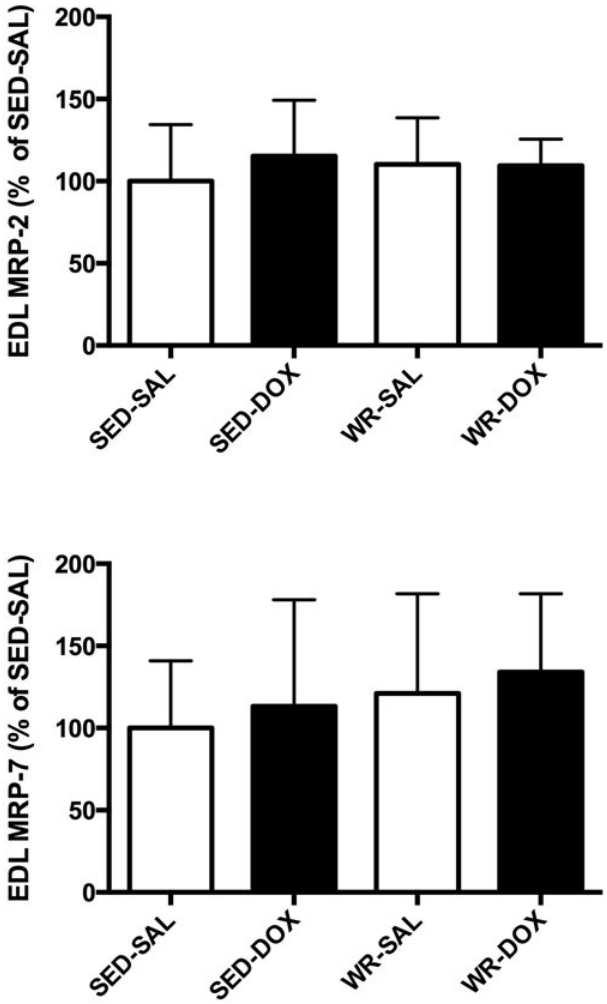


Figure 4: MRP expression as a percentage of SED-SAL in EDL.

MRP-1 was undetectable in the EDL.

Data are mean ± SD. EDL, extensor digitorum longus; SED, sedentary; WR, wheel-run; SAL, saline-treated; DOX, doxorubicin-treated.

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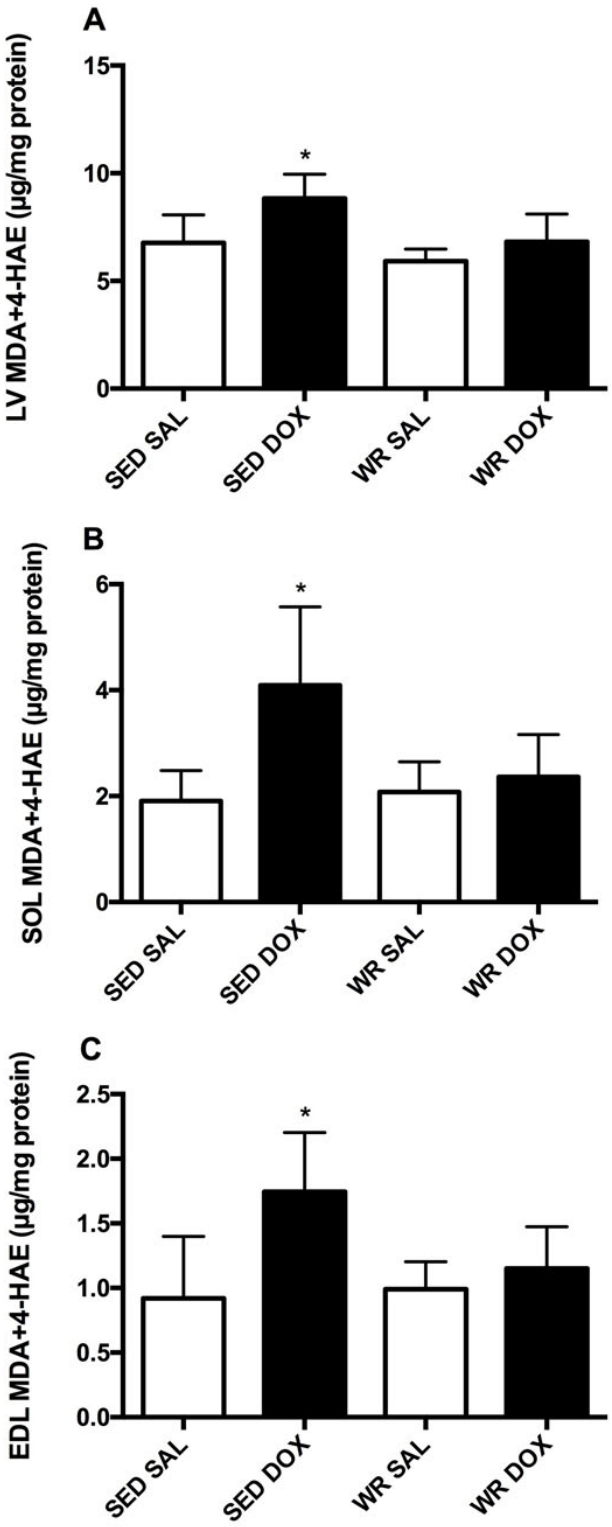


Figure 5: MDA+4-HAE in LV (A), SOL (B), & EDL (C).

Data are mean ± SD. LV, left ventricle; SOL, soleus; EDL, extensor digitorum longus; MDA+4-HAE, malondialdehyde and 4-hydroxyalkenals; SED, sedentary; WR, wheel-run; SAL, saline-treated; DOX, doxorubicin-treated.

* Significantly different than all other groups ($p<0.05$).

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Table 1: Animal Characteristics

	SED-SAL	SED-DOX	WR-SAL	WR-DOX
Heart Mass (g) †	1.515 ± 0.227	1.397 ± 0.081	1.438 ± 0.119	1.290 ± 0.117*
SOL Mass (mg)	0.154 ± 0.016	0.144 ± 0.016	0.163 ± 0.014	0.158 ± 0.031
EDL Mass (mg)	0.147 ± 0.013	0.145 ± 0.018	0.150 ± 0.014	0.162 ± 0.021

Data are mean ± SD. LV, left ventricle; SOL, soleus; EDL, extensor digitorum longus; SED, sedentary; WR, wheel-run;

SAL, saline-treated; DOX, doxorubicin-treated.

* Significantly different as compared to SED-SAL group ($p<0.05$).

† Main drug effect ($p<0.05$).

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Association between Breast Cancer and Alcohol Consumption: Review Articles

By Abdulraouf Lamoshi
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Introduction- About 10% of women will be diagnosed with breast cancer during their lifetime; however in majority of cases the underlying cause is unknown. Across the globe, breast cancer is the second most common type of cancer and the second leading cause of cancer death in women; the risk factors for breast cancer include older maternal age at birth of first child, earlier onset of menses, later age at menopause, increased mammographic density and the presence of specific genetic alterations (Beasley et al, 2010). Both alcohol and tobacco have significant causal roles in numbers of cancers including breast cancer (Kristan, 2003). A prospective follow-up clinical study that tracked 1.3 million women over 7.3 years demonstrated that among women who reported recent alcohol consumption, a 12% elevation in breast cancer risk was observed for each additional drink (Beasley et al, 2010). Notwithstanding studies such as those cited above the impact of smaller amounts of drinking is not documented well. Information on drinking styles, such as regular drinking and heavy episodic drinking, aka binge drinking, is also deficient. Also, it is important to assess the role of alcohol intake at different times in a woman's life (Chen et al, 2011). Consumption of alcohol could be a modifiable risk factor which could reduce the cancer burden among women in the Western world. (Tjønneland et al, 2007).

GJMR-K Classification: *NLMC Code: QW 170*



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The Association between Breast Cancer and Alcohol Consumption: Review Articles

Abdulraouf Lamoshi

I. Introduction

About 10% of women will be diagnosed with breast cancer during their lifetime; however in majority of cases the underlying cause is unknown. Across the globe, breast cancer is the second most common type of cancer and the second leading cause of cancer death in women; the risk factors for breast cancer include older maternal age at birth of first child, earlier onset of menses, later age at menopause, increased mammographic density and the presence of specific genetic alterations (Beasley et al, 2010). Both alcohol and tobacco have significant causal roles in numbers of cancers including breast cancer (Kristan, 2003). A prospective follow-up clinical study that tracked 1.3 million women over 7.3 years demonstrated that among women who reported recent alcohol consumption, a 12% elevation in breast cancer risk was observed for each additional drink (Beasley et al, 2010). Notwithstanding studies such as those cited above the impact of smaller amounts of drinking is not documented well. Information on drinking styles, such as regular drinking and heavy episodic drinking, aka binge drinking, is also deficient. Also, it is important to assess the role of alcohol intake at different times in a woman's life (Chen et al, 2011). Consumption of alcohol could be a modifiable risk factor which could reduce the cancer burden among women in the Western world. (Tjønneland et al, 2007).

When alcohol consumption is initiated during earlier years of life, the cumulative risk can be more serious; in a case-control study of pre- and postmenopausal women, breast cancer was related with alcohol use of < 13 g/day solely for those who consumed alcohol before the ages of 30 years and regardless of recent use, where as no significant increase in risk was associated to recent consumption of a same amount among women with low-to-moderate intake of alcohol before age 30 y (Tjønneland, 2004). There are many examples showing that exposure to some dietary or environmental changes could enhance the development of different cancer types.

Asian and African women are potential victims to such modification because of some changes in their lifestyles. The incidence of breast cancer in the United States was about 6 times higher than in Asian populations this rate gradually has become approximately 3 times higher in US white women than in Asian women; the migration of Asian women to the United States led to increase of breast cancer rates and continue to rise in next generations to the level of rates in white women living in the United States (Brown et al, 2010). According to Adebamowo and Adekunle (1999), in the United States and Canada, the lifetime risk of breast cancer is 10 % for white women and 7.3% for African-American women; some 3.5% will die from breast cancer. The incidence of breast cancer in Nigeria in 1976 was 15.3 / 100 000 but this number jumped to 33.6 / 100 000 in 1992; moreover, from 1960 to 1980, the cancer record of one Nigerian hospital registered 17 496 cases of cancer; by that time breast cancer was the fourth commonest tumor in Nigeria (6%) and the second most common among women, dramatically, from 1980 to 1989 this tumor became the most common malignant cancer in the country (Adebamowo and Adekunle, 1999).

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II. Methods

A MEDLINE search was conducted to identify epidemiological studies on the relationship between alcohol consumption and breast cancer incidence among different ethnic backgrounds from 1980 to 2011. Fifteen papers written in English were reviewed. The objectives, findings, population samples, strengths and weaknesses, and conclusions have been reviewed. Key words: Alcohol consumption, breast cancer

III. Main Features and Comments

Fifteen studies to evaluate the association of breast cancer with alcohol consumption were identified including eight cohort and seven case control studies. The analyzed components of the studies including age range, study period, numbers of women enrolled, the risk of breast cancer due to alcohol drinking and covariates used in adjustment. Studies that presented separate estimates of risk were assessed in terms of: frequency of alcohol drinking, amount of consumed alcohol, type of alcohol, or/and menopausal status, genotype status of the participants, age at consumption, folate as protective factor, dietary intake, smoking, postmenopausal hormones treatment, and the mechanism of causality. The studied populations had some shared and unique characteristics. The first study

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started in 1983 4 and the last one was completed by 2007 9. The youngest participants in these studies was 20 years old 13 whereas two studies analyzed postmenopausal women without defining an upper age limit, however the majority of studies did not go beyond 79 years of age 5. The sample sizes in these studies ranged from 57 12 to 1.3 million women 11.

The participants have different ethnic backgrounds; the majority is Western women, in addition to Asian Americans, Mexicans, Japanese, Chinese, Filipino, and Nigerian 9, 13, 15. The average response rate is about 90%, and the samples had been selected from different resources. All studies consider drinking alcohol as an independent variable in addition to some other variables such as smoking, coffee, folate, vitamin B12, postmenopausal hormonal therapy, antioxidant nutrients, and other dietary and environmental factors 1, 2, 4, 13. Breast cancer is the dependent variable but some studies looked with more details at its different types and the age of diagnosis 6. Alcohol use was studied in terms of the amount of consumption on daily and weekly bases, type of alcohol (beer and/or cider, wine, liquor, spirits, or fortified wine), the duration of use, and the age of consumption 2, 4, 10. To measure the variables most of the studies collected the data about the alcohol use, other dietary and environmental factors, and demographic information through conducting questionnaires either by mail or during face to face interviews 3, 13. The diagnosis of the cancer was carried out in the majority of cases through checking the medical records of the participants; however, there are two studies dependent on the follow up questionnaire to gather that information 2, 11. In one study the patients had been subjected to blood drawing samples to determiner gene phenotype 7.

The main findings of these studies show that there is an increased risk of breast cancer observed in women with a higher absolute and dose dependent intake of alcohol 1, 3, 4, 6, 8. Thirteen out of the fifteen studies demonstrated this correlation regardless of the amount or the age of drinking commencement with different levels of measured certainty 1,2,5,7. Only one study showed that low alcohol intake is not related to an increased breast cancer risk in Asian-American women 13. The final study did not support an association between alcohol intake and alcohol metabolism with breast cancer risk 7. One study pointed out that decreasing recent alcohol consumption, independent of early lifetime exposure to alcohol, may reduce the risk of breast cancer in postmenopausal women 14. Another study that showed positive correlation specified that women who drank more heavily than their average lifetime rate for a period of 6 months or more were at higher risk for breast cancer 4. An important implication of these studies' findings is that even small amounts of alcohol (5 g/day, 3 glasses of wine per week) can increase the risk of breast cancer 1, 5, 9, 11.

Results of many studies did not vary by type of alcohol ingested, and their observations did not show significant change in risk related to any beverage type 1, 6, 11. The increased risk of breast cancer is associated with recent but not lifetime or early total alcohol intake 6. One study pointed out that there is no association between alcohol intake, alcohol metabolism enzymes (Aldehyde dehydrogenases genotype (ADH1C, and ADH1B)) and breast cancer risk among sisters discordant for breast cancer 7. One study connected increased risk for breast cancer with drinking alcohol, taking postmenopausal hormones, and, especially, both together 8. More than one study emphasized that low levels of alcohol intake also can increase the risk for breast cancer among both pre- and postmen opausal women 1, 5, 9. Only one study explored that alcohol use may be more strongly associated with risk of hormone-sensitive breast cancers than hormone-insensitive subtypes, suggesting distinct etiologic pathways for these two breast cancer subtypes 10. One study suggested that moderate alcohol consumption increases some biomarkers of oxidative stress in postmenopausal women which could lead to development of breast cancer 12. Conversely, one article shows that low alcohol intake is not related to increased breast cancer risk in Asian-American women, and neither alcohol nor cigarette use contributed to the elevated risks in Asian-American women associated with migration patterns and westernization 13. Tjønneland's et al study suggests that baseline intake of alcohol is a more important determinant of postmenopausal breast cancer risk than earlier lifetime exposure 14.

