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TABLE OF CONTENT	Page
EDITORIAL	
Call for Research on Health Impacts of Climate Change in Developing Countries Gulrez Shah Azhar	1-3
ORIGINAL ARTICLE	
KAB Study on Leprosy among General Population Sane Roja Renuka, V.S. Harsha Rupak, G. Sukanya, N.R. Vignesh	4-8
Analysis of Design of Free Online Animations in Biochemistry based on Multimedia Learning Principles Ravi Kishore P	9-12
Knowledge about Smoking and Second Hand Smoke among the Male Adults of Abuja, Nigeria Mohammad Umair Siddiqui, Syeda Saiqa	13-19
Evaluation of Availability of Online Animations to Facilitate Visual Learning In Genetics for MBBS 1st Year Students Ravi Kishore P	20-25
Level of Support for Comprehensive Tobacco Measures among the Male Adults Aged 15-49 Years in Abuja, Nigeria Syeda Saiqa, Mohammad Umair Siddiqui	26-31
Cardiovascular Disease Risk Assessment in Type 2 Diabetes: Role of Myeloperoxidase and Apolipoprotein B Mridula Mahindrakar, Shruthi C N, P. Suresh Babu	32-37
Prevalence of Salmonella Typhi (S. Typhi) Carrier State and Altered P53 Gene Expression in Chronic Cholecystitis & Cholelithiasis Patients in Eastern India J Soumik Ghosh, Kalidas Biswas, Atrayee Chatterjee	38-44

CASE REPORT

- A Case Report of Tuberous Sclerosis Complex 45-48
Balakrishna Teli, Sneha Biradar
- A Rare Case of Perineal Leiomyoma in a Postmenopausal Woman 49-51
Radha, Arshad Ikram



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CALL FOR RESEARCH ON HEALTH IMPACTS OF CLIMATE CHANGE IN DEVELOPING COUNTRIES

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With increasing temperatures linked to climate change, the adverse health consequences of heatwaves are likely to worsen across the globe. Climate experts predict that average temperatures will increase by 2°C by the end of the 21st century under all (4.5, 6.0 and 8.5) but one (2.6) Representative Concentration Pathways (RCPs). RCPs are greenhouse gas concentration pathways (total radiative forcing) representing a broad range of climate outcomes. Each RCP has a different set of underlying assumptions and could "result from different combinations of economic, technological, demographic, policy, and institutional futures".[1] It is virtually certain that there will be more frequent hot and fewer cold daily temperature extremes over most land areas as global mean surface temperatures increase. Heatwaves also are very likely to continue increasing in frequency, intensity and duration. The direct consequences of this combination of factors are increased morbidity and deaths. According to the Intergovernmental Panel on Climate Change (IPCC) report, there is a virtual certainty of more frequent hot and fewer cold temperature extremes over most land areas on daily and seasonal timescales as global mean surface temperature increases. Most directly,

heatwaves are very likely to continue increasing in frequency, intensity, duration, and by extension, result in greater morbidity and death. Secondary large-scale effects are also likely, including increased migration, urbanization and food insecurity. [1]

In countries with adequate reporting systems, heatwaves have been documented to have caused a large number of deaths. The 2003 European heatwave killed in excess of 70,000 people over the summer, and the 2010 Russian heatwave and wildfires killed 56,000. North America and California have also faced several similar severe events with many lives lost. [2] While heatwave events have been recorded since historical times, lately they have been on the increase globally. The impact of these events is likely higher in developing countries such as India given a combination of increasing temperatures from climate change and higher population vulnerability.

Though there has been some work in the western settings, many research gaps remain regarding the scale of the problem, the areas impacted and heat adaptation measures especially in developing countries. There are sound arguments that heatwave deaths in tropical developing country settings tend

to be underreported and there is a high likelihood that such mortalities are likely to increase in the future. While many estimations of future deaths have been done in the US and other developed countries, "there are very few or no available projections of future mortality for populations in Africa, the Middle East, South America, and much of northern, central and southern Asia".[3] The case is the same for India, a hot tropical developing country, where a fifth of this planet's population resides.

Loss of lives during a heatwave period is caused by both direct (heatstroke) and indirect effects (exacerbation of preexisting conditions) with the latter being far more common than the former. A review of pathophysiological pathways links at least 27 such pathways linking heatwaves to deaths.

From the limited studies we have, Low and Middle-Income Countries (LMICs) such as India are likely to suffer an extraordinarily high burden of deaths. This is due to tropical locations, high population, dense urbanization, resource shortages, endemic poverty, poor human development and systemic low response preparedness capacity. Unfortunately, many of these countries lack the resources and empirical evidence and are therefore quite underprepared to face these future environmental and health challenges and consequent societal effects.

However, neither reliable national-level data is available nor are the future estimates of this problem. And the reported heatwave deaths seem to be gross underestimates. For example, in the May 2010 heatwave that struck the northern part of the country, newspapers reported "dozens" of deaths. A study of municipal death certificates during that time, however, revealed 1344 excess deaths in that

month just in the city of Ahmedabad.[4] In absence of estimates heatwaves often aren't recognized for their disaster potential.

The changing climate and poor socio-economic conditions increase heatwave vulnerability not just individually but may also have an additive effect, potentiating individual responses. Similarly, their impacts may not be uniform across the country. Various communities are at different risk levels depending on a variety of factors such as their demographic composition, neighborhood vegetation, health status, household amenities etc.[5]

Where evidence is available, heatwave adaptation strategies have been documented to be effective. Simple preparedness strategies and activities like awareness, early warnings and communication have been shown to dramatically lower heat-related deaths. They have also been shown to be extremely cost-effective in western settings. Researchers in Philadelphia found that the cost of running a heatwave warning system for Philadelphia was practically at the "noise" level compared to the economic benefits of saving hundreds of lives.[6] However, there is little evidence of the applicability of suitable adaptation strategies in developing country settings or of their cost-effectiveness.

At the same time, many standard adaptation strategies in the West may not be suitable for India or other developing countries. While there is some evidence of effective adaptation strategies from the west, the applicability of those in the developing world hasn't been studied as much. For example, researchers attribute the entire decline in US heatwave deaths to the adoption of residential air conditioning.[7] This strategy isn't possible in a country with irregular power supply where many do

not have electricity and where most are too poor to be able to afford the costs. Thus, it is essential to also look for local adaptation solutions. These possibly include a whole gamut of options – from lifestyle and behavioral changes to modifications in building designs, water storage, food, clothing, and other local technological innovations.

We urge the research community to focus their efforts on this deadly specter of heat deaths and illnesses.

(This is an abridged version of Chapter 1 from Indian Summer: Three Essays on Heatwave Vulnerability, Estimation, and Adaptation by Gulrez Shah Azhar. Link: https://www.rand.org/pubs/rgs_dissertations/RGSD431.html)

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KAB STUDY ON LEPROSY AMONG GENERAL POPULATION

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ABSTRACT

Background: Leprosy is often referred to as one of the oldest disease known to man. Of all the communicable diseases, leprosy has the most potential to cause progressive and permanent physical disability. This study was undertaken to assess the Knowledge, Attitude and Behaviour (KAB) towards leprosy among general population.

Material and Methods: A cross sectional survey was done among the general population visiting DVL OPD at Sree Balaji medical college and hospital using self reported questionnaire after obtaining the approval of the Institutional Ethics Committee of the Medical College. A sample size of 100 was taken. Data entry was done in SPSS 22.0 version.

Results: It was found that knowledge on leprosy was unfavourable, more than 65% didn't know the cause of leprosy and 75% didn't know about the treatment of leprosy. Regarding attitude most didn't want to shake hands (59%) or share food (63%) with a leprosy patient. Regarding behaviour most felt sympathetic (81%) on seeing a leprosy patient.

Conclusion: This study result shows that the knowledge and attitude on leprosy were unfavorable. In spite of the existence of the National Programme for Leprosy since 1955 the awareness and attitude on leprosy is inadequate. There would be steady improvement in the knowledge and attitude of the population through behaviour change communication (BCC) programs in the community.

Key words: Leprosy, Knowledge, Attitude, Stigma.

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INTRODUCTION:

Leprosy is often referred to as one of the oldest disease known to man. Of all the communicable diseases, leprosy has the most potential to cause progressive and permanent physical disability. In addition the disease and visible deformities there is intense social stigma and social discrimination of the patients^[1]. Rejection, exclusion, blame or devaluation that results from experience, perception or reasonable anticipation of an adverse social judgment about a person or group are the stigmas of leprosy. Based on old religious

traditions leprosy sufferers were isolated to keep them out of sight.

MATERIAL AND METHODS:

A cross sectional survey was done among the general population visiting Dermatology Venereology and Leprosy outpatient department at Sree Balaji medical college and hospital from January to February 2021. Individuals who were between 18 to 60 years of age were included in this study. People who are not willing to participate were excluded.

Study Design: Cross sectional study
 Study Area: Skin Outpatient Department, Sree Balaji Medical College and Hospital
 Study Population: General population
 Study Setting: General population coming to OPD of SBMCH, a tertiary care hospital, No 7, Works road, Chrompet, Chennai, Tamilnadu.
 Study Sample: Sample size of 100 was taken.
 Sample size calculation:
 $N = (Z^2 (1-p) / d^2)$
 $Z = 1.96 =$ Level of confidence i.e
 $p =$ proportion of outcome i.e., 14%
 $q = 100 - p$
 $d =$ precision i.e.12%
 Sample Size: 100

Type of sampling: Purposive sampling
 Data collection method: Using self reported questionnaire. Questionnaire was designed in a manner that the general population could understand and was distributed randomly irrespective of gender.
 Data entry was done on MS Excel and data analysis in SPSS 22.0 version.

RESULTS:

Table 1: Knowledge about Leprosy

Question	Know %	Don't Know %
Do you know the cause of leprosy?	36%	64%
Does leprosy spread from person to person?	71	29
Is leprosy curable?	69	31
Do you know any relative with leprosy?	29	71
Do you know any leprosy centre in your area?	19	81
Do you know about the treatment of leprosy?	25	75

Table 1 shows the knowledge of Leprosy. About 64% didn't know the cause of leprosy. Nearly 71% knew that leprosy can spread (Fig.1). About 69% knew it was curable. Nearly 81% were not aware of any nearby leprosy centres. About 75% didn't know about the treatment of leprosy (Fig.2).

Table 2 a: Attitude towards Leprosy

Question	Normal	Afraid	Sympathetic
How do you feel on seeing a leprosy patient?	47%	38%	15%
How do you feel when a family member is affected with leprosy?	-	19%	81%

Regarding attitude 47% felt normal seeing a leprosy patient. When a family member was affected by leprosy 85% felt sympathetic. (Table 2a)

Table 2 b: Attitude towards Leprosy

Question	Yes %	No%
Do you feel ashamed to tell others that your family member has leprosy?	21	79
Will you shake hands with a leprosy patient?	41	59
Will you share food from the same plate with a treated leprosy patient?	37	63
Will you think that it is difficult for a leprosy patients to get married?	54	46
Will you give job to a treated leprosy patient?	81	19
Will you panic when a leprosy patient approaches you?	31	69
Do you think that leprosy patients should be treated with more compassion?	88	12
Can you imagine yourself working with a leprosy patient?	83	17

71% felt ashamed of tell others that their family member has leprosy. 59% didn't want to shake hands with leprosy patients. 63% didn't want to share food from the same plate of leprosy patient.81% felt that they would give jobs to a treated leprosy patient.69% said they would not panic when a leprosy patient approached them. 83% were ready to work with a leprosy patient. (Table 2b, Fig.3)

Table 3: Practice in Leprosy

Question	Yes %	No%
Do you wear any gloves while handling leprosy patients or the object used by them?	76	24
Do you think leprosy patient requires counselling?	73	27
Do you think leprosy patients must be isolated from normal people?	23	77
Do you think leprosy is associated with shame?	22	78
Do you think leprosy patient requires special footwear to prevent from ulcers?	65	35

Regarding practice 76% wore gloves while handling the patients and 73% believed that leprosy patients require counseling. About 77% said no for isolating leprosy patients. Nearly 78% felt that leprosy is not associated with shame and 65% knew that leprosy patients require special footwear to prevent from ulcer. (Table 3)