A Nigerian study insisted that, acquisition of `western' lifestyle, and the changing socioeconomic patterns of the country contributes to development of breast cancer 15. There are proved significant factors that could contribute to dietary prevention strategies to reduce breast cancer incidence such as alcohol ingestion, which remains one of the few risk factors for breast cancer that can be prevented 3. According to Duffy et al (2009), however the increased risk of breast cancer with alcohol use is relatively small (13% for 1 drink/day), the results emphasize the necessity to include breast cancer risk among the list of consequences for even the low levels of alcohol drinking, which is considered ''safe'' according to the National Institute on Alcohol Abuse and Alcoholism (NIAAA) guidelines 5. Chronic alcohol consumption of moderate amounts of alcohol by healthy postmenopausal women may lead to important changes in biomarkers associated with oxidative stress where high levels of reactive oxygen species can enhance lipid peroxidation, damage cells, and participate in chronic diseases, including breast cancer 12. Berstad's et al study shows that recent collective alcohol ingestion of

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two or more drinks per day may be associated with a high risk of breast cancer before age 50 years 6.

Concerning the strength of the studies, most of the (ten) studies have thousands of participants 1, 2, 4, 5, 6, 8, 9, 10, 11, 14 and high response rate 1, 3, 10, 12. Some studies focused on specific populations 9, 15 whereas others try to explore that association among the Western women in general 1, 2, 3, 10. One study uniquely studied the association of both frequency of alcohol consumption and binge drinking and breast cancer 1. One study was a multi-center study by inclusion of subjects living in countries from all over Europe 2. Only incident cases that had undergone genetic testing for the six most common BRCA gene mutations among French-Canadians were studied in one remarkable research 3. Trained interviewers carried out well designed interviews to decrease the risk of information bias, and the RR of breast cancer did not depend on ever drinking alcohol 4, 6. Duffy's et al, (2009) study did not confirm the protective effect of folate on breast cancer risk for postmenopausal women who are moderate alcohol consumers 5. A study examined full sisters discordant by breast cancer status to see if alcohol, and its potential impact by genotype, could participate in differences in breast cancer risk within families; this approach removes any potential confounding by race/ethnicity due to population stratification 7. The greatest power to examine an interaction effect, with longer follow-up of 20 years was provided by Chen et al, 2002 8. Beasley et al, 2010 used, uniquely, US Department of Agriculture food composition tables for estimating nutrient intake in a Mexican population to control the other breast cancer dietary factors 9. One research had a large size sample, which allows examination of the association of alcohol intake on individual cancer sites 11. Hatman et al, 2005, focused on mechanism of the development of breast cancer because of alcohol use 12. A wide range of lifestyles and acculturation in Asian-American women, including the role of smoking and alcohol consumption, particularly low levels of intake was the core of Brown's et al, 2010 study 13. The timing of the initiation of alcohol consumption was considered both in terms of chronological age and relative to first birth in one research 14. Another study focused on the epidemiological risk factors, including alcohol use, of breast cancer in Nigerian women 15.

In terms of disadvantages and bias of the reviewed studies, eight of them are observational, so alcohol use was not randomly assigned to women 1, 2, 3, 5, 8, 10, 11, 14. Some studies relied on self-reported alcohol use, which may have resulted in recall bias; consequently, women with breast cancer might be more likely to overestimate consumption of certain foods they believe to be related to their disease 1, 3, 8, 9, 15, and, compared with some studies done in Europe, the study does not have as many women with higher levels of alcohol consumption 1. Lash's and Aschengrau's study did not address an age-specific history of alcohol ingestion rates and the proxy respondents may have participated in non-differential misclassification of alcohol consumption rates, which bias the expected results toward the null; the crude assessment of alcohol ingestion may have obscured an underlying dose-response association 4. Alternatively, it may be that the low doses of alcohol consumed by most women in this study are not associated with breast cancer risk, and solely high doses could associate with breast cancer 4. Two studies considered the use of only one baseline measurement of alcohol and folate and underestimated dietary folate intake because of changes in the fortification of cereals and grains which began in the early 1990s and was completed by the end of 1997 as part of a federal policy 5, 10. Short length of follow-up (3-6 years) was one disadvantage of some studies in contrast to other previous cohort studies (10–16 years) 2, 5, 11. In Berstad et al study, the number of control subjects was much smaller than the number of cases; this gave the study limited power to distinguish differences between cases and controls, and its response rate was relatively low (62%) 6, and it could not exclude the possibility of selection bias in this study 6. The participants in two studies were not able to differentiate between red and white wine, and there is some indication that white wine may increase the density of mammary tissue, whereas red wine may reduce the density; they were not able to assess the effect of binge drinking, and they did not obtain data on other dietary intakes 6,9.

One of a study's problems is non differential measurement error of self-reported alcohol consumption and/or genotype, and a low statistical power because of high concordance of genotype and alcohol use 7. Some studies were not able to assess the cancer risk for never drinkers and former drinkers separately because the used questionnaire did not differentiate between lifelong nondrinkers and those who had stopped drinking (not exclude the possibility of non differential misclassification) 11, 13, 14; the increased risk seen in women who were currently nondrinkers compared with small amount drinkers, for many cancer sites, shows that many nondrinkers could be former drinkers who have stopped drinking because of medical problems, and this may have caused a non-genuine high relative risk in this group 11. In the experimental study, the measurement of selected antioxidant nutrients and one marker of oxidative stress in blood may or may not represent what is occurring at the tissue level; these changes were observed over a relatively short time frame 12. Limitation on estimating the influence of moderate to heavy intake of alcohol in this Asian population is the small percentage of subjects who drank 5 or more grams of alcohol per day 13. Adebamowo's and Adekunle's study was a case-

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controlled study but it was not a blind one, so the patients tend to be more interested about any possible risk factor than the controls 15.

Concerning the generalizability, most of the studies, especially, those have large numbers of western women[1, 2, 4, 5, 6, 8], can be generalized. Two studies where too specific and have relatively small numbers 13, 15, thus cannot be safely applied to the rest of the women.

IV. Conclusion

A majority of epidemiologic studies provide evidence for a positive association between breast cancer risk and alcohol consumption but that causality relationship is not proved in case of Asian-American women. Evidence from human biomarkers assay support plausible biological mechanisms where chronic alcohol consumption of moderate amounts of alcohol by healthy postmenopausal women may lead to breast cancer. Significant changes in biomarkers associated with oxidative stress where high levels of reactive oxygen species can promote lipid peroxidation, damage cells, and contribute to chronic diseases, including breast cancer.

Findings of this analytic study suggest that breast cancer is strongly associated with alcohol consumption. These findings may have broad implications for the prevention and reduction of breast cancer. Since the prevalence alcohol drinking is very high, and there is a causal relationship linking breast cancer with alcohol, that has serious public health influences in terms of the number of breast cancer cases due to drinking alcohol. The different incidence of breast cancer across the globe shows that environmental impacts are significant in the etiology. Although the high risk associated with alcohol consumption is relatively small compared with the major risk factors for breast cancer, but it is a modifiable risk factor. Determination of these factors may improve the ability to prevent this type of cancers by providing more focused health education and other prophylactic ways.

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

A Computed Tomography-Aided Clinical Report on Anatomical Variations of the Paranasal Sinuses

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Abstract- Disease of the paranasal sinuses (PNS) is a global public health problem, with the only treatment option available being endoscopic surgery. Previous studies have suggested that anatomical variations of the PNS are common in different populations; however, there is little information available to verify this. Hence, the objective of the present study was to determine the prevalence of different anatomical variations of the PNS and nasal cavity among Sudanese patients who were referred by ear, nose and throat (ENT) surgeons for a computed tomography (CT)-aided study. The total number of patients eligible for the study was 557; of these, 51 were excluded, 317were in the study group, and 189 were controls. The CT images were carefully reviewed and discussed with the involvement of consultant radiologists, an anatomist, and an otolaryngologist. Our results showed that there was extensive pneumatization of the frontal sinus (FS) in 37% of cases, a rudimentary FS in 11%, and absence of the FS in 12%. In addition, the Keros classification showed the FS to be normal in 55%, type I in 27%, type IIin 10%, type III in 6%, and type IV in only 2% of patients. A large ethmoid bulla (EB) was found in 43% of patients, but the remaining 57% had a normal ethmoid sinus.

Keywords: *paranasal sinuses, anatomic variation, computed tomography, sudanese population.*

GJMR-K Classification: *NLMC Code: WN 206*



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A Computed Tomography-Aided Clinical Report on Anatomical Variations of the Paranasal Sinuses

Salah ELdeen Dafalla [α], Mohamed Ali Seyed [σ], Nazik A Elfadil [ρ], Osman M. Elmustafa [ω] & Zakir hussain [¥]

Abstract- Disease of the paranasal sinuses (PNS) is a global public health problem, with the only treatment option available being endoscopic surgery. Previous studies have suggested that anatomical variations of the PNS are common in different populations; however, there is little information available to verify this. Hence, the objective of the present study was to determine the prevalence of different anatomical variations of the PNS and nasal cavity among Sudanese patients who were referred by ear, nose and throat (ENT) surgeons for a computed tomography (CT)-aided study. The total number of patients eligible for the study was 557; of these, 51 were excluded, 317were in the study group, and 189 were controls. The CT images were carefully reviewed and discussed with the involvement of consultant radiologists, an anatomist, and an otolaryngologist. Our results showed that there was extensive pneumatization of the frontal sinus (FS) in 37% of cases, a rudimentary FS in 11%, and absence of the FS in 12%. In addition, the Keros classification showed the FS to be normal in 55%, type I in 27%, type IIin 10%, type III in 6%, and type IV in only 2% of patients. A large ethmoid bulla (EB) was found in 43% of patients, but the remaining 57% had a normal ethmoid sinus. Posterior ethmoid cells showed extensive pneumatization unilaterally in 5%, and bilaterally in 3% of patients; 92% were normal. Extensive pneumatization of the sphenoid was seen in 49%of patients, while the remaining 51% had a normal sphenoid. The sphenoid septum was attached bilaterally to the internal carotid artery in 6% and unilaterally in 21% of patients. Aseptate sphenoid sinus (more than one septum) was found in 33% of patients; the remaining 67%were normal. A bilateral hypoplastic maxillary sinus was seen in 23%, a unilateral hypoplastic maxillary sinus in 3%, extensive pneumatization in 17%, bilateral septation in 17%, unilateral septation in 19%, Haller cells in 33%, and normal anatomy in 67% of patients. To the best of our knowledge, this is the first clinical study describing anatomical variations of the PNS in the Sudanese in detail.

Keywords: paranasal sinuses, anatomic variation, computed tomography, sudanese population.

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I. Introduction

The paranasal sinuses (PNSs) are air-filled, mucosa-lined cavities that develop in the facial and cranial bones and communicate with the nasal airways. However, their function is not known, and has been the subject of much speculation. Some argue that they could be involved in decreasing the weight of the skull and others postulate that they may act as resonators for voice Durr DG et al[1]. In lower animals, they are associated with a more acute sense of smell, and are largely lined by olfactory epithelium that has developed to increase the available surface area for smelling. However, in humans, olfaction is limited to a much smaller surface area, and the presence of the paranasal sinuses is probably a vestigial anachronism.

The PNSs are clinically categorized into the following four paired types a) the frontal pair in the frontal bone. The posterior wall is located adjacent to the anterior cranial fossa. They are usually asymmetrical and occasionally absent. b) The maxillary pair in the maxilla. The superior wall forms the floor of the orbit and the medial wall is formed by the lateral wall of the nose. Inferiorly, the maxillary pairis related to the tooth-bearing area of the maxilla. c) The numerous ethmoid cells in the superior and lateral walls of the nose and the medial walls of the orbits. d) The sphenoid pair, located in the sphenoid bone where the sellaturcica projects into this space Koskenkorva T et al[2]. Infection in the PNSs can cause headache because of obstruction to normal drainage as well as negative pressure created in the sinus. It has been reported that neoplasms growing in the sinuses also may cause obstruction for a prolonged period, and are therefore usually diagnosed at an advanced stage.

Types of anatomical variations in the PNS:

Variable pneumatization of the frontal sinus:

Pneumatization of the frontal sinuses is highly variable and asymmetric. Although extensive pneumatization of the frontal sinuses is very common, aplasia and hypoplasia can be seen in up to 5% of the population Ozpursoy OB et al[3]. It is speculated that pneumatization may extend into the orbital plate of the frontal bone, resulting in development of the anterior ethmoids, and is associated with the absence of an

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ethmoid bulla Momeni AK et al[4]. Frontal cells are an additional reported variation of the frontal sinuses[5] demonstrated that these cells play a role in embryonic development of the sinuses, whereas Van Aleya et al[6] reported that these cells encroach into the frontal recess or frontal sinuses. However, J.M. Delgaudio et al[7] classified these into the following four categories:

Type I: Single frontal recess cell above the agger nasi cells.

Type II: Tier of cells in the frontal recess above the agger nasi cells.

Type III: Single massive cell pneumatization cephalad to the frontal sinuses.

Type IV: Single isolated cell within the frontal sinus.

a) Variable pneumatization of the maxillary sinuses

Four types of recesses are reported in the maxillary sinuses, as follows. (i) Palatine recesses, which extend medially into the hard palate from the inferior aspect of the maxillary antra Lanzieri CF et al[8]. These recesses are bilateral and symmetric. (ii) Alveolar recesses, which are formed by extension of pneumatization into the superior alveolus and are seen more frequently in edentulous patients Bell GW et al[9]. They are close to the roots of the premolar and molar teeth. (iii) Infraorbital recesses are the result of pneumatization along the roof of the maxillary sinus and are medial to the groove for the infraorbital nerve. These extensions are usually confused with infraorbital ethmoid air cells, i.e., Haller cells. (iv) Zygomatic recesses are the result of extension of pneumatization of the maxillary sinus roof into the zygomatic bone.

b) Anatomical variation of the ethmoid sinuses

The most notable anatomical ethmoid variations include the agger nasi cells, which are mostly seen in the anterior ethmoid air cells, and are extramural (i.e., not within the ethmoid bone). Because of variations in anatomic descriptions, the prevalence of these cells also varies widely in nature. For the purpose of coronal CT imaging in this study, agger nasi cells are defined as those located anterior to the superior end of the nasolacrimal duct, because they may cause narrowing of the frontal recess and cause predisposition to ipsilateral frontal sinus disease. The large ethmoid bullae are another variation of the ethmoid sinus; these may compromise the osteomeatal complex at the level of the infundibulum, hiatus semilunaris, or middle meatus. A third anatomical variation is the presence of infraorbital ethmoid Haller cells, which extend along the medial aspect of the orbital floor; they may be present as discrete air cells or drain into the infundibulum or maxillary sinus. These infraorbital cells may cause narrowing of the ethmoid infundibulum, resulting in ipsilateral maxillary sinus disease.

The fourth and final variation is the fovea ethmoidalis. This asymmetric, low-lying ethmoid roof is a potential risk factor for intracranial injury during endoscopic surgery of the ethmoid air cells; focal dehiscence of the fovea ethmoidalis or cribriform plate may be posttraumatic or congenital Krmpotic et al[10].

c) Anatomical variation of the sphenoidal sinuses

Sphenoid pneumatization is highly variable because the sphenoid sinuses can be hypoplastic or extensively pneumatized with aeration of multiple recesses. Lateral extension of pneumatization may occur into the lesser wing of the sphenoid above the optic nerve, or into the anterior clinoid processes inferior and lateral to the optic nerves. Pneumatization of the optic nerve may increase the risk of injury during endoscopic surgery in the region. Pneumatization into the greater wing may extend lateral to a line drawn between the pterygoid canal and foramen rotundum. These extensions excavate into the base of the pterygoid process and are referred to as pterygoid recesses. However, midline extensions may occur anteriorly into the nasal septum or posteriorly into the dorsum sellae. Another variation in the sphenoid sinus is septation. Vertical septations within the sphenoid may traverse the sinus obliquely and terminate posteriorly in the bony covering over the internal carotid artery, predisposing the vessel to injury Lund Sethi et al[11]. Hence, an objective of the present study is to demonstrate the anatomical PNS variations that may occur in the Sudanese population. This study also intends to correlate sinus disease to anatomical variations of the PNS.