Fig. 1: Knowledge on spread of Leprosy

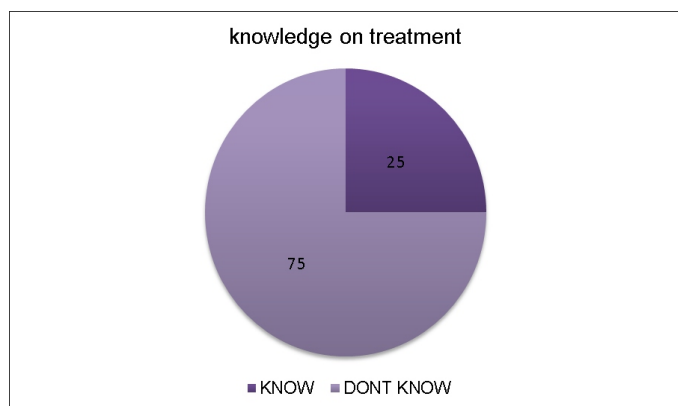


Fig. 2: Knowledge on treatment of Leprosy

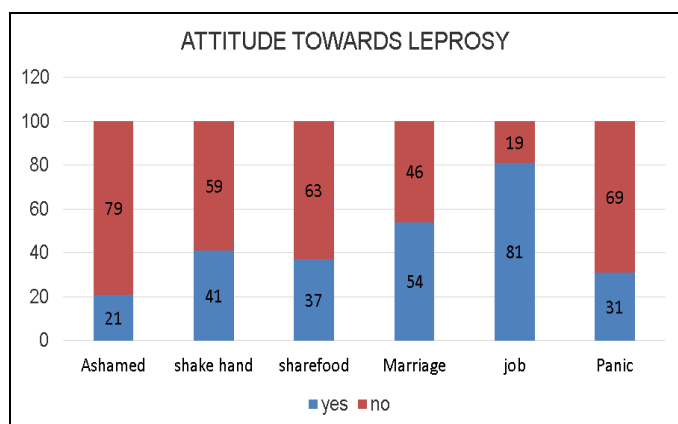


Fig. 3: Attitude towards Leprosy

DISCUSSION:

The causative organism of Leprosy is Mycobacterium leprae. The mode of transmission is usually through droplet infection. It primarily affects skin and nerves^[2]. The clinical spectrum varies from very mild and limited cutaneous disease to very severe one with extensive nerve and systemic involvement. In severe forms of the disease there is various mutilating deformities and disabilities with disability index as high as 55%^[3]. The infection must be treated at initial stages if left untreated it may progress slowly to its severe and complicated form. Therefore, early recognition and prompt therapeutic intervention is prudent to halt the disease at its initial stages and spare the patients from gruesome complications and resultant prejudice of late disease. The social prejudice of leprosy is as grave as its morbid complications like bony deformities^[4].

Knowledge about Leprosy:

This study showed that nearly 45% of respondents had inadequate knowledge of leprosy. A similar finding was reported by a study conducted by Raju MS *et al.*, where 35%–50% of the participants were noted to have adequate knowledge on leprosy, but the attitude was more in negative.^[5] A study done in Eastern Ethiopia found that 56.91% had high knowledge of leprosy.^[6] A study conducted by Mohite RV *et al.* in a rural community in Karad reported that nearly 79% of respondents had good knowledge and 69% had a positive attitude towards leprosy.^[7] Majority of the people (64%) did not know about the causal agent of leprosy and only a few (36%) knew about it. A similar finding was noted in the study by Tesema AA *et al.* where 48.3% reported that leprosy is caused due to bacteria.^[4] A study carried out in Mexico reported 60% of the study participants had the knowledge on the cause of leprosy.^[8] In the study by Mohite RV *et al.*, nearly 66% of respondents had knowledge on the cause of leprosy. Stephen T *et al.* in their study in Tamil Nadu noted that 32% of leprosy patients and 37% of their family members were aware of the cause of leprosy.^[9] Danturty I *et al.* in a study carried out in Secunderabad reported that 44% of the participants knew the cause of leprosy.^[10]

Similar findings were noted in studies carried out in Uttar Pradesh and in Nepal.^{[11],[12]} Regarding the spread of leprosy 71% of people were aware that they spread from one to another. Knowledge about mode of transmission and of any infectious is important in its control and prevention. 67% believes that leprosy is curable but only 25% of people are aware about the treatment of leprosy. In the study carried out in Secunderabad, only 29% of the respondents were aware that leprosy is treatable and curable.^[13] In the Ethiopian study, a higher proportion of the study subjects (93%) responded that leprosy is treatable with modern drugs.^[14] Whereas, the study carried out in Mexico reported that only 31% were aware that leprosy is treatable.^[15] Majority of people were unaware about the multi drug therapy in treating leprosy.

Attitude towards Leprosy:

Regarding attitude, majority of the people felt normal (47%) on seeing a leprosy patient and 81% felt sympathetic when they had a family member with leprosy. 63% were not willing to share food from the same plate, in a similar study in western rajasthan 39% of the people were not comfortable with idea of sharing food with a leprosy patient. and 59% did not want to shake hands with a leprosy patient. 54% of people feel that it is difficult for leprosy patient to get married. 69% did not panic when a leprosy patient approaches them and 89% of people felt that leprosy patients should be treated with compassion. In a study in Karachi More than 90% of respondent mentioned that they will not work with lepers, do not share food or give job to a leper. The various stigmas towards leprosy were avoidance of contact with a diseased person, not sharing personal belongings, refusal to allow children to mingle with the patients' children, or sitting next to the patient. They expressed negative feelings in the form of fear, hopeless reaction, or shame if they were diagnosed with leprosy. These factors could in turn contribute to the cover-up of the symptoms/signs of the disease. Similar findings were noted among the studies carried out by Singh R *et al.*, Adhikari *et al.* and Hejinders ML *et al.*^{[16][17][18]} Owing to these stigmas, leprosy patients tend to suffer from psychosocial problems such as depression and anxiety.^[19]

Practices in Leprosy:

Regarding practice in leprosy, 76 % wears gloves while handling objects used by leprosy patients similar study by Adhikari et al only 50% wear gloves while handling objects^[17]. 73% of people felt leprosy patients require counseling. 77 % people felt that there was no need for a leprosy patient to be isolated from normal people. 65% thinks that leprosy patients require special footwear to prevent form ulcers.

CONCLUSION:

This study shows that the knowledge and attitude on leprosy were unfavorable. In spite of the existence of the National Programme for Leprosy since 1955 the awareness and attitude on leprosy is inadequate. There would be steady improvement in the knowledge and attitude of the population through behaviour change communication (BCC) programs in the community. These programs should be redesigned with the involvement of the community, social activists, local leaders, local family physicians, primary health care providers, and the print and visual media. Educating the population on emphasizing early diagnosis and prompt treatment of leprosy as per the standard treatment guidelines based on NLEP needs to be carried out both in rural and urban communities.

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ANALYSIS OF DESIGN OF FREE ONLINE ANIMATIONS IN BIOCHEMISTRY BASED ON MULTIMEDIA LEARNING PRINCIPLES

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ABSTRACT

Background : MBBS Ist year students are expected to learn concepts in Biochemistry that helps them understand the principles behind the diagnosis and management of diseases. Medical students have been shown to access free online animations to aid learning. Richard E Mayer proposed multimedia learning principles that could be used to design animations to maximise learning. To the best of our knowledge, there are no studies that have evaluated the design of online freely available animations based on Mayer's multimedia learning principles that foster generative processing. In this study, the objective was to evaluate the online animations in Biochemistry based on criteria of multimedia learning principles (that foster generative processing) proposed by Mayer.

Material and Methods : A total of 102 animations belonging to various topics in Biochemistry relevant to MBBS Ist year students, as per the curriculum suggested by National Medical Commission(NMC) were shortlisted through online search using www.google.com and www.youtube.com. These animations were then scored based on their compliance with multimedia learning principles that foster generative processing.

Results : Shortlisted animations had a median score of 10 (interquartile range=0). Only 4% of animations had a score of 15 (maximum score =15). In contrast, 96% of animations had score of 10. Personalisation principle was violated in 96% of animations. Image and multimedia principles were complied with in 100% of the animations.

Conclusions : Majority of online free animations in Biochemistry that we assessed do not comply with all the design principles of multimedia learning that foster generative processing. Creators of online animations should use the Mayer's multimedia learning principles that encourage generative processing, while designing the animations in order to maximise their potential for learning by MBBS students.

Keywords : Mayer's principles, Biochemistry animations, MBBS students, Medical Students

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INTRODUCTION

MBBS Ist year students are expected to learn complex molecular concepts in Biochemistry that helps them understand the scientific basis behind the diagnosis and management of diseases [1]. Animations on Biochemistry topics are freely accessible through popular search engines like www.google.com and www.youtube.com. Online animations are accessed by MBBS Ist year

students in order to learn complex concepts in Biochemistry [2]. Based on Cognitive theory of multimedia learning, Richard E Mayer proposed multimedia learning principles that could be used to design animations to maximise learning [3-7]. Students have been shown to learn better with animations based on multimedia learning principles when compared to graphics [8]. There are hardly any studies that have evaluated the

design of freely available online animations in Biochemistry based on Mayer's multimedia learning principles that foster generative processing. Our objective in this study was to evaluate the free online animations in Biochemistry based on Mayer's multimedia Learning Principles that foster generative processing to determine if they could act as effective learning aids for MBBS 1st year students.

MATERIALS AND METHODS

This is a cross-sectional study involving analysis of online animations. Ethical approval was not needed for this study for two reasons- First, this study does not involve human subjects; second, all the data (animations) used in this study is available online for public. Study Sample included 102 online animations in Biochemistry.

A total of 27 topics under Biochemistry were chosen from 1st year MBBS syllabus prescribed by National Medical Commission (NMC) of India in 2019 [1]. Animations relevant to these topics were searched on www.google.com and www.youtube.com using relevant key words. For the purpose of our study, an Animation was defined as follows- "Animation refers to a simulated motion picture depicting movement of drawn or simulated objects"[9].

Inclusion criteria for the animation to be shortlisted were-

1. Animation should fit the above definition
2. Animation should have content relevant to the topic

An Exclusion criteria was-

1. Animations that require payment or creation of login id for access.

A total of 102 animations were shortlisted based on the above criteria.

Mayer's multimedia learning principles, based on cognitive theory of multimedia learning, are evidence based guidelines that could be used to design multimedia like animations for effective learning. Mayer proposed 9 principles that could be used to design multimedia to facilitate learning. We chose 3 principles amongst these- Personalisation, Image and multimedia principles. Personalisation principle states that words used in the multimedia presentation should be in conversational style rather than formal style.

Image principle suggests that there is no evidence to show that speakers image on the screen would help learning. Multimedia principle states that presenting words and pictures in a multimedia presentation is better than words alone. Voice principle was not included because of lack of strong evidence supporting its use. It has been shown that these principles promote generative processing [3,4]. Generative processing is a type a cognitive processing required for deeper understanding of content of multimedia presentation. It depends on learner's motivation to exert effort. It involves organising the knowledge being presented and integrating it with previous knowledge.

The shortlisted animations (n=102) were evaluated using 3 of the Mayer's multimedia Learning Principles mentioned above. If a principle was complied with in an animation, a score of 5 was given. If a principle was violated, a score of 0 was given. Maximum score for an animation was 15. Total score for an animation was obtained after adding all the scores for individual principles.

Statistical analysis : Descriptive statistics in the form of proportion was used.

RESULTS

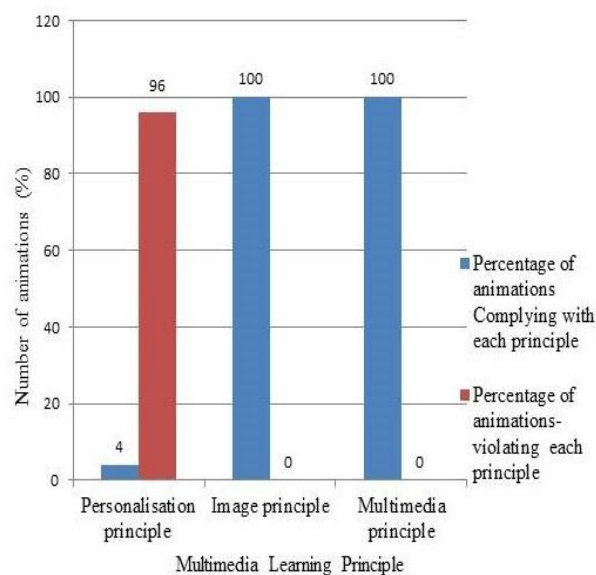
The shortlisted 102 animations had a median score of 10 (Inter Quartile Range=0) out of a maximum score of 15.

Table 1: Distribution of animations based on scoring using multimedia learning principles

No. of Principles	Number of animations (%) (n=102)	Scoring on animations using multimedia Principles (Maximum score=15)
Three	04(4)	15
Two	98(96)	10

Only 4% of the animations complied with all the three principles (personalisation, image and multimedia principles) that foster generative processing. In contrast, 96% of the animations complied with only two principles of the three principles (personalisation, image and multimedia principles). (Table 1).

Figure 1: Distribution of animations based on compliance / violation of individual Multimedia learning principles



Personalisation principle was violated in 96% of animations, as the spoken words in these of the animations were in formal style rather than conversational style. Image principle was complied with in all the animations, indicating that speaker's image was not present in any of the animations. Similarly, Multimedia principle was complied with in all the animations (Figure 1).

DISCUSSION

In this study we evaluated the design of online animations in Biochemistry based on Mayer's multimedia learning principles. Animations have been used by students to learn complex concepts [2]. Even in a classroom setting, students who received lecture instructions with supplemental animations showed better learning outcomes as compared to students that received same lecture instructions without animations [10,11].

The shortlisted animations had a median score of 10 out of a maximum score of 15. Only 4% of animations had an ideal score of 15. Majority (96%) of animations had a score of 10. Only 4% of the animations complied with all the multimedia learning principles. 96% of the animations violated personalisation principle suggesting that students who use these animations may have challenges in organising and integrating

information in these animations potentially resulting in poorer learning. Personalisation involves converting the spoken words in a multimedia presentation from formal to conversational style. One of the major techniques for creating conversational style is to use words like "you" and "I" instead of exclusively using third person constructions. Social cues such as personalisation (ex. using words in conversational style) help the activation of social response in the learner, such as commitment to attempt to understand the content of multimedia presentation. This social response enhances generative processing as the learner tries harder to select, organise and integrate content of multimedia presentation, which in turn leads to better learning outcomes [3]. The other principles- Multimedia and Image principle were complied with in all the animations.

The limitation of the study is that these principles (findings) are more strongly applicable when the topic is complex, pacing of the animation is fast and learners have low prior knowledge of topic [3]. We welcome future studies that could test the effect of these variables on learning through animations.

CONCLUSION

Majority of online animations in Biochemistry that we evaluated using Mayer's multimedia Learning principles do not comply with Personalisation principle of multimedia learning, potentially leading to poorer learning. Medical publishing companies and individuals who create animations could incorporate all the multimedia learning principles during the design of animations in Biochemistry for Ist MBBS students for effective learning.

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KNOWLEDGE ABOUT SMOKING AND SECOND HAND SMOKE AMONG THE MALE ADULTS OF ABUJA, NIGERIA

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ABSTRACT

Introduction : The Framework Convention on Tobacco Control (FCTC) was introduced by the WHO to curtail the global tobacco pandemic that is claiming millions of life yearly. Though Nigeria is a signatory, it has lacked behind in implementation and this to a large extent can be blamed on poor surveillance. Very few studies in Nigeria have assessed knowledge about harmful effects of Second Hand Smoke (SHS). This study was undertaken to determine the level of knowledge of the ill effects of tobacco among the male adults aged 15-49 years residing in Abuja, Nigeria

Material and Methods : This cross-sectional study was carried using a sub-set of the Global Adult Tobacco Survey questionnaire. The WHO 30 X 7 Multi-stage, modified cluster technique was used to obtain a sample for smokers and another for non-smokers. Microsoft Excel was used to create a database.

Results : Smokers were found to be older and less educated than non-smokers. Levels of tobacco-related knowledge were found to be high in Abuja but were higher amongst non-smokers and were also associated with the educational achievement of respondents. Overall, non-smokers were found to be more knowledgeable than smokers about the harmful effects of smoking and SHS.

Conclusion : It observed that male adults in Abuja were very knowledgeable about the harmful effects of tobacco consumption; however, smokers had significantly less knowledge as compared to non smokers. Larger nationwide studies using all the protocols of GATS are further required.

Key words: Nigeria, Knowledge, Second Hand Smoke

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INTRODUCTION

Tobacco is the single most preventable cause of death in the world today". (1) Tobacco use is also known to be causally related to heart disease, emphysema, stroke, peripheral vascular disease and adverse outcomes of pregnancy and healing following surgery.(2) There are currently approximately 1.1 billion smokers around the world (3,4) and this figure is set to rise to 1.6 billion by 2030 if the current trend is left unchecked. (2) Over 80% of smokers today reside in middle and low-income countries. (3,4) Moreover, secondhand Smoke (SHS) or involuntary smoking is known to be carcinogenic

to humans with no safe levels of exposure. (5) SHS is even more dangerous than active smoking because it contains higher concentrations of small particles as it is not "filtered", has toxic gases, and includes exhaled mainstream smoke. Even brief exposures to SHS greatly increase the risk of developing atherosclerosis. (6) Other diseases caused by SHS include acute respiratory infection, sudden infant death syndrome, asthma, middle ear infection and possibly certain learning defects. (5) SHS is estimated to kill about 3000 non-smokers each year in the USA mostly due to cardiovascular disease (2) while in the UK about 11,000 deaths are attributed to the adverse effects

of SHS. (6) This explains to a large extent why more and more countries are enforcing smoking bans to protect the people from SHS. Lopez et al (7) developed a conceptual framework that presents the 4 stages of the Tobacco epidemic, considering the fact that Nigeria is still at stage 1 of the pandemic; there lies a huge opportunity to prevent it from progressing to more advanced stages and hopefully reverse it out of the pandemic by employing aggressive tobacco control strategies. It is important to realize that there has been limited research on smoking in Nigeria and most studies have focused on Knowledge, Attitudes and Practice (KAP) of smoking in subgroups such as Soldiers (8), Physicians (9) and Secondary school children (10, 11). Knowledge about tobacco may be an important determinant of behavior such as initiating and quitting smoking. (12) In Nigeria currently, very little data is available on the amount of knowledge about tobacco use and its harmful effects amongst adult. Aim of the present study was to determine the level of knowledge of the ill effects of tobacco among the male adults aged 15-49 who are resident of Abuja, Nigeria.

MATERIAL AND METHODS

Study design

This cross sectional, descriptive study collected primary data through a survey of a representative sample using a structured questionnaire with close-ended questions.

Sampling Approach

Multi-stage, modified cluster sampling (13) approach was used. This sampling technique includes the following steps: 1) estimating the population of the communities and villages in the study area; 2) dividing each of the communities into non-overlapping Local Government Councils (LGCs) of roughly the same population size; 3) random sampling of 30 clusters, with probability proportionate to the size (PPS) of the population of the LGC in the study area; 4) selection of seven subjects in each cluster to give a total sample size of 210. (14) Since this approach assumes uniformity of the general characteristics of the respondents, and because this study aimed to compare the knowledge between smokers and

non-smokers; two samples of 210 were chosen one for the smokers and one for the non-smokers.

Inclusion and exclusion criteria:

Non-institutionalized male adults aged between 15-49 years residing in the FCT, Abuja were included. Female subjects due to the fact that the prevalence of tobacco use among them is less than 1 % were excluded. (15) Male adults with physical or mental handicap that could prevent them from providing informed consent were also excluded.

Questionnaire design

Data for this study was collected in a questionnaire that was based on a subset of the GATS questionnaire. The GATS questionnaire provides a global standard protocol for the consistent monitoring of tobacco use amongst adults and ensures that the obtained results are comparable with the results obtained from standard tobacco surveys in other parts of the world. (16)

Ethical considerations

Approval from the Health Research Ethics Committee (HREC) of the FCT and that of the University of Liverpool HREC was obtained. A written consent was obtained from each respondent after explaining the essence of the study and the voluntary nature of their participation using the information sheet.

Sampling procedure /Selection of households/ Participants

Table 1 shows the cluster selection. In which all districts and localities in each local government councils (LGC) were obtained from the Abuja Geographic Information Systems (AGIS). A list of all localities by districts and LGCs was created. Each LGC was divided into clusters. In every LGC, each cluster was given a unique number and the 30 required clusters were randomly selected. On the day of the interview, the first identified house was visited followed by the next nearest household, followed by the next until 7 eligible smokers and 7 non-smokers were interviewed.

'Complete knowledge' represented correct response (yes) to all 8 questions; 'good knowledge' represented 5 to 7 correct responses; 'Poor knowledge' represented 1 to 4 correct responses; and 'No knowledge' represented 0 correct responses.

Data Analysis

Microsoft Excel was used to create a database. Questionnaire information was inputted into IBM's SPSS version 18.0 and was used for all analysis. Knowledge about the ill effects of smoking and SHS was analyzed as categorical variables. Statistical significance of the differences between the two groups was analyzed using Chi-square statistics. Next, the questions that identified the level of knowledge about hazardous effects of tobacco use were combined to create a new variable that was used as the dependent variable in multivariate logistic regression analysis. $P < 0.05$ was considered as statistical significant.

RESULTS

The age distribution of the smokers and nonsmokers were very similar, 16 to 49 years and 15 to 48 years, respectively. However, nonsmokers on the average were 2.2 years younger than smokers. Majority of respondents in both groups have received some level of formal education; 95.7% of non-smokers and 91.4% of smokers. Overall, 41.0% of non-smokers reported being unemployed as compared to 35.7% of smokers. Close examination of the employment pattern in the two groups detected no statistical significance. (Table 2)

Table 1: Cluster Selection in the study population

LGCs	Population	Percentage of the total population	Percentage of 30 cluster	Potential clusters in the locality	Number of clusters selected
Abaji	91,168	4.2	1.2	9	1
Bwari	354,438	16.2	4.9	35	5
Gwagwalada	246,108	11.2	3.4	25	3
Kuje	151,884	6.9	2.1	15	2
Kwali	133,898	6.1	1.8	13	2
Municipal-Area-Council	1,214,498	55.4	16.6	121	17
Total	2,191,994	100.0	30.0	219	30

Table 2: Univariate distribution of age, education level and work status

Variables		Nonsmokers		Smokers		Statistic al Test
		Mean	SD	Mean	SD	
Age (years)		26.59	6.84	28.78	7.55	t value = 3.120 p=0.348
Variables		No	Percent	No	Percent	Statistic al Test
Education	No Education	9	4.3	18	8.6	
	Primary	28	13.3	33	15.7	
	Secondary	86	41.0	78	37.1	
	More than secondary	87	41.4	81	38.6	
Work status over past 12 months	Unemployed	86	41.0	75	35.7	Chi-square test = 1.869, p=0.393
	Self-employed	84	40.0	85	40.5	
	Formally employed	40	19.0	50	23.8	
Total		210	100.0	210	100.0	

Table 3: Association between smoking status and background characteristics

Background characteristics		p value	Odds Ratio	95% C.I.N	
				Lower	Upper
Age	15-19 years *	0.205			
	20-24	0.536	1.245	0.621	2.496
	25-29	0.203	1.603	0.775	3.314
	30-34	0.060	2.121	0.968	4.644
	35-39	0.053	2.660	0.986	7.171
	40-44	0.094	2.550	0.851	7.637
	45-49	0.028	4.734	1.183	18.948
Education	No Education *	0.099			
	Primary	0.343	0.628	0.240	1.644
	Secondary	0.098	0.478	0.199	1.146
	>Secondary	0.027	0.365	0.150	0.889
Work	Unemployed *	0.584			
	Self-employed	0.514	0.846	0.513	1.397
	Formally employed	0.729	1.116	0.600	2.075

* = Reference category

Table 3 shows that the likelihood of being a smoker increases with age and respondents between the ages of 45-49 years are almost 5 times more likely to be smokers than non-smokers as compared to respondents aged 15-19 years. The likelihood of smoking declines as levels of education increases. Actually, those who have

completed 'more than secondary education' have significantly lower smokers than the uneducated ones (OR: 0.365, 95% CI: 0.150-0.889). However, none of the odds ratio of the association between the employment and smoking status was found to be statistically significant. Therefore, based on this sample, smokers in the study area are older and less educated.