II. Material and Methods

Study design: This was a retrospective and descriptive hospital-based study that recruited557 patients.

Study location: This study was conducted in the Department of Radiology of the Ear, Nose, and Throat (ENT) Hospital, and in the Antalia diagnostic Center of Alzytona Hospital between January 1, 2012 and January 1, 2015.

Groups, inclusion and exclusion criteria: The present study included 557 individuals. Of these, 368 were referred to the Radiology Department for a CT study of the PNS. We excluded 51 individuals for the following medical reasons: those who were diagnosed with nasal or sinus tumors and those who had a history of surgery of the nose or sinuses, as well as those who had congenital facial anomalies and dysmorphic syndromes. Finally, we included a total of 317 patients with PNS disease (Group B). The control group consisted of 189 randomly selected healthy individuals with no symptoms or signs of PNS (Group A).

a) CT scan equipment

We used a Siemens Sensation 64-slice multidetector CT scanner (Siemens AG, Germany); this is the preferred modality for PNS imaging because it

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provides a precise view of the anatomical structures. The PNS differ between patients, leading to anatomical variations in the general population. In this study, coronal reference views were used for data collection; in some cases, axial views were used for additional details. CT images were analyzed for the presence or absence of anatomical variations in the PNS and also for pathological conditions.

b) Statistical analysis

The collected data were analyzed using SPSS version 16 software (IBM Analytics, USA).

III. Results

The results showed that there is a strong association between PNS anatomical variations and sinusitis in Sudanese patients.

a) Anatomical variation of the frontal sinus (FS) in the Sudanese population

Individuals belong to Group B were characterized by the pneumatization of the FS in about 28%, with a rudimentary FS in 10%, and the absence of FS in 11%. In addition, 51% showed no PNS anatomical variation. Our study also indicated that extensive pneumatization of FS had the highest incidence rate in group B, at about 37%, followed by a rudimentary FS in11%, and absence of the FS in 12%. However, nearly 40% of group B showed no PNS anatomical variation (Table 1). The results indicated a strong correlation ($P < 0.05$; chi-square between anatomical variation and PNS disease.

b) Keros classification of the FS in the Sudanese population

Based on the Keros classification (13, 14), 64% of group A individuals were normal, type I was seen in 19%, type II in 9%, type III in 6%, and type IV in 2%; in contrast, 55%of individuals in group B were normal, type I was seen in 27%, type II in 10%, type III in 6%, and type IV in 2%, as shown in Table 2.

c) Anatomical variation of the ethmoid sinuses in the Sudanese population

i. *The ethmoid bulla (EB)*

In group B individuals, a large EB was present in about 43%, but 57% were normal. However, in group A, about 29% had a large EB, and 71% were normal ($P<0.05$). These results indicated a correlation between a large EB and ethmoiditis (Table 3).

ii. *Posterior ethmoid cells*

In group A, unilateral cells were seen in about 2%, bilateral cells in 1%, and normal anatomy in 96% of patients. In group B, posterior ethmoid cells were extensively pneumatized unilaterally in 5%, bilaterally in 3%, and were normal in 92% of patients (Table. 4).

d) Anatomical variation of the sphenoid sinuses in the Sudanese population

Among individuals belong to Group A, 44% showed extensive pneumatization of the sphenoid sinuses, whereas 56% had normal sphenoids. However, in group B, extensive pneumatization of the sphenoids was found in 49%, and 51% had normal sphenoids (Table 5).

In addition, in group A, the sphenoid septum was attached bilaterally to the internal carotid artery in 16%, and unilaterally artery in 39% of patients. However, in group B, the sphenoid septum was attached bilaterally to the internal carotid artery in 6%, and unilaterally in 21% of patients (Table 6). In group A, a septate sphenoid sinus (more than one septum) was found in 41% of patients and 59% were normal. However, in group B, a septate sphenoid sinus was found in 33% of patients and about 67% were normal (Table 7).

e) Anatomical variation of the maxillary sinuses in the Sudanese populations

In group B, bilateral hypoplastic maxillary sinuses were observed in 23% of patients, and unilateral hypoplastic maxillary sinuses were found in only 3%, with extensive pneumatizationin 17% (Table 8). Moreover, bilateral maxillary sinus septation was observed in 17% and unilateral septation in 19% of patients (Table 9). In group B, Haller cells were found in 33% of patients, while 67% had normal Haller cell anatomy. In group A, Haller cells were observed in only 15% of patients and 85% had normal Haller cell anatomy (Table 10).

IV. Discussion

Anatomical variations of the PNS must be determined before performing any surgery in order to avoid the potential risks associated with functional endoscopic surgery (FESS) Abdullah et al[12]. Before proceeding with endoscopic surgery, the radiographic details of the osteomeatal complex as well as the anatomy of the other Para nasal sinus must be thoroughly verified.

In a previous study Nitinavakarn B et al[13] emphasized that CT of the PNS is usually required prior to any endoscopic sinus surgery because it demonstrates both the extent of disease and the anatomical variations that may predispose the patient to rhinosinusitis, as well as nearby vital structures so that iatrogenic injury can be avoided. Detailed knowledge of anatomical variations in the PNS region is critical for endoscopic sinus surgery as well for involvement by radiologists in the preoperative work-up. Prior knowledge of the anatomical variants and possible accompanying pathological influences are the key to the success of diagnostic and therapeutic management of PNS disease.

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Haller cells are ethmoid air cells that project beyond the limits of the ethmoid labyrinth into the maxillary sinus. They are considered to be ethmoid cells that grow into the floor of the orbit and may narrow the adjacent ostium of the maxillary sinus, especially if they become infected Stallman JS et al[14] Furthermore, failure to diagnose this condition preoperatively can predispose the patient to orbital trauma and violation of the lamina papyracea during middle meatal antrostomy. The incidence of Haller cells in this study was 33%, Lioyd GAS et a[15] but reportedthe incidence as 15%, which was less than that reported previously by palmer JN et al[16] (45.9%), Maru YK et al[17] (36%), and Laine FJ et al[18] (6.8%). Based on the above, it was clear that there is a strong association between maxillary sinusitis and anatomical variations of Haller cells ($P < 0.05$).

The EB is the air cell directly above and posterior to the infundibulum and hiatus semilunaris. Although it is the largest ethmoid air cell, excessive size and pneumatization can impair sinus ventilation and drainage. An EB sometimes grows to a size that completely fills the sinus in the middle turbinate. An excessively large EB is a potential cause of sinus infection, especially if pus, polyps, or cysts are present. However, Danese M et al[19] reported that this variation is difficult to assess and that it may be over diagnosed. The authors also reported that when compared to other variations, an enlarged EB had a lower correlation with sinus disease.

The present study reported a pneumatized EB in 43.2%, whereas Kang SJ et al[20] found a large bulla unilaterally in 12.5%, and bilaterally in 7.4%. The presence of a large EB was strong evidence of maxillary sinusitis in our study ($P<0.05$). Preoperative evaluation of the anatomy of the sphenoid sinus by CT is a routine procedure and can direct surgical decision-making. Conchal pneumatization of the sphenoid sinus was seen in 2%, pre sellar pneumatization in 21%, sellar type in 54.7%, and post sellar pneumatization in 22.3% of patients. Lateral pneumatization of the greater wing of the sphenoid, leading to a capacious sinus, was reportedly seen in83% of patients by Hamid O et al[21], whereas the frequency of pneumatization of the sphenoid sinus was 49.5% in this study.

Asymmetric septation of the sphenoid sinuses (more than one septum) is very important, especially when it shows considerable deviation. As it advances, it marks the line of the internal carotid artery, which may protrude into the posterior ethmoidal cell Zinreich SJ et al[22]. However, the prevalence of septation in this study was about 33.5%; the frequency of presence of a unilateral sphenoid septum was 20.5%, and the septum was attached bilaterally to the internal carotid artery in 6.6%. If the removal of a sphenoid septum is required, it should be done cautiously after CT is performed in order to prevent possible complications in case of attachment to the internal carotid artery or the optic nerve.

The FSs are typically paired, asymmetric, and separated by a central intersinus septum, and have different degrees of pneumatization. Around 10 to 12% of normal adults may have a rudimentary FS or even a complete lack of frontal bone pneumatization on one side. Around 4% of the asymptomatic population has bilateral frontal sinus agenesis. In the present study, we found that extensive frontal sinus pneumatization occurred in 36.9%.

Agenesis of the paranasal sinuses is an uncommon clinical condition that appears mainly in the FS. Bilateral FS agenesis was seen in 11.7%, and unilateral agenesis was seen in only three individuals (11.1%) as reported by Pondé et al[23]. The prevalence of bilateral absence of the FS has been reported to be only 3-4%, but can be 10% in some populations Aydinlioglu A et al[24]. However, this frequency was significantly higher in some populations, including Alaskan Eskimos (25% in males and 36% in females) and Canadian Eskimos (43% in males and 40% in females), as reported by Som PM et al[25]. The frequency of bilateral absence of the FS in this study was 11.7%, which is identical to that in Aydinlioğlu's report, but lower than that reported for most other ethnic populations Binali Çakur et al[26].

Bulla frontalis is a group of variants basically indicating air cells located cranial to agger nasi cells, and extending towards the frontal sinus; they can be isolated and protruding (type III) or not (type I). In the present study, bulla frontalis was found in 31.5%.Frontal cells have been implicated as a cause of frontal recess obstruction. Follows 14.9% had type I, 3.1% had type II, 1.7% had type III, and 2.1% had type IV. The frontal cells in the present study, based on the Keros classification, showed the highest incidence for type I (26.5%), followed by type II (10.4%), type III (5.7%) and type IV (1.6%).

Extensive maxillary sinus pneumatization (EMSP) was defined when the largest horizontal and/ orvertical dimension of the maxillary sinus was equal to or exceeded 90% of the corresponding diameter of the orbit. In a study by Meyer TK et al[27], the prevalence of EMSP was found to be 8% (7% bilateral and 1% unilateral). Extensive pneumatization was found in 17.3% of our cases, Maxillary sinus hypoplasia is an anomaly of the paranasal sinuses occasionally encountered by otolaryngologists. However, the literature lacks a system by which the various types of maxillary sinus hypoplasia can be classified using computerized topographic imaging. The overall prevalence of maxillary sinus hypoplasia was 10.4%. In the present study, we found that the incidence of a hypoplastic maxillary sinus was 26.5%, whereas twice this percentage was reported by Bassiouny A et al[28]

Maxillary septa were found in 47%, and appropriate imaging prior to performing sinus surgery seems justified, since complications and the success

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rate of sinus floor elevation are clearly related to the presence of septa. Bilateral maxillary septa were seen in 22.7% of scans, whereas unilateral septa were seen in 13; the combined total percentage was 35.7%.

V. Conclusion

From the above results, we conclude that anatomical variations in patients have a great impact on the predisposition to inflammatory disease of the PNS, as these variations are largely found in patients with EB, Haller cells, variable pneumatization of the FS (hypo and hyper), frontal cells, and sphenoid sinuses with septation and pneumatization. Imaging of the PNS will aid in diagnosis in individual patients, and also provide a deeper understanding of the manifestations of the disease. In addition, CT topographical imaging of the PNS and anatomical variations enables endoscopic surgeons to be fully prepared for FESS, thus decreasing the risk of complications. This study may alert the endoscopic nasal surgeon to the importance of defining anatomical variations, and the significant impact of these variations on patient care, there by reducing iatrogenic complications of morbidity and mortality.

VI. Recommendations

The authors propose the following recommendations for the improvement of current treatment options available for PNS.

1. Collaboration between endoscopic nasal surgeons and radiologists is very important in proper diagnosis and interpretation of scans of the sinuses.
2. Establishment of a standard protocol for CT scanning of the nose, paranasal sinuses, and skull base, including window specifications, thickness of cuts, and views to increase the precision of detection of pathology and anatomical variations.
3. Regularly scheduled combined radiology and ENT academic meetings are recommended to bridge the gap between radiologists and ENT surgeons, and to provide exchange of knowledge and experience with the aim of improving medical services.

VII. Acknowledgements

The authors express special thanks to the Dental Research Center, Tehran University of Medical Sciences, and Tehran, Iran. The study did not receive any financial support. Author Disclosure Statement No competing financial interests exists.

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Table 1: Anatomical variations of the frontal sinus (FS) in group A and B

Sample	Anatomical variations of the frontal sinus				
	Normal FS	Extensive pneumatization of FS	Rudimentary FS	Absent FS	Total
Group A* No. of cases	51%(96)	28%(53)	10 % (19)	11.% (21)	100% (189)
Group B* No. of cases	40 % (127)	37% (117)	11% (35)	12% (38)	100% (317)
Cases/ control prevalence %			Chi – square		p-value
61.5/37.4%			640		<0.05
Group *A = Healthy individual with no signs or symptoms of PNS Group B* = Patients with PNS disease					

Table 2: Anatomical variations of the frontal sinus karos classification

Sample	Frontal sinus karos classification					
	Normal	Karos I	Karos II	Karos III	Karos IV	Total
Group A* No. of cases	64% (121)	19% (36)	9% (17)	6% (11)	2% (4)	100% (N= 189)
Group B* No. of cases	55% (174)	27% (86)	10 % (32)	6% (19)	2% (6)	100% (N= 317)
ses/ control prevalence %				Chi – square		p-value
27.7 / 16.8 %				147.474		<0.05

Table 3: Anatomical variations of the size of ethmoid bulla

Sample	Size of Ethmoid bulla		
	Normal ethmoid bulla	Large ethmoid bulla	Total
Group A* No. of cases	71 (134)	29 %(55)	100% (N= 189)
Group B* No. of cases	57% (181)	43 % (136)	100% (N = 317)

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Table 4: Anatomical variations of the posterior ethmoid cell Pneumatization

Sample	Posterior Ethmoid Cell Pneumatization			
	Normal	Unilateral	Bilateral	Grand Total
Group A* No. of cases	96% (181)	2% (5)	1% (3)	100% (N= 189)
Group B* No. of cases	92% (291)	5% (16)	3% (10)	100% (N= 317)

Table 5: Anatomical variations of the Pneumatization of sphenoid sinus

Sample	Pneumatization of sphenoid sinus		
	Normal	Pneumatization of sphenoid sinus	Total
Group A* No. of cases	56% (106)	44% (83)	100% (N= 189)
Group B* No. of cases	51 % (162)	49 % (155)	100% (N= 317)

Table 6: Anatomical variations of the sphenoid septum attached laterally internal carotid artery

Sample	Sphenoid Septum Attached Laterally internal carotid artery			
	Normal	Unilateral	Bilateral	Total
Group A* No. of cases	45% (85)	39% (74)	16% (30)	100% (N= 189)
Group B* No.of cases	73 % (231)	21 % (67)	6 % (19)	100% (N= 317)

Table 7: Anatomical variations of the septate sphenoid sinus

Sample	Septate sphenoid sinus (more than one septum)		
	Yes	No	Total
Group A* No.of cases	41% (77)	59% (112)	100% (N=189)
Group B* No. of cases	33% 10.4	67 % (12)	100% (N=317)