Tobacco related knowledge

The level of knowledge that smoking causes serious illness was higher amongst non-smokers as compared to smokers. There was relatively poor knowledge in both groups that smoking could result in stroke; with smokers knowing even less than non-smokers. Overall, knowledge about the four major harmful effects of smoking tobacco that were investigated in the study was consistently higher amongst non-smoker as compared to smokers. (Table 4)

Table 4: Knowledge about the Harmful effects of Smoking

Variables	Smoking Status				Total		Chi-Square
	Non-smokers		Smokers		Count	Percent	
Smoking causes serious illness	Count	Percent	Count	Percent	Count	Percent	
Yes	204	97.1	186	88.6	390	92.9	19.51, p<0.001
No	5	2.4	3	1.4	8	1.9	
Don't know	1	0.5	21	10	22	5.2	
Smoking causes Stroke							
Yes	124	59	98	46.7	222	52.9	8.23, p=0.016
No	44	21.0	68	32.4	112	26.7	
Don't know	42	20.0	44	21.0	86	20.5	
Smoking causes Heart attack							
Yes	184	87.6	162	77.1	346	82.4	10.256, p=0.006
No	8	3.8	24	11.4	32	7.6	
Don't know	18	8.6	24	11.4	42	10.0	
Smoking causes Lung cancer							
Yes	195	92.9	171	81.4	366	87.1	17.141, p=0.000
No	4	1.9	25	11.9	29	6.9	
Don't know	11	5.2	14	6.7	25	6.0	
Total	210	100	210	100	420	100	

Non-smokers were more aware of the fact that SHS can cause serious illness as compared to smokers. The level of knowledge that SHS causes heart disease in adults was higher amongst non-smokers than smokers. Knowledge that SHS causes lung illness in children was higher in non-smokers as compared to smokers. Non-smokers were found to be more knowledgeable about lung cancer being an effect of smoking as compared to smokers. Overall, non-smokers were found to be more knowledgeable than smokers about each of the 4 ill effects of SHS investigated in the survey. (Table 5)

Table 5: Knowledge about the Harmful effects of SHS

Variables	SHS Causes				Total		Chi-Square
	Non-smokers		Smokers		Count	Percent	
SHS causes illness	Count	Percent	Count	Percent	Count	Percent	
Yes	180	85.7	144	68.6	324	77.1	17.630, p<0.001
No	16	7.6	38	18.1	54	12.9	
Don't know	14	6.7	28	13.3	42	10.0	
SHS causes adult heart disease							
Yes	165	78.6	121	57.6	286	68.1	21.482, p<0.001
No	26	12.4	47	22.4	73	17.4	
Don't know	19	9.0	42	20.0	61	14.5	
SHS causes lung illness in children							
Yes	180	85.7	153	72.9	333	79.3	10.940, p=0.004
No	20	9.5	34	16.2	54	12.9	
Don't know	10	4.8	23	11.0	33	7.9	
SHS causes lung cancer in adults							
Yes	166	79.0	124	59.0	290	69.0	19.784, p<0.001
No	23	11.0	48	22.9	71	16.9	
Don't know	21	10.0	38	18.1	59	14.0	
Total	210	100	210	100	420	100	

Table 6: Overall knowledge about the ill effects of Smoking and SHS

Knowledge	Smoking Status		Total	chi square
	Non-smoker	Smoker		
No knowledge	4	14	18	20.17, p<0.001
Poor Knowledge	28	56	84	
Good Knowledge	79	96	148	
Complete Knowledge	99	71	170	
Total	210	210	420	

Overall, non-smokers were found to be more knowledgeable than smokers about the harmful effects of Smoking and SHS and this difference was statistically significant- Therefore, smoking status is associated with the amount of knowledge respondents possess. (Table 6)

Table 7: Factors associated with knowledge about ill effects of active and passive smoking

Knowledge	Degree of Freedom (df)	p value	Odds ratio	95% C.I for Odds Ratio	
				Lower Bound	Upper Bound
Poor Knowledge	Intercept	1	0.085		
	Age in 7 categories	1	0.703	1.082	0.721, 1.623
	Education	1	0.983	0.994	0.564, 1.752
	Work	1	0.331	0.641	0.262, 1.569
	Smoking status	1	0.506	0.649	0.182, 2.314
Good Knowledge	Intercept	1	0.031		
	Age in 7 categories	1	0.980	0.995	0.688, 1.441
	Education	1	0.225	1.384	0.819, 2.340
	Work	1	0.964	0.982	0.442, 2.180
	Smoking status	1	0.037	0.291	0.091, .930
Complete knowledge	Intercept	1	0.109		
	Age in 7 categories	1	0.291	0.816	0.560, 1.190
	Education	1	0.113	1.544	0.903, 2.640
	Work	1	0.263	1.581	0.709, 3.527
	Smoking status	1	0.010	0.218	0.068, 0.699

To determine the association of tobacco-related knowledge with smoking status, age, education and employment status; multivariate logistic regression was used. For this analysis, the reference group was "no knowledge". Smoking status was the only factor that showed statistically significant association with the knowledge about ill health effects of tobacco smoke. Smokers were less likely to have good knowledge (29%) or complete knowledge (22%) about the ill effects of smoking and SHS as compared to having no knowledge. (Table 7)

DISCUSSION

The study found that both groups had similar age distribution. However, smokers on the average were 2.2 years older than non-smokers and the odds of being a smoker increases with increasing age. Furthermore, the modal age of smokers was shown to be 30 years and these findings are similar to the NDHS (2008) finding that indicated the highest proportion of smokers among men in the age group 30-34 was 13%. (15) Helsinki a study by Laaksonen et al (2005) showed that smoking displays clear association with socioeconomic differences, being more common among those with lower occupational, education and income status.(17) While the majority of the sample in both groups was found to have acquired similar proportions of formal education, multivariate regression showed that higher educational achievement is associated with lower odds of being a smoker. This kind of association has also been reported several other studies. A study by Cavelaars et al (2000) of 12 European countries found that in men between the ages of 20 to 44 years smoking rates were higher among lower educated people in most countries. (18) While a cross-sectional study in New Delhi, India found that education was the strongest predictor of smoking, and men with no education were 1.8 (1.5 to 2.0) times more likely to be smokers than those with college education. (19) In the USA, another study found greater education was strongly associated with both never and former smoking. (20) Therefore, the findings of this study are in line with growing evidence that smokers tend to be less educated than non-smokers. This study did not find any statistically

significant association between smoking and the employment status of the respondents and this was surprising as occupational status is closely related to one's educational level (17) and therefore one would expect non-smokers to have better employment by virtue of their better educational achievement. One possible explanation could lie in the definition of unemployment used in the study. In India, men who were self-employed smoking prevalence of 55.7%, compared to 9.5% amongst students. (22) In both groups, lung cancer was the most commonly identified disease that resulted from smoking while stroke was the least commonly identified. No other study from Nigeria provides comparable results, however a study on adults in north-eastern Nigeria by Olufemi et al (2008) show much lower levels of knowledge with only 60.7% of smokers agreeing that smoking was injurious to health. (22) GATS study above asked respondents if they believed SHS caused serious illness, but only the GATS of Thailand asked about specific diseases associated with SHS. (23) Multivariate regression showed that smoking status was the only factor in the study to be associated with the amount of knowledge respondents possessed about the harmful effects of active and passive smoking, while age, educational and employment showed no significant association. Additionally, a study from the USA by Finney et al (2008) showed that tobacco-related knowledge is not equally distributed in the population, that there is a knowledge gap between those with lower and higher levels of education and income, and that non-smokers have more accurate knowledge than smokers. (12)

CONCLUSION

It is found that the male adults in Abuja were very knowledgeable about the harmful effects of tobacco consumption, however, smokers had significantly less knowledge as compared to non smokers and this is also associated with their lower educational achievement. Abuja is only one part of Nigeria and for any control measure to be truly successful, data is required from the whole country. Therefore, future studies should cover the entire nation using all the protocols of GATS and

not only the questionnaire as was the case in this study.

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EVALUATION OF AVAILABILITY OF ONLINE ANIMATIONS TO FACILITATE VISUAL LEARNING IN GENETICS FOR MBBS Ist YEAR STUDENTS

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ABSTRACT

Background: As per the latest "Competency Based Medical Education" (CBME) curriculum prescribed by National Medical Commission (NMC) of India, genetics is an important component of Biochemistry for MBBS Ist professional students. Learning concepts in genetics is important for clinical application of knowledge in diagnosis and management of diseases. Visual media like animations have been shown to assist learning of complex molecular concepts. To the best of our knowledge, there are no studies that have assessed availability of free online animations in genetics. This study aimed to assess the availability of free animations and evaluate their characteristics in relevant topics under genetics for MBBS Ist year students.

Materials and methods: A total of 16 topics in Genetics were shortlisted amongst competencies under Biochemistry as per the NMC curriculum. Keywords containing the title of topics were used to search www.google.com (Google) and www.youtube.com (You Tube) separately to shortlist the animations available. These animations were characterised based on the total views and time duration.

Results: A total of 101 online animations under the shortlisted topics in genetics based on our search criteria were included. Sixty of these animations were shortlisted from YouTube and 41 animations were from Google. These animations had combined views of 3,01,07,775. Thirteen percent and 54% of animations belonged to highly popular and less popular categories, respectively. Majority (85%) of animations had time duration of 6 minutes or less.

Conclusions: It was observed that www.google.com and www.youtube.com can act as online resources for accessing animations to assist visual learning in genetics for MBBS Ist year students. However, students and teachers have to verify the knowledge content of animations before using them as learning resources.

Key words: Genetics animations, animations availability, medical students, online resources, Biochemistry

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INTRODUCTION

As per the Competency Based Medical Education (CBME) curriculum of National Medical Commission (NMC) of India, an Indian Medical Graduate at the end of his undergraduate training course is expected to demonstrate knowledge of normal and abnormal human structure, function and development from a molecular, cellular, biologic, clinical, behavioral and social perspective. Genetics forms an important component of Biochemistry Syllabus as prescribed by NMC [1]. Learning concepts in Genetics is critical for MBBS students to

understand the principles behind the diagnosis and management of many diseases. Furthermore, it is imperative to have a strong foundation in Genetics in order to be able to critically appraise vast amounts of evidence that is being generated in research studies. Many of the critical concepts in Genetics are complex making them difficult to visualize, thereby hindering the understanding and application of concepts in Genetics in health and disease. Animations visualising complex molecular concepts have been created with an aim of making visualising and learning easier. In fact, animations have been shown to improve

understanding and memorisation of molecular processes. Animations used with traditional lectures have been shown to aid memorisation of sequence of molecular events when compared with lectures alone [2,3,4]. Animations explaining various molecular concepts are freely available online and MBBS students have been shown to access these animations to learn complex concepts [5]. However, there have been no studies to the best of our knowledge that have evaluated the availability of free online animations in genetics on internet. Such studies would help us determine the availability of relevant animations and the same could be used in teaching learning processes. In the absence of relevant animations, such animations may be developed by publishing companies and individuals to fill the gap.

In this study, our objective was to assess the availability of free online animations in relevant topics in genetics for MBBS 1st year students.

MATERIAL AND METHODS

This is a cross-sectional study involving availability of online animations. Ethical approval was not needed for this study for two reasons-First, this study does not involve human subjects; second, all the data (animations) used in this study is available online for public. Competencies as mentioned in MBBS 1st year Syllabus in Biochemistry prescribed by NMC in 2019 were used to shortlist complex and difficult to visualize concepts in Genetics [1]. As a next step, online resources for accessing animations on internet were shortlisted. Since www.google.com (Google) and www.youtube.com (YouTube) happened to be top two search engines, we selected these two search engines for our online search [6]. Using the title of topics as key words search for animations were performed. Boolean operators "AND" was used as needed. For example, search terms used for DNA Replication were "DNA Replication AND animation". We did not deliberately make the search criteria complex as our intention was to simulate the usual search strategy of learners/students. A total first 30 search results in Google and first 50 search results in YouTube were scanned for the presence of animations on shortlisted topics. If relevant animations were not found, further search of 30 and 50 results on

Google and You tube was performed, respectively.

For the purpose of our study, an Animation was defined as "A collection of still images called "cels" that when viewed in rapid succession gives the impression of movement" [3].

Inclusion criteria for the animation to be shortlisted were-

1. Animation should fit the above definition
2. Animation should have content relevant to the topic

An exclusion criterion was:

1. Videos that have only transitioning images in a slideshow.
2. Animations that were used as marketing tool to promote a certain product.
3. Animations that require payment for access.

Statistical analysis: Data was represented using median, interquartile range and frequency distribution (proportion).

RESULTS

A total of 16 topics in Genetics were shortlisted amongst competencies under Biochemistry as per the NMC curriculum. We found a total of 101 online animations under the shortlisted topics in genetics based on our search criteria. Of these animations, 41 were accessed on Google and 60 animations were accessed on You Tube (Table 1). Date of access was December 4th and 5th, 2021.

Table 1: Topics and corresponding number of animations accessed on Google and You Tube

Topic of Animation	Number of Animations on You tube	Number of Animations on Google
Structure and function of DNA	6	3
Structural Organisation of DNA to form Chromatin	3	1
Cell cycle	6	3
DNA replication	5	3
DNA repair mechanisms	4	2
DNA transcription	5	3
Translation (Protein synthesis)	3	2
Regulation of Gene expression	4	5
Protein targeting	2	1
Gene Therapy	3	4
Recombinant DNA technology	3	1
DNA cloning	3	2
Polymerase Chain Reaction	4	6
Blotting Techniques	4	1
DNA Microarrays	3	3
Human Genome Project	2	1
Total animations	60	41

Table 2: Online resources and corresponding views of animations

Online resource	Total number of animations	Animations with viewership data (n)	Total number of views
Google	41	7*	28,16,034
YouTube	60	60	2,72,91,741
Google + YouTube	101	67*	3,01,07,775

*Data on views was not available for all the animations accessed on Google.

Only 17% (7 out of 41 animations) on Google had data on views, whereas 100% of animations on YouTube had viewership data. (Table 2)

Table 3: Descriptive statistics of animations on different online resources

Online resources	Variable	Minimum	Maximum	Median	Interquartile Range
Google	Views (n=7)*	705	27,34,411	12,468	32,355
	Duration of animations (n=37)**	00:23	08:20	03:06	01:57
YouTube	Views (n=60)	132	68,76,053	93,533	3,79,969
	Duration of animations (n=60)	00:48	25:67	03:11	02:52
Google + YouTube	Views (n=67)	132	68,76,053	59,655	3,63,564
	Duration of animations (n=97)	00:23	25:67	03:08	02:16

*Data was not available for all the animations

** Four Animations accessed through Google did not have time duration data, as they were user controlled i.e. Users had to click to move to a subsequent step and hence, play time would be dependent on individual users.

Note: Duration of animations is in mm:ss format

Google videos had a median duration of 3 minutes 6 seconds [Interquartile range (IQR)=1 minutes 57 seconds], with median views of 12,468 (IQR=32,355). For google animations, data regarding views was available for only 7 animations and regarding duration was available for 37 animations (out of 41 animations). YouTube videos had a median duration of 3 minutes 11 seconds (IQR= 2 minutes 52 seconds), with median views of 93,533 (IQR=3,79,969). (Table 3)

Table 4: Distribution of animations based on duration

Category of animation based on duration	Duration of animation (mm:ss)	Number of animations	Percentage in each category
Short	00:01-03:00	44	45
Medium	03:01-06:00	39	40
Long	>06:01-30:00	14	15

Majority of the animations (85%) belonged to 'Short' and 'Medium' duration category with a runtime of 6 minutes or less; whereas 15% of the animations belonged to 'Long' duration category, with a runtime of more than 6 minutes and up to 30 minutes. (Table 4)

Table 5: Distribution of animations based on views

Category of animation based on views	Duration of animation	Number of animations	Percentage in each category
Less popular	1-1,00,000	36	54
Popular	1,00,001-10,00,000	22	33
Highly popular	10,00,001-1,00,00,000	9	13

More than half the animations (54%) belonged to 'less popular' category having views ranging from 1-1,00,000, whereas 13% of animations were in 'highly popular' category having views ranging from 10,00,001-1,00,00,000 (Table 5).

Table 6: Number of Views of Individual Animations belonging to 'highly popular' category (YouTube)

Topic	Number of Views
DNA Microarrays	10,45,671
DNA Replication	68,76,053
Structure of DNA	25,80,252
Translation	10,39,888

Note: The above table includes a few examples of topics of animations in the 'Highly popular' category.

Examples of such animations/topics with their corresponding individual viewership include DNA microarrays, translation etc. (Table 6)

DISCUSSION

In this study, we evaluated the availability of free online animations for various topics in Genetics as prescribed by NMC [1]. We found the 101 animations pertaining to Genetics on Google and YouTube. Animations have been shown to aid learning of complex molecular concepts [3]. Medical students use online animations to learn complex concepts [5].

These animations accessed in our study were hosted by various entities including medical publishing companies, entities sponsored by academic organisations and individuals. Table 1 indicates a representative sample of online animations on various topics in Genetics based on search results and is not meant to show list of all available online animations under a particular topic. An alternate independent search at a different point of time by a different person may yield different results, since the search algorithms of Google and YouTube are determined by various factors, which include person performing the search, timing of search and other factors [7]. This study does not intend to document all the animations in Google and YouTube, as that is not intended purpose of this study. To the best of our knowledge there have been no studies that have evaluated the availability of animations pertaining to genetics for Medical students. A total of 101 animation animations belonging to 16 topics in genetics were found through specific search criteria used in our study on www.google.com and www.youtube.com. Animations were found in all the shortlisted topics. Data related to views was present in only (17%) of animations accessed through Google. In contrast, 100% of animations accessed through YouTube exhibited the views data by default, allowing the users to assess the popularity of animations. Though YouTube had higher aggregate viewership than Google for the given animations, the two cannot be compared for two reasons- first, viewership data is not available for Google for 83% of the animations; second the number of animations with viewership data is far lesser for Google (7 animations) as compared to YouTube (60 animations). Combined total views of animations were 3, 01, 07, 775 suggesting the huge popularity of online animations related to concepts in genetics among learners/students.

Animation duration

Majority (85%) of the animations belonged to "short" and "medium" duration categories (: 6 minutes). In contrast, a minority (15%) of the animations belonged to "long" duration category (> 6 min). The possible reasons for the short duration of animations may be: First, animations with brief duration are more likely to be viewed by learners. Second, these animations tend to focus on illustrating the core concept, without delving into details, as it happens in lecture sessions. Third, creating animations is an expensive process.

Animation Views

Majority (54%) of the animations belonged to "less popular" category, whereas a minority (13%) of the animations belonged to "highly popular" category. Some examples of animations in the highly popular category belonged to topics like "DNA microarrays", "DNA replication", "Structure of DNA" and "Translation (protein synthesis)". Popularity of these animations may be determined by many factors including complexity of the topic, duration of animations, popularity of the channels/websites hosting animations and quality of the animations.

Advantages of Google and YouTube as access points for animations

There are certain advantages of accessing Google and YouTube for animations. Most of the animations are free for access. An animation related to any topic can be easily searched based on a combination of relevant key words. Learners have an option to choose to view a particular animation amongst multiple animations.

Limitations of Google and YouTube as access points for animations

Nevertheless, there are few limitations of these online resources. First, the content accessed through YouTube and Google is dynamic. Learners have no control over it; ex. a good animation that is available today may not be available tomorrow. In addition, an animation that is ranked higher in search results today may not be at the top of the search results at a later point of time, posing challenges to access. Ease of access does not guarantee the quality of animations in terms of design and knowledge content, as concerns have been raised regarding quality of online educational

videos [8,9,10,11]. Moreover, the quality of content is determined by the person/organisation creating it and Google/YouTube has no role to play in it. In addition, entry of search term containing key words relevant to topic may sometimes return huge number of searches, which may include irrelevant search results, putting the onus on the learner to scrutinise and select an appropriate animation resulting in expenditure of time and energy.

To circumvent this problem, teachers can systematically search animation online for topics of interest using relevant key words, shortlist animations based on appropriate design and accuracy of knowledge content and create an online database of links of such animations. Such a database can be shared with students, so that best of animations are readily available for learning.

Strengths of the study

To our knowledge, this is one of the first studies that has explored the availability of online genetics animations for MBBS students. We have used two most popular searching engines for shortlisting online animations, thereby reflecting to some extent the search patterns of students[6]. Sixteen topics in genetics were chosen for shortlisting animations thereby reflecting a wide spectrum target topics for animations. These topics were chosen from MBBS Ist year syllabus thereby making the findings relevant for MBBS students and teachers [1].

Limitations of the study

Given the dynamic nature of online resources like Google and YouTube as discussed above, these search results are applicable on the date of access. An alternate search at a different point in time by a different person may yield different results [7]. Though we evaluated the availability of online animations in genetics, we did not focus on detailed analysis of knowledge content of animations, as it is beyond the scope of the study. Future studies can be done to assess the content in these animations to decide whether these animations can serve as credible learning aid for MBBS Ist year students.

CONCLUSION

MBBS Ist year students can use search engines- www.google.com and www.youtube.com for accessing free online animations for learning concepts in genetics. However, students should verify the knowledge content of these animations before using them as a learning aid.

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LEVEL OF SUPPORT FOR COMPREHENSIVE TOBACCO MEASURES AMONG THE MALE ADULTS AGED 15-49 YEARS IN ABUJA, NIGERIA

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ABSTRACT

Introduction : Tobacco control monitoring is vital but needs attention. Very few studies in Nigeria have assessed tobacco-related knowledge and none have assessed levels of support for tobacco control measures; which are very important for the planning and implementation of control measures. This study was planned to determine the amount of support for each of the cost-effective Tobacco control measures proffered by the World Bank among the male adults aged 15-49 years in Abuja, Nigeria.

Material and Methods: This cross sectional, descriptive study was conducted in Abuja using a structured questionnaire. A written consent was obtained from each respondent after explaining the essence of the study. The WHO 30 X 7 Multi-stage, modified cluster technique was used to obtain a sample for smokers and another for non-smokers.

Results: The average age of smoker and non smokers were 28.8 years and 26.6 years respectively. Overall, the support for all the control measures was higher amongst non-smokers compared to smokers and the differences were statistically significant. A ban on smoking in public places was a very popular support measure amongst the respondents.

Conclusion: Level of support for comprehensive tobacco measures was found to be high in both groups, but significantly higher amongst non-smokers. Moreover, the government needs to focus more on implementation of the FCTC guidelines that are the most-cost-effective measures and are already very popular amongst the study population.

Key words: Nigeria, Framework Convention on Tobacco Control, Global Adult Tobacco Survey.

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INTRODUCTION

Tobacco kills almost half of all its users, and kills more than 8 million people each year which is more than HIV/AIDS, Malaria and Tuberculosis combined. (1) It is estimated that if not controlled this number will rise to 10 million deaths a year by 2030. (2) The overall smoking prevalence was highest in Europe and central Asia (35%) and lowest in sub-Saharan Africa (18%). Men in East Asia and the Pacific had the highest prevalence at 63%, while those in sub-Saharan Africa had the lowest at 29%. (3) Among females, the smoking prevalence was highest in Latin America, at 24%, and lowest in East Asia

and Pacific as well as Middle East and North Africa, at 5%. Knowledge about tobacco will affect the support for control policies as increasing education about smoking is one of the reasons reported for improved support.(4) It is also important to assess the level of support the various control measures enjoy among the public, because data from 4 countries (Australia, Canada, the UK, and the USA) shows that support for smoke-free policies is important for their successful implementation. The study also suggests that after the initial opposition to such policies; compliance and public support, even among smokers increases with time, as they begin to

appreciate the rationale behind implementing the policies and experience their benefits. (4) However, as a European study has shown, smokers tend to be less supportive of smoke-free and taxation policies than non-smokers, and this is understandable as such policies tend to affect them more directly than non-smokers. Public support is a key element in the design and implementation of smoke-free policies. (5) In developing countries just like high-income countries, support for smoking bans increased after its implementation. (6) In Nigeria currently, no data exists about the level of support tobacco measures enjoy amongst adults and these are important pieces of information for planning and executing effective tobacco control. The present study was planned to determine the amount of support for each of the 6 cost-effective Tobacco control measures proffered by the World Bank among the male adults aged 15-49 years in Abuja, Nigeria.

MATERIAL AND METHODS

This cross sectional, descriptive study was conducted in Abuja using a sub-set of the GATS questionnaire. A written consent was obtained from each respondent after explaining the essence of the study. Approval from the Health Research Ethics Committee (HREC) of the FCT was obtained from the University of Liverpool. Subjects were selected from the non-institutionalized male adults between the ages of 15-49 years residing in the FCT, Abuja. Female subjects (due to very low prevalence of smoking reported⁷), male adults who were physically or mentally handicapped, male who did not consider the visited household their primary residence and those who refused to participate were excluded from the study.

Sampling Approach/procedure

Multi-stage, modified cluster sampling approach was utilized for this study.(8) This technique that was originally developed by the WHO for assessment of vaccination coverage is composed of a sample of 210 individuals in 30 clusters each of which includes 7 individuals. A map of the FCT showing all districts and localities in each local government councils (LGC) was obtained from the Abuja Geographic Information Systems

(AGIS). List of all localities by districts and LGCs was created. To approximate the population of localities; land survey officials in each of the LGCs were contacted. Each LGC was divided into clusters with an approximate population of 10,000 each. Some localities had more than 1 cluster; while some localities were combined to create a cluster. In every LGC, each cluster was given a unique number and the 30 required clusters were randomly selected.