Table 8: Anatomical variation of maxillary sinus

Sample	Anatomical variation of maxillary sinus				
	Normal	Expensively	Bi Hypoplastic	UniHypoplastic	Total
Group A*	54%	17 %	28%	2 %	100%
Group B*	57%	17 %	23%	3%	100%
Cases/ control prevalence %			Chi – square		p-value
61.5/37.4 %			278.079		<0.05
Conclusion			Very strong evidence		

Table 9: Anatomical variations of the maxillary sinus septa

Sample	Maxillary sinus septa			
	Normal	Bi Septate	UniSeptate	Total
Group A* No. of cases	74% (140)	12% (23)	14% (26)	100% (189)
Group B* No.of cases	64% (203)	17% (54)	19% (60)	100% (317)

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Table 10: Anatomical variations of the Haller cells

Sample	Haller cells		
	Yes	No	Total
Group A* No. of cases	15% (28)	85% (161)	100% (189)
Group B* No. of cases	33% (105)	67% (212)	100% (317)
Cases/ control prevalence %		Chi – square	p-value
61.5/37.4%		113.834	<0.05
Conclusion		Very strong evidence	

List of Figures



Figure 1: Variable pneumatization of the

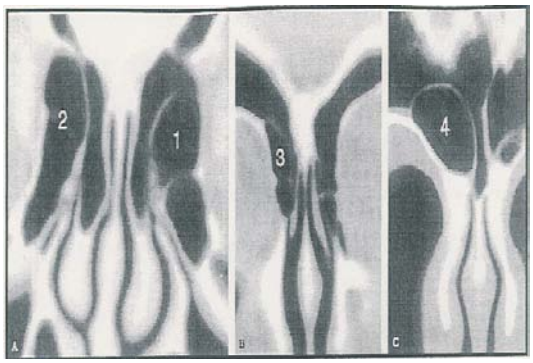


Figure 2: Coronal views of frontal cells frontal sinuses as shown in coronal (four types)

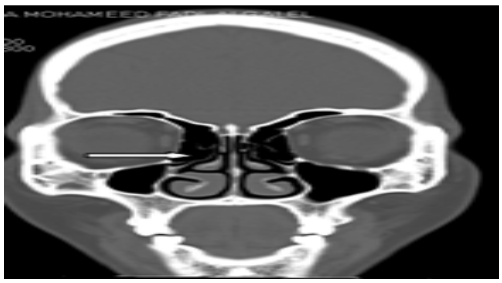


Figure 3: Ethmoidal bulla delineated

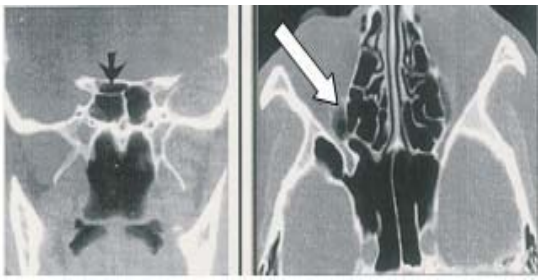


Figure 4: Posterior ethmodi cell shown in both by coronal scan coronal and axil section [Black and white arrows]

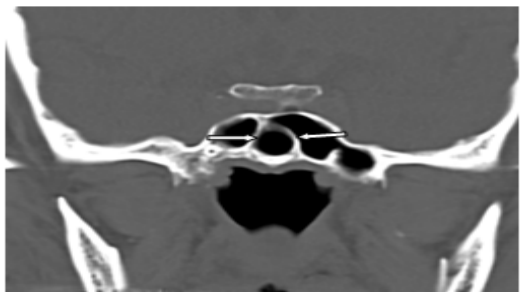


Figure 5: Sphenoid pneumatization (white arrow)

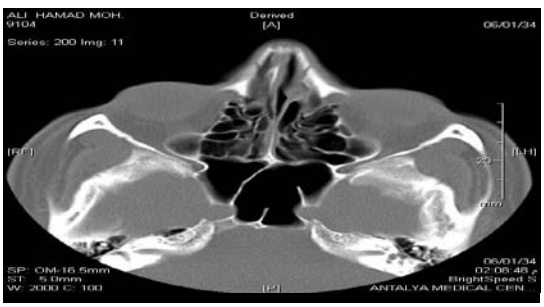


Figure 6: sphenoid septum attached laterally to internal carotid artery

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Figure 7: Septation of the sphenoidal sinus

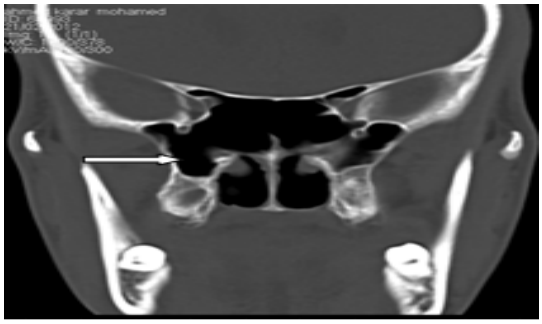


Figure 8: Anatomical variations of maxillary sinus



Figure 9: Maxillary sinus right unilateral

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Global Journal of Medical Research: K Interdisciplinary
Volume 16 Issue 6 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Assessment of Barriers of Behavioral Change to Stop FGM Practice among Women of Kebri Beyah District, Somali Regional State, Eastern Ethiopia

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Objectives: To assess barriers of behavioral change to stop FGM practice among women of Kebri Beyah district in Somali region, where the high prevalence of FGM is documented.

Methods: A community-based cross-section study design was applied. Both quantitative and qualitative methods were employed to generate relevant evidence. A total of 633 households drawn from five randomly selected kebeles involved in the quantitative part of the study. Participants identified purposefully were involved in the qualitative study. While survey data was analyzed by SPSS version 21. Multiple logistic regressions were carried out to examine the existence of a relationship between intention to stop FGM and socio demographic characteristics.

GJMR-K Classification: *NLMC Code: WJ 700*

ASSESSMENTOFBARRIERSOFBEHAVIORALCHANGETOSTOPFGMPRACTICEAMONGWOMENOFKEBRIBEYAHDISTRICTSOMALIREGIONALSTATEEASTERNETHIOPIA

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Results: This study revealed that 69.5% of the respondents intend to stop FGM. Religion was the major reasons for the perpetuation of this practice. About 50.3% of circumcision was performed by traditional birth attendants. 87.4% of participants responded that FGM was being practiced in that area. More than 79.9% of participants were undergone Sunni type of circumcision. More than 79.9% of participants were undergone Sunni type of circumcision. Most of respondents were found have good knowledge and negative attitude towards FGM. Divorced respondents were 4.35 (AOR=4.35, 95% CI (1.03, 18.33) times more likely intending to stop FGM than widowed and those who have television were 0.57 (AOR=1.76, 95% CI: (1.18, 2.63)) times less likely intending to stop FGM compared to those who have not television.

Conclusion: The study shows that the intention to stop female genital circumcision was high in Kebri Beyah district. The prevalence of FGM/FGC is still high in the study area. Most of women in this study have good knowledge and negative attitude towards FGM. Most of them undergo Sunni (clitoridectomy) type of circumcision. Traditional birth attendants were the main operators of female circumcision. Intention of women to stop FGM showed association with marital status and television.

Recommendation: Religious organizations should have to explain to the community that there is no religious justification for the practice female circumcision. Local organizations, Community and religious leaders should play significant role in the process of change within the entire community by arranging training, workshops, media campaign, public speech and outreach for awareness creation programmers.

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I. Introduction

a) Background

All societies have behaviors and norms based on age, life style, gender and social class. The norms often referred to as group-led beliefs about how the member behaves in a given context that may be beneficial or harmless but some may be harmful. However, culture is not static, it is a constant flux, adapting and reforming that people will change their behavior when they understand the hazards and indignity of harmful practices and when they realize that is possible to give up harmful practices [1]. Among harmful traditional practices, Female genital mutilation/ cutting (FGM/C) is one of mostly practiced worldwide and affecting almost all ethnic groups. It involves various procedures either partial or total removal of the female external genitalia for non-medical reasons. It is deep-rooted traditional practice; this practice is rooted in religious, personal and societal beliefs within a frame of psycho-sexual and social reasons such as control of women's sexuality and family honor, which is enforced by community mechanisms [2, 3].

While reasons for the practice vary across cultural groups, social reasons may include FGM/C as an initiation act for girls into womanhood, as an act of social integration and for the maintenance of social cohesion, socio-economic reasons include beliefs that FGM/C is a prerequisite for marriage or an economic necessity in cases where women are largely dependent on men, Religious reasons rest on the belief that it is a religious requirement, Hygienic and aesthetic reasons for FGM/C include beliefs that the female genitalia are dirty and unsightly, and health reasons include beliefs

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that FGM/C enhances fertility and child survival. FGM/C may also be an important source of income for circumcisers [4].

Any type of FGM is considered as a violation of the human rights of girls and women, it is known to be harmful to girls and women in many ways; the removal of or damage to healthy, normal genital tissue interferes with the natural functioning of the body and causes several immediate and long-term physical, psychological and sexual consequences [5].

It is estimated that about 100–140 million girls and women worldwide have undergone FGM, and each year a further two million girls and women are at risk of this practice. It is performed on girls aged 4–12 years and in some cultures as early as a few days after birth or as late as just before marriage [6].

Most of the girls and women affected live in 28 African countries, but also in the Middle East and Asia. They are also increasingly found in Europe, Australia, New Zealand, Canada, and US, mostly among immigrants from cultures where FGM is a tradition [7].

The prevalence of FGM according to figures from African countries shows a prevalence of more than 70% in Burkina Faso, Djibouti, Egypt, Eritrea, Ethiopia, Guinea, Mali, Mauritania, Northern Sudan, and Somalia. However, there is great variation in prevalence between and within countries, reflecting ethnicity and tradition [4].

b) Statement of problem

Over the past decades, the traditional removal of vital and normal external genital tissue of girls (called female circumcision (FC) and female genital mutilation or cutting (FGM\C) has become a major concern, and there has been an international consensus to take all possible measures to abolish a practice that is internationally deemed as a serious human rights and public health problem that concerns all sectors of society [9].

Like many other countries, FGM/C is a widely practiced in Ethiopian and it is one of the major socio-economic development problems of the country. The negative health implication of this practice increases the chance of maternal mortality during childbirth [10].

FGM is widespread across Ethiopia and is practiced in the majority of regions and ethnic groups. According to EDHS2005 the highest FGM practiced is the Somali region, the rate is 97.3% and the least is Gambela region 27.1% [11].

Yet 74.3% of Somali women believe FGM should continue, which is the highest percentage of women in any region in Ethiopia to think so. This is despite 60.9% knowing of the harmful consequences of FGM [12].

Ethiopia outlawed female genital mutilation in 2004, but still the practice is deeply rooted; the penalties for the practitioners range from a minimum of three months to a maximum of life in prison or monetary fines 14].

Laws can act as one tool to end the practice because they can empower the women and girls to refuse undergoing mutilation. Experts on the subject of FGM, states clearly “In some cases people are informed about the practice and are well educated but they cannot stand the belief that women can live with their clitoris not cut.” She adds that” the law is not meant to break up families and generations but “it sets the standards and informs what is morally right or wrong” [15].

When people lack an awareness of how their behavior affects their health and wellbeing, they have little reason to put themselves through the misery of changing the risk behaviors they have engaged in for many years. Although increased knowledge creates a precondition for change, yet additional communal or self-influences are needed to overcome the impediments to adopting and maintaining new behaviors [16]

It was commonly reported that most of those who went through the process FGM/C and the public at large claim to know about the problem entailed in FGM/C and disapprove the practice; Nevertheless, recent studies attest that despite relatively widespread awareness about consequences of FGM/C and disapproving attitude, four in five women reported to have circumcised their daughters. Besides, still there are mothers who come out proactively to support the practice in connection to sanitary reasons, to avoid shame and to respect cultures [12].

According to UNICEF’s report, Education is misleading factor to explain variation of FGM/C practice since the procedure takes place way before a girl is enrolled to school. Yet, it’s important to note the fact that mother’s level of education is believed to determine daughters FGM/C status [10].

A study done in Kersa Demographic Surveillance and Health Research Center field site revealed that, only one third of the respondents stated that they knowing of FGM being practiced in their community. Local healers were the main performers of FGM. Women knew about the negative reproductive health effects of FGM and also experienced these themselves. However, only a few had tried to stop the practice and the majority had taken no steps to do so. This may be attributable to the fear of becoming alienated from the cultural system and fear of isolation [19].

Among the Somali refugee community in Eastern Ethiopia, there was a considerable support for the continuation of the practice particularly among women. The findings indicate a reported shift of FGM from its severe form to milder clitoral cutting. More men than women positively viewed anti-FGM interventions, and fewer men than women had the intention to let their

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daughters undergo FGM, indicating the need to involve men in anti-FGM activities [13].

Several measures like IEC activities focusing on informing, promoting, motivating and teaching on FGM, workshops and seminars, community outreach, anti-FGM lessons in literacy schools, Religious education, media campaign and legal approach have been taken to bring about awareness on the harmful consequences of FGM and to put an end over the past decade at international, regional, and national levels.

In spite of these efforts, update reports reveal there was still widespread of FGM in Somali regional state. And this makes the study relevant and timely that plays important role to examine the barriers of behavioral change to stop FGM practice in this community.

c) Significance of study

When people lack an awareness of how their behavior affects their health and wellbeing, they have little reason to put themselves through the misery of changing the risk behaviors they have engaged in for many years. Although increased knowledge creates a precondition for change, yet additional communal or self-influences are needed to overcome the impediments to adopting and maintaining new behaviors There are large numbers of behavioral change theories, but changing the behavior of FC requires a unique approach as it is a communal rather than individual behavior. One of the main characteristics of FC is that even if each individual in the intermarried group thinks of abandoning the practice, no single individual acting alone can succeed [30].

In Ethiopia, there are a number of FC programmes underway. Including IEC activities programs, which is considered as essential steps for reaching behavior change in FGM, but there is no evidence as to whether or not these programmes have changed people's positive attitudes toward the practice of FC, the aim of this study is to identify the barriers of behavioral change to stop FGM practice. In addition to this, the study outcome will help policy makers, local government, public health practitioners and other interesting organizations to design appropriate intervention to stop FGM practice for the future female generation. Moreover, it serves as baseline for further researches.

II. Literature Review

a) Historical overview of FGM

The historical roots of FGM practice are not known but it appears in the ancient Egypt during the time of Pharaoh's. The "FC" used in the 1980 mostly by western writers and it was indorsed by inter African committee (IAC), on traditional practices affecting health of women and children, because of severity and irreversible of the damage inflicted on the girl's body that has been termed FGM/C. This is currently the term used in all official documents of united and other international documents instead over the use of female circumcision [21].

There is also little that can be said with certainty about the origin of different types of FGM. It seems most unlikely that the practice spread initially from any single location. One possibility suggested by Seligman is that FGM in the African and Arabian area are derived from ceremonies enacted by the Hamito-Semitic inhabitants of the Red Sea Coast .As for infibulations, its distribution throughout the Sudan-Ethiopia-Somalia region might indicate a relation with the Cushitic. Although often perceived to be a Muslim practice there can be no doubt that FGM in Egypt, Sudan and Ethiopia dates from long before Islam or Christianity [23, 24].