Selection of households/ Participants

In each of the selected clusters, a central landmark was identified prior to the data collection date. The closest intersection to the landmark was chosen and a bottle was spun to randomly select a direction to enumerate. All houses from the center of the area to the edge of the area were counted and 1 number was randomly selected from all the counted houses and served as the first household to visit. The first identified house was visited followed by the next nearest household, followed by the next until 7 eligible smokers and 7 non-smokers were interviewed. Smokers were selected either from the same household as nonsmokers when present, or from another household in the same cluster.

Statistical Analysis

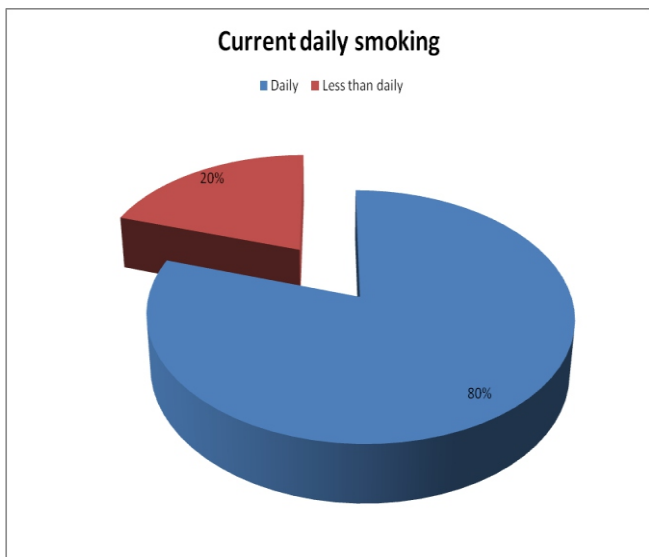
Microsoft Excel was used to create a database. Questionnaire information was inputted into IBM's SPSS version 18.0 and was used for all analysis. Graphical representation depicts the number and percentage of variables. Support for each of the control measures was compared between the 2 samples using cross-tabulation and tested for statistical significance using chi-square test. Multivariate logistic regression was used to determine the association of smoking status with support for tobacco control. $P < 0.05$ considered as statistically significant.

RESULTS

This cross sectional study included 420 male adults aged 15-49 years in Abuja, Nigeria. In which 210 were smokers and 210 were non smokers. Average age of smokers and non smokers were 28.8 years and 26.6 years respectively. The youngest study subject was 15 years old in the study. More than 90% study

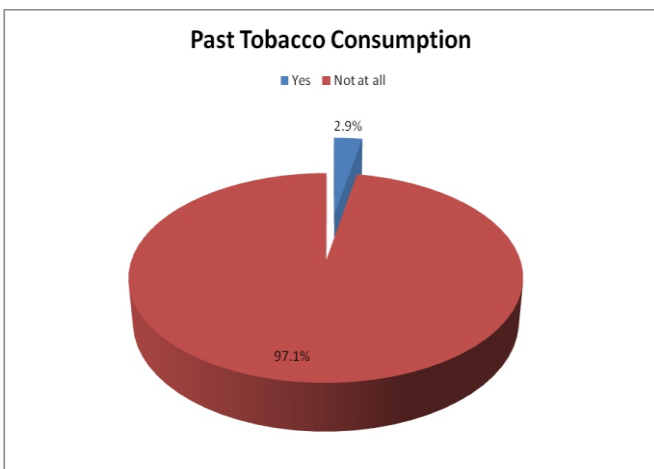
subjects were educated in smokers group and only 4.6% were illiterate in the non smokers group.

Figure 1: Current tobacco smoking status of smokers



* Daily = smoking at least one cigarette every day in the past one month or more. Less than daily = smoking at least one cigarette but not every day in the past one month or more.

Figure 2: Past tobacco smoking status of non smokers



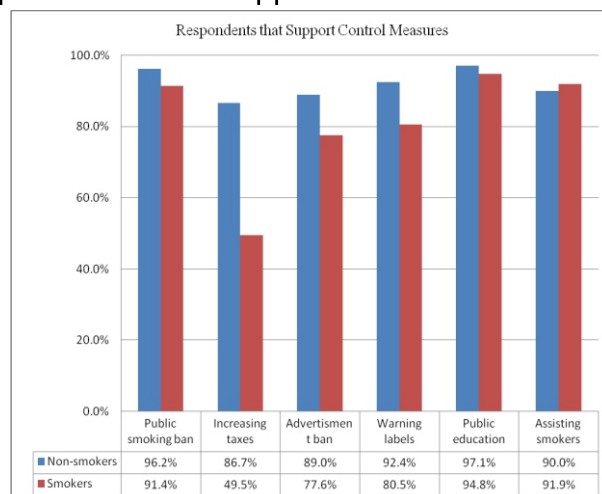
Past smoking= Smoked at least one tobacco product every day or nearly every day over a period of a month or more in the past, but who currently don't smoke Not at all = not smoking any cigarette in the past one month or more

Figure 1 and figure 2 shows that 97.1% of non-smokers have never smoked before. Eighty percent of those who were grouped as smokers were daily smokers.

Support for Control measures

A ban on smoking in public places is a very popular measure amongst respondents. More non-smokers seemed to support this measure as compared to smokers. Smokers were significantly less supportive of tax hikes on tobacco products when compared to non-smokers. Overall there was a high level of support in both the groups for more tobacco-related public education, with slightly higher levels observed amongst non-smokers. Most respondents in both the groups believe that the government must help smokers to quit tobacco, the difference being statistical insignificant. Whereas, non-smokers were more supportive of laws prohibiting tobacco advertisements than smokers and support for printing of health warning was found to be higher amongst non-smokers than smokers and this difference in support was statistically significant. (figure 3)

Figure 3: Respondents that responded “yes” to questions about support Control measures



Percentage of subjects that responded yes to support for control measures

Overall Support for control measures

To determine how the two groups, compared in regards to their overall support for the control measures; a new composite variable was generated. This combined responses to questions about support for the 6 control measures and comprised of 4 categories where 'complete support' represented correct response (yes) to all 6 questions; 'good support' represented 4 to 5 correct responses; 'Poor support' represented 1 to

3 correct responses; and 'no knowledge' represented 0 correct response. (table 1)

Table 1: Overall support for control measures

Support	Smoking Status		Total	Pearson's chi square
	Non-smoker	Smoker		
Good support	67	117	184	45.238 (p<0.001)
Complete support	137	70	207	
Total	210	210	420	

Overall, the support for all the control was higher amongst non-smokers compared to smokers and the differences were statistically significant. (Table 1)

To determine the association of smoking status with support for tobacco control and other variables such as age, education and employment status have on support: multivariate logistic regression was used. The two samples were combined to achieve meaningful comparison and the newly computed composite support variable was used as dependent variable and regressed against age, education, employment and smoking status as covariates. (Table 2)

Table 2: Factors associated with support for comprehensive tobacco control measures

Variables		Degree of freedom (df)	p value	Odds Ratio	95% C.I. for Odds Ratio	
					Lower Bound	Upper Bound
Good support	Intercept	1	0.017			
	Smoking status	1	0.119	0.467	0.180	1.216
Complete support	Intercept	1	0.321			
	Smoking status	1	0.000	0.136	0.052	0.355

For this analysis, the reference group was "poor support". Statistically significant association was detected only among those who had complete support for tobacco control. People who smoke were 0.136 times as likely to supportive i.e., smokers were rather unlikely, less than 15%, to support tobacco control efforts. (table 2)

Eighty percent of male adult smokers in Abuja smoked cigarettes on a daily basis, which is similar to GATS from other countries. In this study, 3.9% of current non-smokers had smoked in the past while other countries have shown higher percentages.(9-12) The study found that all comprehensive tobacco control measures are supported by most male adults in Abuja, though the levels of support may vary. The present study showed that the ban of smoking in public places was very popular with no significant difference between the two groups. Though other studies have detected high levels of support for public bans; they have also found difference between smokers and non-smokers. For example, in Australia, Trudy et al found a statistically significant difference in the beliefs about and support for anti-public smoking legislation between smokers and non-smokers. (13) Similarly, in Greece, Lazuras et al concluded that smokers tend to be less supportive of smoke-free and taxation policies than smokers.(6) The high levels of support in Abuja may be due to the ban on smoking in public places in the FCT since 2008 .(14) In present study agreement with the increase in taxes on tobacco products was significantly lower amongst smokers when compared to non-smokers. These findings are similar to a cross-sectional household survey in England which showed that non-smokers were more supportive than smokers, and furthermore the level of support decreased with increasing frequency of cigarette consumption. (15) Furthermore, research by Chaloupka et al found sufficient evidence of effectiveness of increased tobacco excise taxes and prices in reducing overall tobacco consumption. (16) The present study also found that the support for a law prohibiting all advertisements for tobacco products was significantly higher amongst non-smokers than smokers. But the fact that tobacco advertisement is quite rampant in Nigeria cannot be denied, as nearly half the students in the Nigerian 2008 GYTS saw billboards bearing tobacco advertisements. (17) Smoking behavior was also found to be significantly associated with cigarette advertisement in a study of secondary school students in a rural community of southwest

Nigeria. (18) Agreement with printing health warning labels on cigarette packets was found to be higher amongst non-smokers than smokers in the present study. This may be explained by the lower levels of literacy among smokers and this may make reading health warning more difficult, giving the fact that tobacco warnings do not carry pictorial warnings and cover only 15% of display areas against the 50% recommended in article 11 of the Framework Convention on Tobacco Control (FCTC). (17,19) The nature of the labeling does have a significant effect on the amount of health knowledge among smokers as shown in a study by Hammond et al, who found positive association between noticing the health warnings on cigarette packages and health knowledge. (20) Support for the initiation or increasing public education about the harmful effects of tobacco was equally high in both the groups in this study. Comprehensive smoke-free laws were associated with reduced risk of initiation and reductions in days smoked per month for all trajectories other than occasional users in a recent study in youth and youth adults of America. (21) Studies with comparable results could not be traced in Nigeria or from the results of GATS elsewhere. But as stipulated in Article 12 of the FCTC, the authorities should employ all measures and tools in improving the tobacco-related knowledge of the population through effective and comprehensive educational and public awareness programs. (19) Most respondents in the present study think that the government must help smokers to quit. This may indicate that smokers as well as non-smokers believe the government is not doing enough in term of smoking cessation assistance, as there are currently no cessation treatment programs in place to help quitters in Nigeria. (17) The support for all the six control measures combined was found to be higher amongst non-smokers compared to smokers.

CONCLUSION

Level of support for comprehensive tobacco measures was found to be high in both groups, but significantly higher amongst non-smokers. It can therefore be concluded that the government has to do more towards the full implementation of the

FCTC, as therein lies the key to effective tobacco control through measures that are highly popular amongst the population. The reason why respondents support or oppose the different control measures as this highlights important issues such as their perception and believes about the control measures and this invariably requires qualitative methods which should be used in further research.

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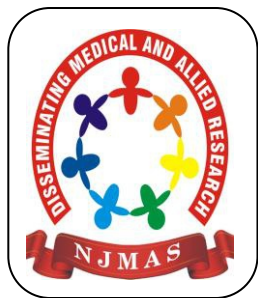
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CARDIOVASCULAR DISEASE RISK ASSESSMENT IN TYPE 2 DIABETES: ROLE OF MYELOPEROXIDASE AND APOLIPOPROTEIN B

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ABSTRACT

Introduction : Cardiovascular death accounts for more than 75% of all deaths among persons with diabetes mellitus. This is generally attributed to the adverse effects of hyperglycaemia and oxidative stress on vascular biology. By measuring the serum levels of MPO, apolipoprotein B, and HbA1c we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and initiate glycaemia control measures to prevent cardiovascular complications in type 2 diabetes patients. This study was undertaken to observe the relationship between serum myeloperoxidase with apolipoprotein B and also with that of serum glycated hemoglobin in type 2 diabetic patients and healthy controls.

Materials and Methods A case-control study was done taking 40 cases of type 2 diabetes mellitus and 40 age and sex-matched healthy controls. In all the subjects, concentrations of HbA1c, serum apolipoprotein B, and serum MPO were estimated. HbA1c was measured by turbidimetric method and serum apolipoprotein B by an immune turbidimetric method using semi-auto analyzer CHEM 5 Plus. Serum MPO was measured by ELISA method using ELISA reader.