The practice is primary found in area where there is high poverty, child mortality, illiteracy, poor sanitation and access to modern health care facilities. Religion, tradition, poor economic and social status of women are among the most common factors reported to play a role for the practice to continue and exist [22].

Although the damage to female sexual organ and their function is extensive and irreversible, yet the true magnitude of the problem is still underestimated due to limited information and mystery of the practice [25].

This practice is considered as one of major international and national problem as it does not only affect the physical, mental and social life of women but also socio-economic development of many countries [10].

b) Female circumcision globally

FGM/C is universally practiced all over the world. According to UNICEF report based on a survey completed by selected countries FGM is known to be prevalent in 27 African countries, Yemen and Iraqi Kurdistan where 125 million women and girls have undergone FGM. FGM/C is major public health issue, majority of women worldwide have under gone the procedure. It is practiced in one form or another, in around 40 countries, mostly in east and West Africa and also parts of Arabian Peninsula. As a result of migration from these areas it is now also practiced in Europe and Australia and united state of America [9].

The highest prevalence rates are in 30 African countries, in a band that stretches from Senegal in West Africa to Ethiopia on the east coast, as well as from Egypt in the north to Tanzania in the south. On the other hand Egypt has the world's highest total number with 27.2 million women having undergone FGM, while Somalia has the highest prevalence rate of FGM at 98% although, estimates about the prevalence of FGM vary by source [10].

A study shows that the percentage of circumcised women was 99.3%, infibulations is the

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commonest type of circumcision used (75-78 %) Kenya 78 %, Eritrea 95.5 %, Egypt 97 %, Djibouti 98 %, Sudan 90 %, Ethiopia 70-90 % and the least prevalence countries including Uganda 5 %, Zaire 5 %, Togo 12%. The age of the circumcision performed varies from one community to other, it may be done during infancy or childhood or adolescent and at the time of marriage. It is thought that mainly performed between ages of 4-15 years average being 7.5 years, demographic health survey of respective countries [8].

c) Female circumcision in Ethiopia

The distribution of female genital cutting in Ethiopia varies depending on ethnic origin and region. The National Committee on Traditional Practices in Ethiopia carried out a national baseline survey to determine the prevalence of this practice. Some 44,000 people were interviewed in a study reaching 65 of Ethiopia's 80 ethnic groups (urban and rural) in all ten regions of the country. The results show that 72.7% of the female populations have undergone a form of circumcision. Regional statistics from the survey revealed that prevalence ranges from 27.1% in the Gambella region to 99.7% in the Somali region, and to more than 50% in the capital, Addis Ababa. Throughout the country, half of all women who have undergone FGM have had clitoridectomy, and the remaining cases have had their clitoris and/or labia minora cut. Nationwide, 6% of females affected by the procedure have undergone infibulations. More than 80% of women in the Somali region are suffering as a result of infibulations, whereas the prevalence is 60% in Afar [27, 28].

A study conducted in JIGJIGA town revealed that the proportion of women who were genitally mutilated was 96% with 52% of them undergone the most severe type of FGM – infibulations. The rest 48% of women had undergone either FGM Type I or Type II; i.e., they were genitally mutilated but not infibulated [29].

A study conducted at national level in revealed that, the support for the continuation of females' genital mutilation decreased from 42.8% (no education) to 2.0% (higher education). It was also observed that the support for the continuation of the practice ranged from 76.0% (Somali) and 69.0% (Afar) to 13.3% (Dire Dawa) and 5.9% (Addis Ababa), respectively [30].

d) Knowledge and Attitude of FGM Practice

There are four types of female genital cutting generally categorized as; clitoridectomy (Sunni) type which involves the dissection and removal of the clitoral hood or fore skin of the clitoris; excision type which is more severe involving the total removal of the clitoral, partial or total removal of the labia minora (small lips) leaving the vulva open. Infibulations which is the crude form of gynecological operation involves excision and in this procedure the clitoris and the labia minora are removed, the inner wall of the labia majora excised or scraped to produce a raw surface [31].

All types of female genital mutilation involve removal or damage to the normal functioning of the external female genitalia and can give rise to a range of well documented physical complications. They are irreversible and their effects last a lifetime. Studies on health effects of FGM shown this practice has negative consequences for delivery, first sexual intercourse, and during menstruation. Studies on the psychological effects of FGM are scarce and need to be given due emphasis, given that FGM is one of the reported risk factors for post-traumatic stress disorder in women [32, 33, 34–38].

A study done in Sudan mentioned that 45 person of women interviewed and believed that as it is a good production because of it is promote cleanliness, and keep virginity, more than 80 % of men and women were against and 40 % respondent unaware that FGM/C is banned by law.8.3% of female respondents thought FGM would increase their chance of marriage, while 78.1% of female were unsure as to whether this would affect their chance of marriage, 74.8% of male preferred to marry uncircumcised women, 65% of respondents said that mothers were responsible in taking the decision, while grand mother and father were responsible in 52% and 25% of cases respectively. 94.2% of female aware of the complication caused by FGM, 50% of female especially the Muslim respondents claimed that FGM is recommended by their religious and 88.1% of women had negative attitude towards FGM practice [52].

According to source of genital mutilation affords young women status in their societies and assures that they will be acceptable bridges. This practice continues even though men who have had sexual intercourse with mutilated and intact women prefer the experience with later. Another factor that contributes to the continuation of the mutilation practice is that, the only autonomous profession open to women in many societies, are those of traditional midwife and circumciser [6].

According to study in entitled female genital mutilation a new challenge for health service; most children or women are circumcised by local women and traditional midwives often the intervention is part of cultural rituals that make the transition to womanhood and preparation for marriage (10).The highest rate of use of medical personnel to perform FGM can be found in Egypt (61%), Kenya (34%), and Sudan (36%), with rates of 9% and 13%, respectively, in Guinea and Nigeria. Similarly, 90% of FGM is performed in Guinea and Eritrea by traditional/ local healers [18].

Study done by Egyptian care society, show that 39% of study women perpetuated FGM/C due to custom. 80% believed that practice should continue. 15-20% refuses to give opinion on FGM/C.

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60% believed that FGM/C was religious practice. 72% husbands prefer to wives to FGM/C. 45% believes that it prevented adultery [6].

A study done in Bale zone revealed that 26.7% of the respondents had intention for the continuation of FGM. Religion, safeguarding virginity, tradition, and social values were the major reasons for the perpetuation of this practice [19].

The majority, however, are village midwives who either interested in their living by performing operation or enjoying a position of status in their village and able to wide considerable influence over women. The reason and the motivation why the practice is continue for allowing girls to undergo FGM/C given present for confusion, they are contradictory to each other and in contradiction of biological facts. On the other hand, a study done in Hargeisa district revealed that 88.8% of the respondents were aware of the possibility of HIV transmission [2, 22].

47.9% of mother had favorable attitude to continue FGM were and with unfavorable attitude were 52.1% that is to discontinue the practice. The mechanism by which FGM might cause adverse obstetric outcomes is unclear, but they can be predicted according to the type of FGM performed; more severe complications are anticipated after type 2 than type 3 FGM. The presence of scar tissue which is less elastic than the perineal and vaginal tissue following the procedure would cause obstruction, tears, and/or a need for episiotomy [39, 40].

A World Health Organization study in six African countries revealed that the annual cost of FGM related obstetric complications amounted to $3.7 million and ranged from 0.1%–1% of government spending on health care for women aged 15–45 years; This poses a significant economic burden on poor countries, when forced to spend a huge amount of money on outcomes of a traditional non medical procedure [6].

A study done in JIGJIGA town revealed that Episiotomies occurred among 61% of women who were delivering for the first time and 28.1% of women delivering for the second time. The rates of instrumental and cesarean deliveries among the first-time deliveries were 6.6% and 3.1%, respectively; while they were 3.2% and 1.3% among the second-time deliveries, respectively. Among primi-parous 36.2% women reported having had complicated postnatal period; 22.5%, prolonged lab our; 10.3%, perineal tear and 9.8%, heavy bleeding. [29]. In addition to the above mentioned complications, FGM/C procedure has also the potential to transmit HIV and cause fistula.

In addition to the negative health consequences of FGM/C, it is vital to highlight that the practice reflects a gender inequality that establishes an extreme form of female discrimination. Progress towards its abandonment may therefore contribute to the empowerment of women (MDG 3), an improvement of maternal health (MDG 5) and a reduction in child mortality (MDG 4). Accordingly, for the good health and human rights of women and children, the United Nations has denounced all forms of the practice, rejecting any shift towards accepting milder forms as well as towards the medicalization of the practice [40].

e) Intervention to stop FGM/C

The first programme for the prevention of female genital mutilation (FGM), which started in the mid-1970s focused on promoting, informing, motivating and teaching on the adverse health effects of FGM, in order to break the taboo surrounding this harmful traditional practice. The efforts to stop practice of FGM, used information, education and communication (IEC) materials, such as leaflets, booklets, training manuals and guidebooks for professionals. These IEC activities were often conducted with a focus on awareness rising rather than behavior change and thus focused on short time results, since behavior change takes time [3].

Health education intervention had a positive impact on the attitude of women towards FGM. However, for sustainable behavioral change that will lead to end FGM practice placing FGM elimination efforts within a comprehensive development strategy and larger context of reproductive health is needed [67].

While the provision of continued and informed care for women who have been affected by FGM/C is crucial, the key to improving the health and lives of women at risk rests in the numerous prevention and eradication strategies that have been implemented worldwide in an attempt to bring the practice of FGM/C to an end. Some have proven more effective than others [41].

Changing behavior to FGM (or any other undesired practice such as smoking or the practice of unsafe sex which might lead to HIV infection) requires a particular approach. To better understand this process of behavior change, several theories have been developed that explain individual or community behavior change [42].

BCC is also used to promote, sustain and maintain individual, community and societal behavior change; it is recognizes that skill building might be needed in order to sustain the change in behavior, for example on how to resist pressure, and how to establish community support [44].

Recent developments in communication recognize the need to move beyond top-down communication towards horizontal and participatory approaches. Such approaches incorporate the concept of enabling environments (e.g. breaking the taboo/silence) and contextual factors (e.g. pressure of grandmother to excise a girl) and are framed by the concept of communication for social change [45].

While legal and political measures are fundamental to ending FGM/C, community based

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eradication and prevention initiatives in conjunction and consultation with NGOs have now become a key component of campaigns worldwide. "While government action is necessary to create a political and legal environment that deters people from practicing FGM/C, it is ultimately the women, their families and their communities who must be convinced to abandon the practice"[15].

FGM is a dangerous and potentially life-threatening procedure to with women and girls in many countries are subjected has been viewed as a human right violation in many countries. More recently, parliamentarians from all over Africa met in Dakar, to push for a continent-wide ban on FGM and calling on UN to pass a general assembly resolution appealing for a global FGM ban, as it violates human rights they argued members of parliament from African nation also exchange lessons learned and action to take to achieve the ban and resolution some 17 African states have banned FGM, among them Ethiopia, Burkina Faso, Togo, Senegal and Uganda [10].

Programmatically, there are a number of institutions that are engaged in the fight against FGM/C in Ethiopia. These institutions have joined hands by establishing networks against FGM/C. there are more than forty six local CSOs that have one or more FGM/C focused intervention. Despite this the persistence of FGM/C is believed to be associated with individual, social and cultural factors, interventions were not particularly focused. This may also affected the whole endeavor of stopping the practice of FGM/C [17].

Kembatta menti gezzime (KMG) is an Ethiopia-based indigenous human right and development NGO that envisions a society where women are free from all forms of discrimination and violence and where they are able to attain justice, equity and equality for themselves, their families and their communities [12].

III. Objectives

a) General objective

- To assess barriers of behavioral change to stop FGM practice among women of reproductive age group in Kebri Beyah district.

b) Specific objectives

- To assess level of Knowledge of women towards FGM practice.
- To describe the level of attitude to and practice of FGM among women.
- To identify factors affecting behavioral change towards FGM prevention.
- To identify preference of setting and means of communication of information related to FGM.

IV. Methodology

a) Study area

The study was conducted in Kebri Beyah district, Fafaan zone, Somali Regional State of Ethiopia, which is located in lowland part of Eastern Ethiopia It is located at 50 kilometers east of Somali regional state capital city (Jigjiga). Based on CSA2007 population of Kebri Beyah district were 165,518 in which 89,703 were men and 75,815 were women [48] and it composed of 29 kebeles. It has six health center and 27 health post. It is selected since various ethnical group exist that manifest cultural diversity which may contribute valuable information. A widespread practice and considerable support for the continuation of the FGM practice were also reported.

b) Study design and period

A community based cross-sectional study with quantitative and qualitative methods was conducted among women of reproductive age groups at Kebri Beyah district from August 2014 to June 2015.

c) Source population

The source populations were all women of reproductive age group living in KEBRI BEYAH district.

d) Study population

The study participants were women of reproductive age group who met the inclusion criteria.

e) Inclusion criteria

All women of reproductive age group (15-49years old) in the district

f) Exclusion criteria

- Critically sick
- Mentally ill
- Unable to hear.

g) Sample size determination

The sample size was determined by using a single population proportion formula and calculated by Epi info.

$$N = \frac{(Z\,\alpha/_2)^2 p\,(1-p)}{d^2}$$

Assumption: In order to obtain adequate sample size

P = Expected proportion of behavioral change 50%;

Since there was no previous studies that estimate the level of change in FGM practice in connection to behavioral change.

$Z\,\alpha/_2 = 1.96$ of significance $\alpha = 0.05$,

d= the margin of error was 0. 05

$$N = \frac{(1.96)^2 * 0.5\,(1-0.5)}{(0.05)^2}$$

$$= 384$$

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Contingency 10 % for refusal and absenteeism was added. 384+10%*1.5= 633

Design effect was considered 1.5.

h) Sampling procedure

A total of 633 households sample were selected by using Multi- Stage Sampling procedure;

- All 29 kebeles of KEBRI BEYAH districts were grouped into five categories based on their direction
- From each category, one kebeles was selected randomly based on the resources in our hand by using simple random sampling by lottery method
- The total sample size was distributed to the selected five kebeles proportional to their total households
- From each kebeles households, households were selected using systematic sampling until the allocated sample size achieved
- Individual aged 15-49 years old in the households were randomly selected for interviewed
- When there was more than one reproductive age women in one household only one person was selected using lottery method
- If the specified age was not found in the household or not available that time three repeated visit were made, then the nearest household was replaced.

Purposive sampling technique was utilized for qualitative approach.

i) Data collection procedures

i. *Quantitative method*

Data was collected by trained female local data collectors who completed grade 10 and had previous experience in data collection using face to face interview administered questionnaire which was developed from reviewing others studies and modified according to variables then translated into local language (Somali). Training was given for data collectors and supervisor on collection technique and objective of the study, Questionnaire, sampling methods and securing informed verbal consent from the study participants for three day at KEBRI BEYAH by investigator.

The questionnaire used in this survey was addressed socio-demographic characteristics of respondent, knowledge related to FGM, types and side effect of FGM, sources of information to stop FGM, preferred settings (home, health institution, religious organization and community base organization) and means of communication (drama, song, news and discussion), attitude and intention to stop FGM practice as well as to identify barriers to stop FGM practice.

The respondents were interviewed at home by interviewers and the data collection process based on self-report and no inspection of genitalia was performed. The questionnaire was pre-tested on 10% of total sample size at other kebeles & the necessary arrangements & corrections were made to standardize & ensure its validity.

ii. *Qualitative method*

Focus group discussion (FGD) was conducted to obtain deep information related to; participant's knowledge on FGM, sources of information for positive behavioral change, altitude and intention to stop FGM and finally to suggest any means that bring positive behavioral change to stop FGM practice. A total of three FGD session (two for women separately and one for community leaders) were hold for 60minutes for each session and moderated by principal investigator with assistance of trained note taker and tape recorder and later transcribed. The discussion was held in private setting and quit environment. Semi structured topic guide was used to guide the discussion.

j) Data quality management

Before embarking upon data collection, pretest was conducted in another kebele to ensure the validity of the survey tool & to standardize the questionnaire. Supervisors & the principal investigator were made frequent checks on the data collection and each discussion was taken note and tape recorded. Finally, the investigator transcribed the tape record after each section. The transcription was checked by interviewees to ensure the completeness & consistency of the gathered information.

k) Data Analysis

Quantitative data was entered and cleaned by using Epi data version 3.11 and analyzed by using SPSS version 21 package. The data will be coded on pre arranged coding sheet by the principal investigator, after all the necessary data collected and checked their completeness. Descriptive statistics were used to calculate the mean and standard deviation for continuous variables and frequency for categorical variables. Bivariate analysis and cross-tabulation was used to see the relationship and effect of the identified factors on FGM prevention with their crude OR association. Multiple logistic regressions were performed to see the effect of independents variables on dependent variables while controlling effect of others.95% C.I with Adjusted odds ratios were used to interpret the result. The qualitative data, the different ideas in the text were merged in their thematic areas, and a thematic analysis was employed manually. Then, the result was presented in narration by triangulating the quantitative finding.

l) Variables

i. *Dependent variable*

- Knowledge towards FGM
- Attitude towards FGM
- Intention to stop FGM practice

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ii. *Independent variables*

Socio demographic characteristics

- Age
- Marital status
- Religion
- Educational status
- Occupation

Communication factors

- Source of information
- Type of information
- Frequency of information
- Means of communication

Family factors

- Number of children
- Type of child
- Decision about FGM

Community enforcement

- Social pressure
- Society norms
- Legal action
- Fear of divorce
- Stigmazation

m) Measurement of variables and Operational definitions

- *Barriers:* Factors considered by the individuals to be obstacles to sop FGM practice.
- *Knowledge:* Eight questions were asked to assess knowledge of FGM and correct answer was given score 1 while incorrect answer was given score 0. The score varied from 0-8. The sum was computed and those who scored above the mean were labeled as having 'good knowledge' while those who score below mean labeled as 'poor knowledge'.
- *Attitude:* Individuals' predisposition to respond in a favorable or an unfavorable manner towards the prevention FGM. lickert scale were used to assess attitude of participants and five question were asked and the scale were measured as follow;

Choice	Positive	Negative
Strongly agree	5	1
Agree	4	2
Neutral	3	3
Disagree	2	4
Strongly disagree	1	5

The score varied from 5-25 and the sum was computed and those who scored above the mean were labeled as having ' positive attitude' while those who score below mean labeled as 'negative attitude'.

- *Practice:* An overt behavior or habit of women towards FGM and being circumcised is evidence for FGM practice.
- *Intention to stop FGM:* The women's plan to carry on with practice by subjecting their own daughter to FGM in the future. Measuring an intention using a single variable may not be practical for this study, Seven questions were presented to the study participants specially Q501, Q504, Q506 and Q507 are considered and correct answer will be given score 1 and incorrect answer 0. And great change=2, low change=1 and no change=0 was given score. The sum was computed and those who scored above the mean were labeled as having 'intention to stop" whiles those who score below mean labeled as "intention to practice."

n) Ethical consideration

Ethical clearance was obtained from the ethnical clearance committees of the School of Public Health, AAU. Official letter was written by School of Public Health to Somali Regional Health Bureau and other concerned bodies to allow implement the study. The objective and importance of the study was explained & informed consent was obtained from each participant. Privacy and confidentiality was maintained at all levels of the study. Participant's who were unwilling to participate in the study & those who went to quit from the study at any juncture were informed to do so without any restriction.

V. Result

a) Socio-demographic characteristics of respondents

A total of 633 households were planned for study and 620 households were successfully interviewed with an overall response rate of 98%.

Most of the respondents 261(42.1%) were in age group of 15-24 year and 244(39%) were in age group 25-34. The mean age of study participants was 27.14 ± (7.67) years, with minimum and maximum value 15 and 49 years, respectively. The majority of the respondents, 576 (92.9%) were Muslim, while 27(4.4%) were orthodox.

Majority of respondents were Somali, 513 (82.7%) followed by Amhara, 50(8.1%), Oromo, 48(7.7%) and others, 9(1.5%). Finding on marital status, shows that, 403 (65%) of respondents were married, while 151(24.4%) were single.

Education accomplishment of participant shows that nearly, 343(55.3%) were unable to read and write,

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while, 146 (23.5%) could read and write and 86(13.9%) had elementary school education. Occupational status of the respondents shows that 286(46.1%) were housewife, 195(31.5%) were farmer, 76(12.3%) were Student.

Findings on ownership of radio and television shows, 463 (74.7%) were found to have radio while 295 (47.6%) had television at household level to access FGM related information. Characteristics of the respondents were summarized as follows:

Table 1: Socio-demographic characteristics of respondents, Kebri Beyah district, May 2015, (n=622)

Variable	Frequency	%
Age group(years)		
15-24	261	42.1
25-34	242	39
35-49	117	18.9
Religion		
Muslim	576	92.9
Orthodox	27	4.4
Protestant	17	2.7
Ethnicity		
Somali	513	82.7
Oromo	48	7.7
Amhara	50	8.1
Others	9	1.5
Marital status		
Single	151	24.4
Married	403	65
Divorce	54	8.7
Widowed	12	1.9
Literacy		
Can't read and write	343	55.3
Can read and write	146	23.5
Grade 1-8	86	13.9
Grade 9-12	45	7.3
Occupational status		
Farmer	195	31.5
House wife	286	46.1
Civil servant	25	4
Daily laborer	38	6.1
Student	76	12.3
Radio		
Yes	463	74.7
No	157	25.3
Television		
Yes	295	47.6
No	325	52.4

b) Communication related to FGM prevention

Four hundred thirty nine (70.8%) of respondents heard FGM messages on radio. Among this, most of the respondents 169(38.4%) listened FGM at least once in two weeks in three weeks on radio, followed, at least once in two weeks, 100(22.7%).

Two hundred sixty one (42.1%) of respondents heard FGM messages on television. Among this, 89(34.1%) were watched television at least once in two weeks in three weeks, followed, 54(20.7%), at least once in two weeks, 52(19.9%). Four hundred forty three (71.5%). of respondents have not read printed materials related to FGM for the last four weeks.

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Table 2: Communication towards FGM messages in Kebri Beyah district, May 2015

Variable	Frequency	%
Ever heard FGM on radio		
Yes	439	70.8
No	181	29.2
How often heard FGM on radio (n=439)		
At least once a week	54	12.3
At least once in two weeks	100	22.7
At least once in two weeks in three weeks	169	38.4
At least once in three weeks in four weeks	73	16.6
Ever watched FGM on television		
Yes	261	42.1
No	36	57.9
How often watched FGM on television(n=261)		
At least once a week	54	13.8
At least once in two weeks	89	20.7
At least once in two weeks in three weeks	52	34.1
At least once in three weeks in four weeks	30	19.9
Read printed materials		
Yes	177	28.5
No	443	71.5

c) *Knowledge of women towards FGM*

Five hundred ninety five (96%) of respondents mentioned FGM have complication. Almost 387(62.4%) of respondents have learned something about FGM for the last 12months. Among this 160(41.3%) learned about the impacts of FGM, 136 (35.1%) learned about how to stop FGM and 89(23%) learned about types of FGM.

Two hundred ninety two (47.1%) know that FGM cause HIV/AIDS to females. Four hundred ninety seven (80.2%) of respondents mentioned FGM cause excessive bleeding, 475(76.6%) of participants believed that FGM cause difficult of lab our during child birth.

About 500(80.6%) of respondents responded FGM negatively affect the future sexual relation of women. And 503(81.1%) of respondents thought that FGM is harmful tradition and should be stopped. Among this 259(52.5%) considered health education were the possible means to stop FGM, followed 207(41.1%), legal action. Almost half of the respondents, 313(50.5%) perform FGM for religious purpose, followed, 229(36.9%) for culture, 74(11.9%) to ensure virginity. Computing the knowledge score of study participants, 557(89.9%) has good knowledge, while the remained 63(10.2%) have poor knowledge regarding to FGM.

Table 3: Knowledge related to FGM in Kebri Beyah district, May 2015

Variable	Frequency	%
Learned anything about FGM for last 12 months		
Yes	387	62.4
No	233	37.6
Know types of FGM		
Yes	447	72.1
No	173	27.9
FGM cause complication		
Yes	595	96
No	25	4
FGM cause difficult during lab our		
Yes	475	76.6
No	145	23.4
FGM cause bleeding		
Yes	497	80.2
No	123	19.8
FGM affect future sexual relation		
Yes	500	80.6
No	120	19.4
FGM cause HIV/AIDS		
Yes	292	47.1
No	328	52.9

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FGM is harmful should be stopped		
Yes	503	81.1
No	117	18.9
Knowledgeable		
Good	557	89.8
Poor	63	10.2

Most of participants divided FGM into two types; Sunni (gudniinka sunniga) and pharaonic (gudniinka fircooniga). Several reasons are used to justify FGM practice, religious and custom are the commonest reasons for FGM practice. In addition to this, there is also an expectation that men desire to marry only circumcised women, while virginity and an intact hymen is given high respect and considered as marriage prerequisite and most of them know the negative health effects of this practice and some of them have experienced these problem.

*"FGM is a long period standing traditional practice and most of people classify into two types Sunni (removal of the tip of the clitoris) and phraonic (removal of prepuce), it's religiously recommended as well as culturally required, so that majority of this community respect and practice FGM."(*29 years old married woman).

Some of participants mentioned that men prefer to marry women that were subjected FGM whether it's Sunni or pharaonic types.

"I remember that, one of my friends has lost her future, because of she was not circumcised. Her fiancé asked her whether she was circumcised or not, one day before Nikah (engagement) and I realized that men desire to marry only circumcised women."

(28 years old married woman)

Most of study participants believe that; FGM is religiously recommended in order to protect premarital sex (sinada) and this also increases the chance of marriage.

"FGM is religious recommended and mandatory so we are expected to perform because there is various hadith that commend us to practice, the following hadith to argue that it is required as part of the Sunnah or Tradition of the Prophet: 'Um Atiyyat al-Ansariyyah said: A woman used to perform circumcision in Medina. The Prophet (pbuh) said to her: Do not cut too severely as that is better for a woman and more desirable for a husband's." (25 years old unmarried woman)

41 years old married woman also stated the following reason, *"I believe that FGM practice whether it's sunni or phraonic because I inherited from my grandparents. I consider as mandatory according to my religion and it's also one of my cultural identities that I should have to maintain."*

Majority of the participants mentioned that, Traditional birth attendants were the main performers of this practice.

*"The traditional birth attendant's were circumcising our daughter by unhygienic procedure in our home and this increase the risk of infection and other sever disease."(*32 years old married woman).

Most of the study participants knew that FGM had health-related problems, including recurrent pain, pain during first sexual intercourse, the retention of urine and menstruation, and infection and complications during delivery.

"I experienced various problem during menstruation I feel sever pain and the blood doesn't come out properly and on my first delivery, I developed prolonged lab our and infection." (26 years old married woman)

d) Source of information to increase knowledge related to FGM

The commonest source of information that helped the participants to increase their FGM related knowledge were health professionals 242(39%), followed, family 116(18.7%) and religious leader 65(10.5%).

Table 4: Source of information on FGM in Kebri Beyah district, May 2015

Variable	Frequency	%
Source of information		
Family	116	18.7
Peers	53	8.5
Religious leader	65	10.5
Health professionals	242	39
Radio	57	9.2
Television	27	4.4
Teacher	14	2.3
Anti- FGM clubs	45	7.3
Others	1	0.2

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e) *Preference of settings and means of communication of information related to FGM*

Three hundred forty eight (56.1%) preferred health institutions for adoption of positive behavioral change. while, 199(32.1%) were preferred religious organization and 71(11.5%) were preferred community based organizations.

Most of the study subjects 226(36.5%) mentioned drama as best means of delivering information related to FGM to bring positive behavioral changes. while 132(21.3%) were preferred group discussions.

Table 5: Preference of settings and means of communication about FGM information in KEBRI KEYAH district, May 2015

Variable	Frequency	%
Preference of setting to obtain information		
Health institution	348	56.1
Religious organization	199	32.1
Community-based organization	71	11.5
Schools	1	0.2
At home level	1	0.2
Preference of means of communication		
Song	93	15
Drama	226	36.5
News	37	6
Speech	119	19.2
Discussion	132	21.3
Others	13	2.1

Participants are asked their source of information used to stop FGM practice in their community. Majority of the participants stated that radio is the commonest source of information while some of the participants mention health professionals, religious leaders, women's organizations, schools and community leaders also deliver information related to FGM to the community, while some of them criticized EIC activities.

For instance a 21 years old unmarried woman mentioned that*; "Workshops and training are always given to health professionals, community leaders, religious leaders and youth in order to sensitize all harmful effect of FGM."*

*"I have never attended any training but I heard from the radio that FGM is harmful should stopped."(*32years old married woman)

*"I have seen Community leaders and other organize public speech and outreach activities, sometimes in market and public area to create awareness on harmful effect of FGM to bring positive behavior to the community."(*19years old unmarried woman)

*"I heard FGM related massages like, FGM stories, drama and songs were broadcasted on radio once in every two or three weeks. I also heard few religious leaders give public lecture Friday prayer (qhudba) to confirm female circumcision particularly pharaonic type is not recommended in Islam."(*26years old married woman)

And limited efforts of IEC activities were used deliver information in order to bring positive behavior to stop FGM.

*"There is no doubt that IEC activities are only performed by only health workers and it is not holistic approach because, community members are not included."(*36years old married woman)

"Once, I was participated a training toward FGM awareness most of trainer criticized IEC work activities, it were not focusing on rural area where information gap exist, the rural people have not access to attend trainings and workshops which is important to bring positive behavioral." (35years old married woman)

f) *Practice of FGM among women*

Majority of study participants 542(87.4%) reported FGM is currently practiced in their community, while 483(77.9%) of respondents have undergone FGM themselves. Among those undergone FGM, 265(54.8%) were circumcised at age 5-9 years, followed by, 149(30.8%) who were circumcised at age10-15 years. Most of the respondents reported to have gone through Sunni type of circumcision 386(79.9%) and the remaining 97(20.1%) were subjected pharaonic type.

More than half of respondents, 312(50.3%) reported that traditional birth attendants were the main circumciser, followed by, 224(36.1%), village women and 67(10.8%), health professionals. Commonest type of instruments used to perform FGM were blade razor 395(63.7%), followed, knife 152(24.5%), scissor 69 (11.1%) and 4(0.6%), others. And most of respondents 435(70.2%) reported that mother decide to perform FGM to their daughters, followed by, 153(24.7%), father and 32(5.2%) for both mother and father.