Results The mean concentrations of HbA1c, serum apolipoprotein B and Serum MPO are significantly increased in type 2 diabetic cases when compared with healthy controls. HbA1c concentration is significantly positively correlated with serum apolipoprotein B and serum MPO in type 2 diabetic cases but there is no significant correlation between serum MPO and serum apolipoprotein B levels.

Conclusion: The present study suggests that chronic hyperglycemia and endothelial dysfunction are the major causal factors for the pathogenesis of macrovascular complications in type 2 DM. The future risk of CVD can be detected by evaluating the levels of apolipoprotein B and MPO in type 2 diabetic patients which can be prevented by adequate control of glycemia.

Keywords: Diabetes Mellitus; Endothelial dysfunction; dyslipidemia; CVD; hyperglycemia.

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INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin.¹ In the presence of chronic hyperglycemia haemoglobin undergoes glycation by the non-enzymatic process to form glycated haemoglobin. In diabetic patients, HbA1C indicates glycemic control over the previous few months. Increase in blood sugar by

30 mg/dl increases the HbA1C by 1%, approximately^{2,3} Hyperglycemia and oxidative stress increase the risk of cardiovascular disease in diabetic patients. Myeloperoxidase (MPO), which is present in the granules of leukocytes, macrophages and monocytes is released at the inflammatory sites. It stimulates the increased production of reactive oxygen species. These reactive oxygen species cause endothelium

dysfunction. MPO is implicated in the initiation, progression, and the complications of atherosclerosis.⁴ Total mass of atherogenic particles like VLDL, IDL, and LDL is indicated by apolipoprotein B. Its levels will increase in cardiovascular disease⁵

By measuring the serum levels of MPO, apolipoprotein B and HbA1c we can assess the future risk of cardiovascular disease in type 2 diabetic patients at an early stage and initiate glycemia control measures to prevent cardiovascular complications in type 2 diabetes patients. Therefore the present study aims to evaluate the serum levels of MPO, apolipoprotein B and HbA1c as early markers of cardiovascular risk in type 2 diabetic patients. In addition, MPO and apolipoprotein B levels will be correlated with glycated hemoglobin levels. This study was undertaken to observe the relationship between serum myeloperoxidase with apolipoprotein B and also with that of serum glycated hemoglobin in type 2 diabetic patients and healthy controls.

MATERIAL AND METHODS

This study is carried out to estimate the levels of serum Myeloperoxidase (MPO), glycated hemoglobin (HbA1c), apolipoprotein B in type 2 diabetic patients and healthy controls, for one year. Patients were selected from the district hospital. Each patient gave an informed consent and the study was approved by the ethical and research committee. The patients and controls voluntarily participated in the study.

Inclusion Criteria:

Cases: 40 proven cases of type 2 diabetic patients without complications, on treatment with no time duration, in the age group of 30 - 80 years.

Controls: 40 cases of age and sex-matched healthy controls will be compared.

All patients suffering from type 2 diabetes without complications, on treatment with no time duration diagnosed and confirmed by a physician with FBS and PPBS according to American Diabetes Association criteria (FBS ; 126 mg/dl & 2-hour PPBS ; 200 mg/dl)

Exclusion Criteria: Patients with congenital heart diseases, hypertension, diabetic complications, systemic diseases, other endocrinal disorders, malignancies, hemoglobinopathies, drugs that

interfere with serum levels of myeloperoxidase, apolipoprotein lipoprotein B, and glycated hemoglobin.

Collection of Blood Sample:

After obtaining informed consent, about 6ml of fasting venous blood samples were drawn under aseptic precautions into a sterile bulb from selected subjects. 4 ml of blood was taken into a plain vacutainer, and serum is separated by centrifugation, which was used for estimation of serum myeloperoxidase and apolipoprotein B. 2 ml blood was taken into EDTA containing vacutainer and used for estimation of HbA1c. Glycated hemoglobin was estimated by immunoturbidometric method⁶, Serum apolipoprotein B was estimated by Immunoturbidometric method,⁷ serum myeloperoxidase by Enzyme Linked Immuno Sorbent Assay⁸.

Statistical analysis was done using SPSS software, version 17.0. Descriptive data were presented as mean \pm SD and range values. Results were subjected to appropriate statistical analysis. An unpaired t-test was used to compare the various parameters between cases and controls. For all the tests, a probability value (p-value) of less than 0.05 was considered statistically significant. Correlation analysis was done to assess the relationship between different variables. (Pearson s correlation coefficient). Receiver Operating Curve (ROC) analysis was done to obtain Area Under Curve (AUC) and optimum cut-off value for Myeloperoxidase.

RESULTS:

In the present study total of 80 subjects were included. They were divided into 2 groups.

Controls: It consisted of 40 age and sex-matched, healthy subjects.

Cases: 40 proven cases of Type 2 DM disease patients without complications, on treatment with no time duration in the age group of 30-80 years attending the medicine department of district general Hospital were included in this study. The parameters were estimated were Glycated hemoglobin (HbA1c), Serum apolipoprotein B, Serum Myeloperoxidase

Table 1: Age and gender-wise distribution of controls and type 2 diabetic cases

Number of subjects		Controls	Cases	p-Value
		40	40	
Age (years)	Mean ± S.D	47.5 ± 8.4	48.4 ± 7.02	> 0.05 NS
	Range	33 - 63	36 - 62	
Gender	Male	19	18	> 0.05 NS
	Female	21	22	

NS- Not significant

Among 40 controls, 19 were males and 21 were females with a mean age of 47.5 ± 8.4 years. Among the 40 diabetic cases, 18 were males and 22 were females with a mean age of 48.4 ± 7.02 years. There is no significant difference among controls and cases for the age (p > 0.05).

Table 2: Levels of HbA1C, apolipoprotein B, Myeloperoxidase in healthy controls and diabetic cases

Groups		HbA1c (%)	Serum apolipoprotein B (mg/dl)	Serum MPO (pg/ml)
Controls	Mean	4.98 ± 0.59	120.62 ± 29.78	10105 ± 2954.6
	SD			
	Range	4.0 - 6.01	57.2 - 183.0	4000 - 15000
Cases	Mean	7.01 ± 1.12	234.53 ± 51.07	21225 ± 7885.7
	SD			
	Range	5.0 - 8.9	125.0 - 373.3	4800 - 36600
Controls and Cases	Mean Difference	2.03	113.9	11120
	t value*	10.07	12.18	7.44
	p value	< 0.001	< 0.001	< 0.001

* Unpaired student, t' test

p value: > 0.05 not significant, < 0.05 significant, < 0.001 highly significant.

The mean levels of HbA1c, serum apo B and serum MPO in cases are in the range of 7.01 ± 1.12 %, 234.53 ± 51.07 mg/dl and 21225 ± 7885.7

pg/ml respectively, which are significantly higher than controls (p < 0.001).

Table 3: Pearson's correlation between biochemical parameters in type 2 diabetic cases

CORRELATION ANALYSIS		
Relationship between	r value	p value
Serum MPO and HbA1c	+ 0.52	< 0.05*
Serum MPO and apolipoprotein B	+ 0.33	> 0.05
HbA1c and apolipoprotein B	+ 0.52	< 0.05*

r: Pearson s correlation coefficient; < 0.05*- Significant; > 0.05- not significant

The Pearson s correlation analysis shows statistically significant positive correlation between serum MPO and HbA1c with r value + 0.52 with (p < 0.05), no significant correlation between serum MPO and serum apo B with r value 0.33 with (p > 0.05) statistically significant positive correlation between HbA1c and serum apo B with r value + 0.52 with (p < 0.05).

Table 4: ROC curve to determine the cut off level MPO activity (pg/ml) in diabetics

Cut-off	Sensitivity	Specificity	AUC	p-Value
15200	80	100	0.88	<0.001

The area under the curve at the cut off level of 15200 pg/ml is about 0.88 (p < 0.001) with a sensitivity of 80% and specificity of 100%.

DISCUSSION

Diabetes mellitus is a multisystem disorder characterized by a relative or absolute insufficiency of insulin secretion or resistance to the metabolic action of insulin on the target tissue. Recently diabetes mellitus has become a major health burden. ⁴ Every year about 5% of the patients die due to diabetes worldwide. ⁹

Persistent hyperglycemia in uncontrolled diabetics can cause inflammation and increased production of reactive oxygen species from glucose auto-oxidation which can predispose to detrimental consequences in diabetes mellitus.

Cardiovascular complications are due to the adverse effects of hyperglycemia and oxidative stress on vascular endothelium. Approximately about 75% of deaths in diabetic patients are caused due to these cardiovascular complications.^{10,11} In the present study, it is found that the concentration of HbA1c is increased in cases when compared with healthy controls, which is statistically highly significant (p-value < 0.001). This is in accordance with the studies of Pasupathi Pet al.¹², Mohsen AF et al.¹³ and Silbernagel G et al.¹⁴ HbA1C is produced by the covalent binding of glucose with haemoglobin. Since the red blood cell membrane is highly permeable to the glucose, the quantity of HbA1C formed is directly proportional to the average blood sugar. There is little effect on the HbA1C levels by short-term elevation of plasma glucose.¹⁵ HbA1C levels also help in the predicting development and progression of macrovascular complications.¹⁶ Advanced glycation end products lead to the long term complications. They also cause endothelial dysfunction, plaque formation and finally atherosclerosis. HbA1C level should be less than 7 % for reducing the risk of cardiovascular complications in type 2 diabetes mellitus. Approximately 1% increase in the HbA1C increase the estimated risk of CVD by 18%.^{17,11} It was found out that there is a positive correlation between HbA1c level and serum apolipoprotein B which is statistically significant (p-value < 0.05). Insulin resistance in diabetes leads to the accumulation of triglyceride rich lipoproteins. Altered glucose metabolism and dyslipidemia are the two important features of diabetes.^{18,19} The severity of the dyslipidemia increases with an increase in the HbA1C.²⁰ There is a significant reduction in cardiovascular risk by improved glycemic control. Reduction in the HbA1C levels by 0.2% could decrease the mortality by 10%.¹⁷ In the present study it was found that the concentration of serum apolipoprotein B is increased in cases when compared with healthy controls, which was

statistically highly significant (p-value < 0.001). These results are in accordance with the studies done by Sniderman AD et al,²¹ Martin SS et al²², Ley SH et al,²³ and Kanani FH et al.²⁴ Dyslipidemia in diabetes consists of an increase in triglycerides, triglyceride-rich lipoproteins, decreased HDL, increased LDL and cholesterol depleted small dense LDL. The main component of atherogenic lipoproteins is apolipoprotein B.⁵ Insulin resistance is the cause for the increase in triglyceride concentration and apolipoprotein B in diabetes mellitus.²³ Apolipoprotein B is required for secretion of VLDL from the liver and it is bound to it until VLDL is cleared from the circulation as IDL or LDL.⁵ Furthermore, correlation was done between serum apolipoprotein B and serum MPO levels in type 2 diabetic cases. And we found out that there is no significant correlation between serum apolipoprotein B and serum MPO levels in type 2 diabetes patients (r-value + 0.33) with p-value (> 0.05). In the present study, it is found that the concentration of MPO is increased in cases when compared with healthy controls, which is statistically highly significant (p-value < 0.001). It is in accordance with the studies carried out by Shetty S et al.⁴ Wiersma JJ et al.⁶³ and Vit JA et al.²⁶ Endothelial dysfunction precedes clinically detectable atherosclerosis. Decreased levels of nitric oxide and oxidative stress are the major contributing factors for the development of endothelial dysfunction and atherosclerosis. Increased insulin levels stimulate the activity of the neutrophils. Myeloperoxidase is released from the granules of activated neutrophils, monocytes, macrophages at the inflammatory sites.²⁵ Myeloperoxidase oxidizes chloride in the presence of H₂O₂ to form HOCl. Compounds like Chlorinated L-arginine, chlorotyrosine, and HOCl-modified LDL are formed by the reaction of HOCl with amino acids and proteins. Increased levels of these compounds are found in the atherosclerotic lesions.⁴ Myeloperoxidase stimulates the formation of oxidized LDL which promotes atherosclerosis. It also oxidizes HDL which in turn decreases its capacity for the reverse cholesterol transport. It also decreases the bioavailability of endothelial derived nitric oxide.^{27,28} We found out that there is a significant

positive correlation between HbA1c and serum MPO levels in type 2 diabetes patients (r-value + 0.52), p-value (< 0.05). This suggests that as the HbA1c values were increasing, there is an increase in the serum MPO values in diabetic cases. Thus it can be concluded from our study that the future risk of CVD can be detected by evaluating the levels of apolipoprotein B and MPO in type 2 diabetic patients and can be prevented early by adequate control of glycaemia.