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Table 6: Practice of FGM among women in Kebri Beyah district, May 2015

Variable	Frequency	%
Current FGM practice		
yes	542	87.4
No	78	12.6
Circumcision status		
Yes	483	77.9
No	137	22.1
Age at circumcision(years) (n=483)		
1-4	69	14.4
5-9	265	54.8
10-15	149	30.8
Type of circumcision		
Sunni	386	79.9
Pharaonic	97	20.1
Performed by		
Traditional birth attendants	224	50.3
Village women	312	36.1
Health professionals	67	10.8
Others	17	2.7
Type of instruments		
knife	152	24.5
Razor	395	63.7
Scissor	69	11.1
Others	4	0.6
Decision of FGM		
Father	153	24.7
Mother	435	70.2
Both	32	5.2

g) Attitude towards FGM

Majority of respondents, 418(67.4%) were agreed that FGM increase chance of marriage, 423(68.2%) were agreed FGM is religious requirement and should be maintained, 416(67.1%) were agreed FGM prevent premarital sex, 421(67.9%) were agreed uncircumcised women are out of social norms while 293(47.2%) were rejected women to actively participate pro FGM activities. Computing the attitude of study participants towards FGM, 410(66.1%) of respondents have negative attitudes, while 210(33.9%) have positive attitude toward FGM.

Table 7: Attitude towards FGM in Kebri Beyah district, May 2015

Variable	Frequency	%
FGM increase chance of marriage		
Strongly agree	281	45.3
Agree	137	22.1
Neutral	7	1.1
Disagree	62	10
Strongly disagree	133	21.5
FGM religious requirement		
Strongly agree	243	39.2
Agree	180	29
Neutral	9	1.5
Disagree	75	12.1
Strongly disagree	113	18.2
FGM prevent premarital sex		
Strongly agree	271	43.7
Agree	145	23.4
Neutral	9	1.5
Disagree	41	6.6
Strongly disagree	154	24.8

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Uncircumcised women are Out of social norm		
Strongly agree	243	39.2
Agree	178	28.7
Neutral	12	1.9
Disagree	44	7.1
Strongly disagree	143	23.1
Women should actively participate pro FGM activities		
Strongly agree	83	13.4
Agree	180	29
Neutral	64	10.3
Disagree	240	38.7
Strongly disagree	53	8.5
Attitude score		
Positive	210	33.9
Negative	410	66.1

h) Intention among women to stop FGM practice

Five hundred two 81% of study participants have changed their previous attitude, after received various information related to FGM. While 370(59.7%) of respondents were believed that circumcision make women physically clean and hygiene when compared to uncircumcised women. On the other hand, most of the respondents, 389(62.7%) have plan to circumcise their daughter in the future to maintain this practice. Majority study participants, 431(69.5%) have the intention to stop FGM practice. interestingly, 189(30.5%) of participants have intended to practice FGM in the future.

Table 8: Intention to stop FGM practice in Kebri Beyah district, May 2015

Variable	Frequency	%
Change of previous belief after received FGM awareness		
Yes	502	81
No	118	19
Circumcision make women physically clear and hygiene		
Yes	370	59.7
No	251	40.3
Experienced negative effect of FGM		
Yes	493	79.5
No	127	20.5
Perceived change of behavioral		
Great change	28	5.7
Low change	333	67.5
No change	132	26.7
plan to circumcise their daughter		
Yes	389	62.7
No	231	37.3

Regarding the negative health effect of FGM, 493(79.5%) of respondents were experienced negative health effects related to FGM. Among this, 333(67.5%) had developed low change of behavior to stop FGM, 117 (23.7%) indicated that they have no change, only, 28(5.7%), had developed great behavioral change and 15(3%) did not report their level of the behavior change after encountered the negative health effect of FGM.

i) Identified barriers of intention to stop FGM practice

One hundred forty four (23.2%) of the respondents reported that the barriers to stop FGM were related to low involvement of community in FGM prevention and controlling programs, followed by, 125(20.2) were due to presence of gender inequality, 104(16.8%) were fear of social stigma and pressure, 98(15.8%) were blamed due to lack of legal measure to stop FGM, 63(10.2%) were low involvement of stop FGM, 63(10.2%) were low involvement of community leaders, 61(9.8%) were low involvement of religious leaders to bring desired change of behavioral towards FGM practice in the local community.

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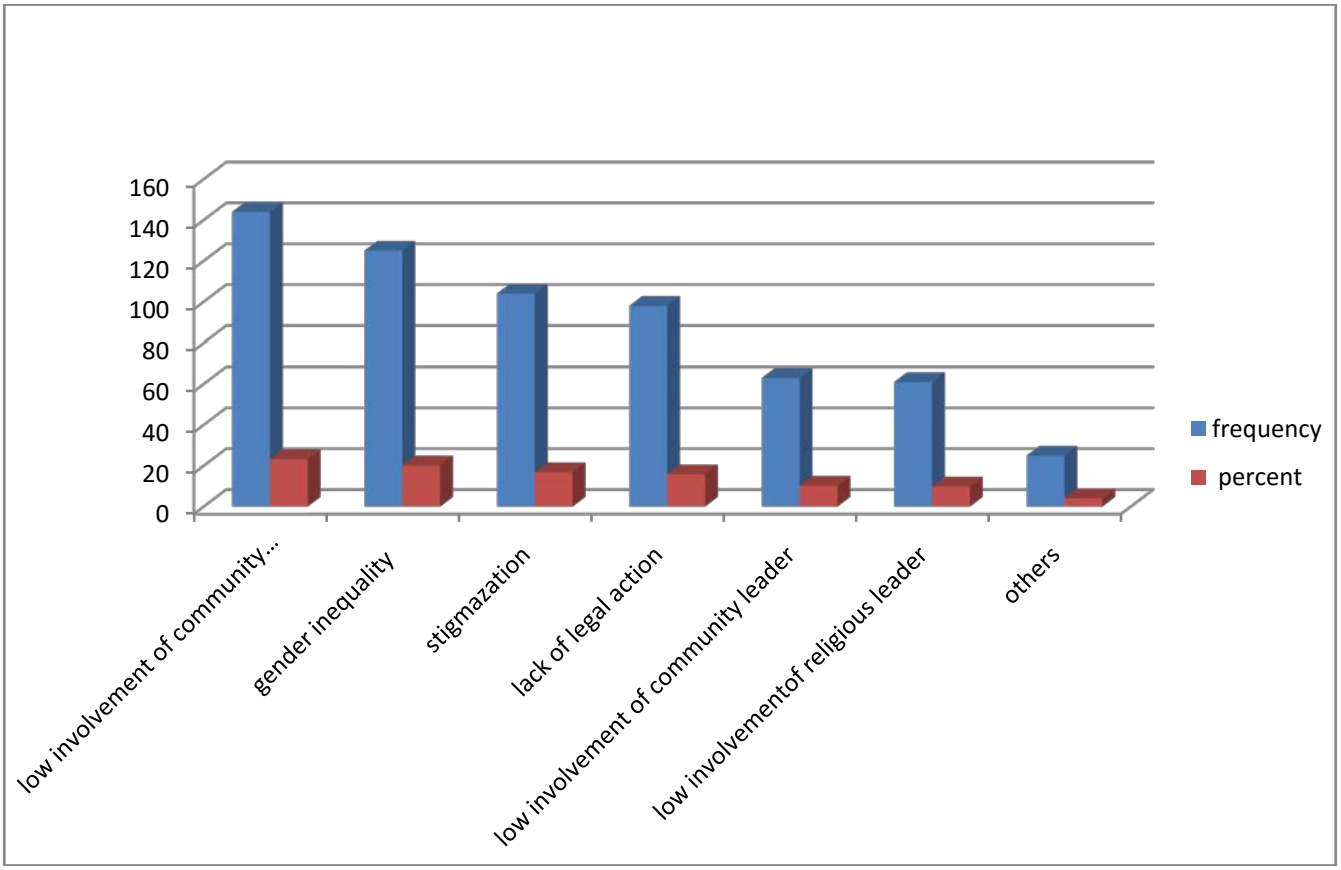


Figure 1: Identified barriers of intention to stop FGM in Kebri Beyah district, May 2015

The majority of the study participants were highly supporting the continuation of female circumcision either Sunni or pharaonic and intended to circumcise their daughters in the future to fulfill their obligation of being Muslim, to avoid shame and social stigma and to protect their family's dignity while some of them think that stopping FGM is foreign driven agenda.

"I believe, we inherited this practice from our mother's because it has its own importance and respect. Therefore, we have to maintain and transfer o next generation." (31 years married woman)

*"Uncircumcised girl can't control her sexual desire and maintain her virginity until she gets married compared to circumcised girl, if she lose her virginity, she will lose her chance of marriage."(*42years old married woman)

"The circumcision of both girls and boys are mandatory aspects. According to our sheria, parents are expected to circumcise their children. If not so they haven't fulfilled their obligation as being Muslim."(36years old married woman)

*"I have four daughters, I already circumcised the oldest one, and the remaining three are still too young to be circumcised but I plan to circumcise them in the future."(*27years old married woman)

*"There is strong traditional that existed for long period of time and if the government ban all forms of circumcision (Sunni and pharaonic) through legal means it may develop conflict and that people think that, government is against their religion and tradition and this may put the administration at risk."(*19years old unmarried woman)

Suggestions of means of bringing positive behavioral change

Majority of study participants are recommended; IEC efforts, religious leader mobilization, legal action, establishing Anti-FGM clubs, strengthening community participation, providing printed materials to rural area and women's active participation, while others, emphasize women's education to stop FGM practice.

"Religious leader should be mobilized and go to outreach to deliver messages relate to health effect of FGM and to correct the misunderstanding of that FGM is religious demanded, workshop should be designed to deliver information related to FGM prevention mechanisms to bring positive attitude to the community, establishing Anti-FGM committee consists of members of different sectors for instance; local administration, NGO, teachers, youth, women's origination, health care providers and religious leader and public discussion should be made to the community by using all forms of media channels (TV, radio and printed materials)." (19 years old unmarried woman)

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"Anti-FGM clubs should encouraged by providing technical, material as well as financial support, strengthening community participation for each and every activities designed to stop and bring positive behavioral change towards FGM practice, and effective and smooth working relation with religious leader and local administration should be created."(36years old community leader man)

"Legal measures are the only ways to stop FGM practice in our community because for the last five years different activities were conducted to create awareness about the negative health effect of FGM but some members of the community are still performing this practice therefore, those who are willing to FGM and those who are performing should be taken legal action in order to show this practice is legally prohibited."(29years old community leader man)

*"Education is the only powerful tool that can bring positive behavioral change towards FGM prevention because the education status of female contributes great to her attitude whether to practice or to stop FGM."(*23years old married woman)

j) Relations of socio-demographic variables and intention to stop FGM practice among women of reproductive age group

In binary logistic analysis, Somali ethnic group have less (COR = 0.61; 95% CI: 0.37, 0.99) intention to stop female FGM compared to those who were amhara and Oromo.

According to marital status, women who were single 1.69 times more intention to stop female genital cutting (COR = 1.69; 95% CI: 1.10, 2.6) compared those who were married. Illiterate women have lower odds of intention to stop female genital cutting (COR = 0.47; 95% CI: 0.33, 0.67) than those who were literate. Odds of intending to stop of FGC were also high among those who have radio at their home (COR = 1.62; 95% CI: 1.10, 2.34) compared to women who have not radio at home. While those who have television at home were 2.33 times more intention to stop FGM (COR = 2.33; 95% CI: 1.63, 3.33) compared to those have not television at home. Multivariate analysis of socio demographic variables in relation to intention to stop the female genital cutting showed that odds of intending to stop FGC was 0.62 times less likely among illiterate women compared to literate (AOR=0.62; 95% CI: 0.42, 0.91). Those who have television at home have 1.89 times more intention to stop FGM than those who have not television at home (AOR=1.89; 95% CI: 1.29, 2.77).

Table 9: Relations of socio-demographic variables and intention to stop FGM practice among women of reproductive age group in Kebri Beyah may 2015

Variable	Intention		Odd ratio	
	n=431	n=189	COR	AOR
Age group				
15-24	71(27.2%)	190(72.8%)	1.55(0.97-2.47)	1.25(0.76-2)
25-34	75(31%)	167(69%)	1.29(0.81-2.05)	1.22(0.75-1.9)
35-49	43(36.8%)	74(63.2%)	1	1
Religion				
Muslim	180(31.3%)	396(68.8%)	0.56((0.26-1.2)	0.86(0.33-2.23)
Non Muslim	9(20.5%)	35(79.5%)	1	1
Marital status				
Single	34(22.5%)	117(77.5%)	1.69(1.10-2.6)**	1.4(0.88-2.23)
Married	155(33%)	314(67%)	1	1
Ethnicity				
Somali	165(%)	348(%)	0.61(0.37-0.99)**	0.71(0.38-1.32)
Others	24(22.4%)	83(77.6%)	1	1
Occupational status				
Employed	15(23.8%)	48(76.2%)	1.45(0.79-2.66)	1.22(0.65-2.3)
Unemployed	174(31.2%)	383(68.8%)	1	1
Educational status				
Illiterate	128(37.3%)	215(62.7%)	0.47(0.33-0.67)	0.62(0.42-0.91)**
Literate	61(22%)	216(78%)	1	1
Radio				
Yes	129(27.9%)	334(72.1%)	1.62(1.1-2.3)**	0.75(0.5-1.13)
No	60(38.2%)	97(61.8%)	1	1
Television				
Yes	63(21.4%)	232(78.6%)	2.33(1.63-3.33)**	0.52(0.36-0.77)**
No	126(38.8%)	199(61.2%)	1	1

** =significance **CI**= confidence interval **COR**= crude odds ratio **AOR**= adjusted odds ratio

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k) Relation of socio demographic variables and knowledge of women towards FGM

Among socio demographic variables, marital status, education, having radio and television shows that association with knowledge towards FGM. It shows that respondents who were single were 0.36 times less likely to acquire good knowledge towards FGM compared to married women. Similarly being illiterate shows 3.13 times more likely to acquire good knowledge towards FGM compared to being literate. Participants who have radio were 0.55 times less likely to access good knowledge towards FGM compared to those who have not radio. Moreover those who have television 0.2 times less likely to acquire good knowledge towards FGM compared to those have not television. Being having television at household shows significant association in multiple logistic regressions; it shows those who have television at home 0.27 times less likely to access good knowledge towards FGM compared to those have not television at home (AOR=0.27, 95% CI (0.13-0.55)).

Table 10: Relation of socio demographic variables and knowledge of women in Kebri Beyah may 2015

Variable	Knowledge		Odd ratio	
	n=557	n=63	COR	AOR
Age group				
15-24	245(93.9%)	16(6.1%)	0.44(0.21-0.93)	0.61(0.28-1.33)
25-34	210(86.8%)	32(13.2%)	1(0.53-2)	1.14(0.57-2.26)
35-49	102(87.2%)	15(12.8))	1	1
Religion				
Muslim	514(89.2%)	62(10.8%)	5.18(0.7-38.3)	3(0.31-28.9)
Non Muslim	43(97.7%)	1(2.3%)	1	1
Marital status				
Single	144(95.4%)	7(4.6%)	0.36(0.16-0.8)**	0.54(0.23-1.27)
Married	413(88.1%)	56(11.9%)	1	1
Ethnicity				
Somali	455(88.7%)	58(11.3%)	2.6(1.02-6.64)	1.55(0.52-4.56)
Others	102(95.3%)	5(4.7%)	1	1
Occupational status				
Employed	61(96.8%)	2(3.2%)	0.26(0.64-1.12)	0.38(0.08-1.66)
Unemployed	496(89%)	15(12.8%)	1	1
Educational status				
Illiterate	294(85.7%)	49(14.3%)	3.13(1.7-5.8)**	1.9(0.99-3.67)
Literate	263(94.9%)	14(5.1%)	1	1
Radio				
Yes	423(91.4%)	40(8.6%)	0.55(0.31-0.95)**	0.78(0.43-1.4)
No	134(85.4%)	23(14.6%)	1	1
Television				
Yes	284(96.3%)	11(3.7%)	0.2(0.1-0.4)**	0.27(0.13-0.55)**
No	273(84%)	52(16%)	1	1

** =significance **CI**= confidence interval **COR**= crude odds ratio **AOR**= adjusted odds ratio

l) Relation of socio demographic variables and attitude of women towards FGM

Among the socio demographic variables, only ethnicity shows a weak association in binary logistic regression. It shows that Somali respondents were 1.53 times more likely to have negative attitude towards FGM compared to Oromo and Amhara ethnic group. And the socio demographic variables did not show significant association with attitude of respondents under multiple logistic regressions.

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Table 11: Relation of socio demographic variables and attitude of women towards FGM in Kebri Beyah may 2015

Variable	Attitude		Odd ratio	
	n=410	n=210	COR	AOR
Age group				
15-24	95(36.4%)	166(63.6%)	0.84(0.53-1.33)	0.93(0.57-1.5)
25-34	77(31.8%)	165(68.2%)	1(0.64-1.65)	1(0.65-1.69)
35-49	38(32.5%)	79(67.5%)	1	1
Religion				
Muslim	191(33.2%)	385(66.8%)	1.53(0.82-2.85)	1.1(0.5-2.3)
Non Muslim	19(43.2%)	25(56.8%)	1	1
Marital status				
Single	59(39.1%)	92(60.9%)	0.74(0.5-1.58)	0.8(0.53-1.2)
Married	151(32.2%)	318(67.8%)	1	1
Ethnicity				
Somali	165(32.2%)	348(67.8%)	1.53(1.11-2.3)**	1.41(0.83-2.4)
Others	45(42.1%)	62(57.9%)	1	1
Occupational status				
Employed	23(36.5%)	40(63.5%)	0.88(0.51-1.51)	0.93(0.54-1.62)
Unemployed	187(33.56%)	370(66.4%)	1	1
Educational status				
Illiterate	113(32.9%)	230(67.1%)	1.1(0.73-1.53)	1(0.69-1.43)
Literate	97(35%)	180(65%)	1	
Radio				
Yes	155(33.5%)	308(66.5%)	1(0.73-1.56)	1.11(0.74-1.64)
No	55(35%)	102(65%)	1	1
Television				
Yes	105(35.6%)	190(64.4%)	0.86(0.62-1.2)	0.87(0.61-1.25)
No	105(32.3%)	220(67.7%)	1	1

** =significance **CI**= confidence interval **COR**= crude odds ratio **AOR**= adjusted odds ratio

VI. Discussion

This community based-cross sectional study has attempted to identify the barriers of behavioral change to stop FGM practice and their associated factors among women of reproductive age in Kebri Beyah district.

Our study finding showed that 69.5% of the respondents had intention to stop FGC practice in the future. This finding is consistency with other findings although the figure greater than these findings [5]. These might be due to adequate knowledge about negative health effect of FGM among women in our study. Our study findings, is also comparable to study from Tanzania revealed that 76% of the circumcised women were in favor of not performing FGM on their daughters, while 24% did, another study in Guinea revealed that, support for the continuation of FGM was significantly higher among women 68% than among men 51%, indicating more attitudinal support for FGM discontinuation among men than women [61, 62].

The present finding shows that 89.8% of respondents have good knowledge towards FGM, while 10.2% of the women have poor knowledge about FGM. This finding is comparable with a study done in Addis Ababa which was 92% of women had good knowledge. On the other hand a study done in Somalia revealed that, about 66.9% of women had good knowledge on the effects of FGM [50, 56]. In contrast to our current findings, a study done in northwest Ethiopia shows that 46.2% of women had good knowledge about the ill health effect of FGM and 53.8% of the mothers had poor knowledge about the ill health effect of FGM [57]. This discrepancy might be due to the difference of the operational definition of the studies.

In our current study finding shows that, the most health effect more than 80.2% of respondents reported, FGM cause excessive bleeding, while 76.6%, difficult of lab our in addition to this infection, painful menstruation and painful sexual contact were also reported as negative effect of FGM. Similar study done in Somalia showed that, infection 60%, bleeding 20%, and 68% difficult of labour to be the main ill effect of FGM [53]. This is consistency with our study finding.

The current study revealed that, Attitude of women towards FGM practice was 28.5% have positive/favorable attitude towards FGM practice meaning less than half of them believe to continue FGM practice among their daughters and 68.5% have negative/unfavorable attitude towards FGM this implies that majority of them believe to discourage FGM practice. Similarly a study done in eastern Ethiopia shows that, 47.9% of women have positive/favorable attitude, while 52.1% of women have unfavorable attitude against FGM practice [57]. This discrepancy

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might be due to combination efforts from different stalk holders against FGM in this region.

The present study revealed that 87.4% of women reported FGC was largely practiced in the study area. This finding is comparable with the other finding in Ethiopia which reported the prevalence of FGC among the women to be 98%, this shows the prevalence of FGC were still high in this area [49].

In our study findings, the type of FGM most commonly practiced was clitoridectomy (Sunni type), and a few women were also subjected infibulations (pharaonic) type, which is the most severe form of FGM. Our finding is similar with another study that revealed; almost half of all victims of FGM in Ethiopia had undergone clitoridectomy. Nationwide, 6% of females affected by FGM have undergone infibulation and more than 80% of women in the Somali region have been victims of the most severe form of FGM [19]. These findings are inconsistency with our study finding which shows, 20.1% had undergone the severe form of FGM (pharaonic type).These might be due to current anti-FGM intervention carried out in the area.

About 77.9% of interviewed women were subjected to FGM, almost all undergone the procedure before the age of 10years. FGM initiated at age between 5-9yaers and this finding is comparable to other surveys that have found 90% of girls who have undergone FGM aged 5–14 years, although practices vary from country to country, FGM are generally done among girls younger than 10 years. When subjected to the procedure, and another study shows that, half of cutting in Ethiopia, Mali, and Mauritania were initiated before age of 5 years, whereas in Yemen about 76% of cutting were started at not more than two weeks of age[1].

In this study, more than half FGM procedures were performed by traditional birth attendants, mostly by using unhygienic procedures in the community, this increasing the risk of infection and later reproductive complications in women undergo FGM. And out present findings are comparable to the Ethiopia DHS 2000 report; more than 92% of the practices were performed by traditional circumcisers [6]. On the other hand, in some countries, medical personnel, including doctors, nurses, and certified midwives perform FGM under anesthesia in health care facilities, even though it is forbidden and subject to prosecution in the west. The highest rate of use of medical personnel to perform FGM can be found in Egypt (61%), Kenya (34%), and Sudan (36%), with rates of 9% and 13%, respectively [49]. These findings were inconsistency with our present study findings.

Our current study shows that about 63.7% of the procedure was performed by using blade razor. This finding is consistency with other findings shows that, the cutting is mostly done with razor blades but some continue to use knives in the country side as in the old days when razors were not available in Somali region [22].

The present study found that religious requirement 50.1% were the most common reasons for FGC practice among the study participants. FGM is performed for various reasons, including preventing women from hyperactivity in sexual practice and early initiation of sexual intercourse before marriage. This finding is in line with other findings were reported by 30% of Kenyan women who supported this practice to ensure virginity. Similarly, more than half of Egyptian women believed that FGM would prevent adultery and that it is proof of a girl's virginity and perceived that it improves marriage prospects for unmarried girls in Nigeria. This shows that traditional and religious reasons for practicing FGM are also widely accepted by females in the societies in different regions. However, the association between religion and FGM needs further research in Ethiopia. It is quite evident that the perception and acceptance of harmful traditional practices, including FGM, is widespread across all regions, regardless of religious practices [19, 53].

In this study about 37.1% of respondents perceived that FGM exposes a woman for HIV. Our finding is inconsistency with a study done in Hargeisa district, Somalia, revealed that 88.8% of the respondents were aware of the possibility of HIV transmission [22]. This discrepancy might be considered only women as our study subjects. Furthermore, there might be variation in accessing sources of information among these study subjects.

Many women still belief that the chance of uncircumcised women to be married is very low and they are directly or indirectly forced to circumcise their daughters or support the practice. In a traditional society like Ethiopia or Somalia, marital decisions are mainly made by men or by the parent of the girl. In such society, men prefer to marry circumcised women and mothers worry about their daughters thinking that an uncircumcised girl would not be married and becoming less attractive to men in terms of intactness, similarly a study in Eastern Ethiopia depicted the preference of men to be married to circumcised woman, this suggests that it would be very easy for women to abandon any type of FGM, if the husbands do not expect it, indicating the importance of involving men in anti-FGM campaigns. Although men generally prefer to marry women who have undergone FGM, there are studies in some settings that have shown the preference of men to marry women who have not undergone the procedure [51].

Our study also attempted to ascertain women's feelings after experience serious outcomes of FGM which many women have faced various problems through out there life. 79.5% of women who had experienced the negative health effect of FGM, among these women, 67.5% of them developed low behavioral

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change while others approached it as a normal phenomenon and none had attempted to stop the practice in their community. Although, most of women had adequate knowledge about the potentially serious reproductive outcomes of FGM and this finding is comparable to other studies [46, 68].

In this study finding health institution were the preferred setting of communication and drama was the commonest means of delivering FGM related information and this finding is comparable to a study done eastern Ethiopia shows that Anti- FGM committee, health institutions and training on anti-FGM activities were mentioned as the major sources of information about possible immediate and long-term risks of health complications associated with FGM. Another study done in Nigeria also showed that exposure to multimedia campaigns had a significant impact on changing attitudes and promoting the intention to stop FGM, another study also revealed that radio and visual education material were the preferred channels to deliver information related to FGM to bring positive behavioral change [26, 63].

The identified barriers were lack of community participation in Anti FGM activities. Although various organizations are operating in this area, the community members were not incorporated the interventional activities to end FGM practice. In addition to this, lack of encouragement and commitment of community leaders inappropriate, IEC work, social pressure and stigmazation associated with uncircumcised women for considering that they are not eligible for marriage, Gender inequality, because women alone can't take action against FGM, due to their limited scope and dependence to their husbands. This finding is inconsistency with a study done in Somalia that show community member were given first priority, "community can create suitable solution for their own problem" and this might bring positive intention towards FGM practice [26].

The current finding shows that, socio demographic variables of this study have not shown significant association with attitude of women towards FGM practice. On the other hand our study findings indicate, among the socio-demographic variables, educational status and having television at home were significantly associated with intention of stopping FGM. Nowadays television became the most powerful tools for communication but most of this community has not television at household level to access information related to FGM and this also have significant association with knowledge of participants. While other studies also showed that the level of education of women has a decisive role on the practice of FGM [51, 59]. This indicates that targeting the education of women is important in this population.

VII. Strengths and Limitations of Study

Strengths

Use of both quantitative and qualitative methods of data collection

Gives baseline information for further study

Limitations

Bias related to social desirability; since the study is self reporting, there is more likelihood of the participants to give culturally acceptable answer.

Lack of standardized questionnaire related to this specific topic.

VIII. Conclusion

The findings of the current study have indicated that intention of women to stop FGM can be influenced by some socio demographic characteristics like marital status and having television at household to access information related to FGM.

This study shows prevalence of FGM is still high in KEBRI BEYAH district although most of study participants have positive intention to stop FGM practice for the future.

In this study most of the respondents justified for the continuation of this practice as religion demand and custom. In addition to this some of them circumcise their daughter to avoid social pressure and stigma. And practice can disappear if the current interventional activities directed towards the alleviation of stigmazation.

This study shows that majority of respondents have good knowledge and negative attitude towards FGM practice. And these are good indicators to bring positive behavior, while conclude education and ethnicity of participants were inversely associated the intention of women to stop FGM in the future and this indicates the importance of women education to end FGM.

We conclude the findings of this study mass media was the major sources of information pertaining to end FGM particularly radio was cited as the commonest source of information used bring positive behavioral change towards FGM practice likewise previous studies and health institutions and religion organization were most preferred settings to achieve the desired behavioral change to stop female circumcision while Drama and group discussion were considered to be the best means of communicating FGM messages to bring positive behavior, on the other hand, IEC activities were criticized for not addressing rural area.

Lack of community member participation in community based anti FGM interventional programs, lack of community leader commitment, Inappropriate IEC work and stigmazation as well as social pressure to uncircumcised women were considered to be the major

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challenges to bring the desired intention to stop FGM practice in this community.

IX. Recommendation

- Anti- FGM interventions should be directed towards the alleviation of stigma at the community level and encouraged by providing technical, materials and financial at community level.
- Religious leaders and community leaders should play significant role in the process of changing the behavior of the entire community by arranging training, workshops, media campaign and outreach to bring desired behavioral change to stop female circumcision.
- Community members should actively take part community based program that are planned to end FGM.
- The government should have to ensure the appropriateness and effectiveness of EIC strategy, which is vital in removing the barriers to bring positive behavioral change towards the elimination of FGM among the population
- The local administration should have to make continued effort to bring positive behavioral change against FGC practice and encouraging the existing negative attitude, hence sustaining concrete information and incorporating the suggestion given by the respondents which possibly enhance the activities of FGC prevention, since solution or change must come from within community.
- The local organizations should encourage the desired change of intention towards FGM practice by establishing anti-FGM clubs in rural area.
- Further research is recommended to explore the religious aspects to bring positive behavioral change towards FGM practice.

Acknowledgement

First and foremost, I would like to express my deepest gratitude to my advisors Dr. Mirgissa Kaba and Mr. Mulugeta Tamire for their unreserved supports and guidance during the whole process of my study.

I would also like to extend my deepest thanks to all my colleagues and experts from different institutions that helped me in providing valuable information and supports to undertake my study and also the ADDIS ABABA university libraries staff for providing me relevant literatures.

I would like to acknowledge the Somali regional health office, KEBRI BEYAH administration office and respective offices of the selected kebeles deserve my appreciation for letting me undertake the study in the district and facilitating everything for smooth implementation of the study.

Last but not the least I would like to pass my appreciation to supervision, interviewer and the entire study respondents as well as focus group discussants for their full participation on this study.

List of Abbreviations

AAU: Addis Ababa University
BCC: Behavioral change communication
CI: Confidence interval
CSA: Central statistical agency
CSO: Civil service organization
SPH: School of Public Health
EDHS: Ethiopian Demographic and Health survey
FGM/C: Female Genital Mutilation/Cutting
FGD: Focus group discussion
IEC: Information, Education and Communication
MDGs: Millennium Development Goals
OWDA: ogaden welfare and development association
WHO: World Health Organization

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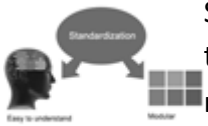


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The board can also take up the additional allied activities for betterment after our consultation.

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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Complete support for both authors and co-author is provided.

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Based on potential and nature, the manuscript can be categorized under the following heads:

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Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

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(a)Title should be relevant and commensurate with the theme of the paper.

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(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

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(h) Brief Acknowledgements.

(i) References in the proper form.

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Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

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- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

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Acknowledgements: Please make these as concise as possible.

References

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1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

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15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.



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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

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Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

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· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

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Abstract:

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An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

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The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
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Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.



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Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
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- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896



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