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PREVALENCE OF SALMONELLA TYPHI (S.TYPHI) CARRIER STATE AND ALTERED P53 GENE EXPRESSION IN CHRONIC CHOLECYSTITIS & CHOLELITHIASIS PATIENTS IN EASTERN INDIA

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ABSTRACT

Background-In this study the main aim and objective was to establish the percentage of Salmonella Typhi positivity in the operated samples of chronic cholecystitis and cholelithiasis patients and among those patients alteration of p53 expression was studied.

Material & Method - The S.Typhi carrier state in Chronic Cholecystitis and Cholelithiasis patients was analyzed by the presence of S.Typhi DNA isolated and demonstrated from gall bladder tissues by Polymerase chain reaction (PCR). p53 expression alteration was also studied in these cases by immunohistochemistry. After analysis, there was a significant number of positive expressions of the p53 gene through IHC examination were found in the S.Typhi carrier state of these samples.

Results-From the samples examined in this study a significant positivity of S. Typhi carrier state was found (~10%) and among those S. Typhi, positive samples alteration in p53 expression was quite high (~70%).

Conclusion-In this study the prevalence of S.Typhi carrier state in eastern India is high than the worldwide prevalence observed. Again the alteration of p53 expression is also significant in S.Typhi positive chronic cholecystitis and cholelithiasis cases.

Keywords- S.Typhi, p53, chronic cholecystitis, cholelithiasis.

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INTRODUCTION

Typhoid fever is a systemic infection caused by Salmonella enterica serotype Typhi (S. Typhi). It is a human-specific disease. It occurs worldwide, primarily in developing nations whose sanitary conditions are poor. The disease remains an important public health problem in developing countries [1]. Typhoid fever is endemic in Asia, Africa, Latin America, the Caribbean, and Oceania, but 80% of cases come from Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, or Vietnam [1]. Within those countries, typhoid fever is most common in underdeveloped areas. Typhoid fever infects roughly 21.6 million people (incidence of 3.6 per 1,000 population) and

kills an estimated 200,000 people every year [2]. Northern India has the maximum prevalence of S.typhi cases & carriers [3]. Eastern India is among the endemic zone of S.typhi infection [4]. The main source of infection is the feces & urine of the case & carrier. The carrier may be a convalescent carrier or chronic carrier. A convalescent carrier excretes bacilli for 6 to 8 weeks after which the number of bacilli decreases rapidly. And the chronic carrier excretes bacilli for more than a year after a clinical attack [4]. The clinical diagnosis of typhoid cases & carriers has been a medical challenge due to its similarity to many other infective illnesses.

The outcome of blood culture is limited by the previous use of antibiotics and a low bacterial load [5]. The continued high incidence of typhoid is due to the dissemination of the disease via typhoid carriers. Widal test, the commonly used serological test is positive only in the latter part of the disease. Moreover, its interpretation in endemic areas is fraught with disadvantages [6]. Hence there is an urgent need to increase the chance of detecting the carriers to decrease the risk that they pose to the communities. Polymerase chain reaction (PCR) is a sensitive and specific method used for the diagnosis of several infectious diseases. In typhoid carrier states, it can be an effective tool because it can be used even in cases where antibiotic therapy has been started or the pathogen load is very low. A nested PCR makes the detection more sensitive and can detect the presence of even 3-5 bacilli [7]. Therefore, nested PCR in the laboratory can be used as a diagnostic tool in culture-negative cases of typhoid fever and carrier. Carcinoma of the gall-bladder (CaGB) is the sixth most common gastrointestinal tract cancer and is endemic in several countries [8]. It is the most common malignant tumor of the biliary tract and a particularly high incidence is observed in Chile, Japan, and India. In the northern and eastern zones of India, it is the third most common gastrointestinal malignancy [9]. Gallbladder cancer is one of the most aggressive malignancies with an extremely poor prognosis. Surgical resection remains the only chance of cure but is possible in only a small percentage of patients with CaGB. The 5-year survival rate for cancers confined to the gallbladder is 32% and for advanced-stage cancers is 10% [10, 11]. This emerges the necessity for identifying predisposing factors and the tools for early diagnosis and treatment. CaGB has been co-related with various predisposing factors. Genetic disorders such as multiple familial polyposis (Gardner's syndrome), Peutz-Jegher's syndrome, porcelain gall bladder, and anomalous pancreaticobiliary ductal communication as seen in the choledochal cyst. Long-standing gall stones also predispose to the development of gall bladder cancer [12, 13]. The association between various chronic infections and the development of a malignant state is a fact.

Various mechanisms have been postulated to explain this association. In the case of the gall bladder, a chronic typhoid carrier state is established to be a high-risk factor for the development of carcinoma of the gall bladder due to chronic infection and inflammatory sequelae [14, 15]. Because of genetics, mutation of p53 and K-ras gene is seen in gall bladder cancer. Mutant p53 is found in about 92% of invasive CaGB and 28% of dysplastic epithelium. K-ras mutations are seen in 39% of CaGB. The over-expression of the p53 gene and K-ras gene have been well observed in CaGB cases. This can be demonstrated by alteration of gene expression in the immunohistochemical examination. [16, 17].

In this study, we evaluated the percentage of positivity of S.typhi carrier state in chronic cholecystitis and cholelithiasis cases in Eastern India. Also, we evaluated the association with alteration of the p53 and K-ras gene expression in chronic cholecystitis and cholelithiasis cases in Eastern India.

MATERIAL & METHODS

Gall bladder samples from the patient of Chronic Cholecystitis and Cholelithiasis were collected. A thorough histological examination was carried out with hematoxylin- and eosin-stained tissue preparations. One hundred paraffinized blocks of gall bladder tissue samples of histologically proven Chronic Cholecystitis and Cholelithiasis were taken into the study. Among them, thirty-six samples were of Chronic Cholecystitis (Male-6, Female- 30) and sixty-four were of Cholecystitis with Cholelithiasis (Male-9, Female-55). p53 gene expression for these was performed at the Department of Pathology, MCH, Kolkata [20- 23]. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical staining. Sections of 5 μ m thick from paraffin-embedded blocks were deparaffinized in xylene and rehydrated in a graded series of ethanol [24]. The sections were pretreated with autoclaving at 121°C for 15 min in 0.01 mol/L citrate-buffered saline (pH 6.0) for antigen retrieval. Endogenous peroxidase activity was blocked by incubation with 3% H₂O₂ for 30 min at room temperature. The sections were incubated with 10% normal goat serum for 1 h to block nonspecific binding of

the immunological reagents. After incubation with mouse monoclonal antibodies against p53 (Clone DO-7; DakoCytomation, Glostrup, Denmark) at 4°C overnight, streptavidin-biotin complex and horseradish peroxidase was applied, and reaction products were visualized using the Histofine SAB-PO (M) immunohistochemical staining kit (Nichirei, Tokyo, Japan), according to the manufacturer's instructions. The peroxidase labeling was developed by incubation of the sections in diaminobenzidine tetrahydrochloride for 1 minute. Finally, nuclear counterstaining was done using Mayer's hematoxylin solution. Two blinded observers (K. A. and Y. Z.) independently examined the immunostained sections.

Percentage of positivity of p53 staining	Result considered with Grade
< 10 %	Negative
11-33%	Weakly Positive
34-66%	Moderately Positive
>66%	Strongly Positive

DNA EXTRACTION:

For S.Typhi DNA PCR, normal-sized tissues a single 5,mm section (average collected volume 1-44 mm³), and biopsy specimen sized tissues a single 10, um section (average collected volume 0,034 mm³) were cut and processed according to the following method: After dewaxing and rehydration, the tissue section was resuspended in 50micL of digestion buffer (50 mM KCl, 1-5 mM MgCl, 10 mM TRIS-HCl, 0-5% TWEEN 20, pH 9 at 25°C) containing 200, ug/ml proteinase K (Merck, Darmstadt, Germany) and was incubated at 55°C for 3 hours.⁹ The proteinase K was inactivated by boiling for eight minutes. Then we added an equal volume of buffer saturated phenol (Fisher, BP1750I-400) and mixed it by inversion. Spun for at least 5 minutes at 14,000 rpm in a microcentrifuge, transferred the aqueous layer to a new tube. Noted the interphase: if they're a lot of white material or not. Repeated steps 1 and 2 on the aqueous fraction until the interphase was clear (typically 3 or more times). Performed back extractions* when the interphase is fuzzy to increase final yield. Once the interphase is clear, added an equal volume of phenol:chloroform: isoamyl alcohol (25:24:1) (Fisher, BP1752I-400)

to the extracted aqueous fraction of my sample. Mixed for 5 minutes and then spun for 5 minutes at 14,000 rpm. This reduced residual phenol and further sharpened the interphase, facilitating the extraction of the aqueous layer. Removed the aqueous layer to a new tube and treated with RNase A at 100 g/ml for 1 hour at 37°C. Repeated steps 1 to 4 to remove any remaining RNase A and collected the aqueous fraction. One should only need 1 or 2 buffer saturated phenol steps as there should be much less protein to remove than in the initial lysate. To perform back extractions added 50-100 l of dH2O to the sample tube containing the interphase and organic portion. Inverted the tube to mix, and spin the sample at 14,000 rpm in a microcentrifuge for 5 minutes. Collected the aqueous phase and added it to the previously acquired aqueous extraction. Continued back extractions until the interphase was clear. Then we estimated the volume of my collected aqueous layer. We added 1/10 the volume of 3 M sodium acetate pH 5.2 and 1 volume of 100% isopropanol (v/v) molecular biology grade (or 2.5 volumes of 100% ethanol)mixed well and put on ice or in a -20°C freezer for 30 minutes. Spun at maximum speed (14,000 rpm) at 4°C in a microcentrifuge for 10 minutes, discarded the supernatant, washed the pellet with 70% ice-cold ethanol to remove unwanted salts, resuspended the pellet in the buffer of choice, dH2O. All DNA samples were quantified by fluorometry using Qubit² dsDNA HS Assay Kit (Life Technologies, Carlsbad, California, US) on Qubit² 3 Fluorometer (Invitrogen, Life Technologies)as per the manufacturer's instructions, and assessed for purity by NanoDrop 8000 Spectrophotometer (Thermo Scientific) 260/280 absorbance ratio measurements, in triplicate. Apart from the Qubit assay, for the quality control analysis of DNA, Infinium HD FFPE QC Assay (Illumina, Inc.) was used by performing a quantitative PCR of FFPE DNA on the CFX96² Real -Time PCR Detection System (BioRad). Subsequent data analysis was performed as per the Manufacturer's instructions. The Cq was calculated to evaluate the quality of isolated DNA, since values Cq > 5 are not suitable for further downstream processing for Infinium HD FFPE Restore Protocol (Illumina,

Inc.) and Infinium MethylationEPIC array (Illumina, Inc.). A value $Cq < 5$ ensures the better quality of isolated DNA that is suitable for various targeted and genome-wide analyses.

PCR detection of *Salmonella* spp. Were done using primers. *Salmonella typhi* strain was retrieved from frozen stock culture and grown in 3 mL Luria-Bertani (LB) broth overnight at 37 °C (aerobically). DNA extraction from pure cultures was achieved by boiling bacterial cells, which were resuspended in 1% (v/v) Triton X-100, as described before (Wang et al., 1996). Without isolation of the DNA, 2 mL of the samples were directly added to the PCR mixture. The set of primers was designed from the *sdiA* gene sequence available from the GenBank database. The multiple sequence alignment was processed using CLUSTAL W software (Thompson et al., 1994). We focused on the *Salmonella typhi* strain. PCR amplification was carried out in a 25 mL reaction mixture containing 2.0 mM of each deoxynucleoside triphosphate (dNTPs), 1 PCR buffer [67 mM Tris-HCl, pH 8.0, 16.6 mM, (NH₄)₂SO₄, 0.05% Tween 20], 3 mM MgCl₂, 25 pmol of each primer (SdiA1, SdiA2), 1 U Taq Polymerase (HyTest Biotechnology Ltd, Cambridge, UK) and 2 mL aliquot of the sample DNA. 1.0 mg mL⁻¹ of nonacetylated bovine serum albumin (BSA, Ambion) was added in the reaction mixture. The cycling program consisted of denaturation at 94.1°C for 5 min, followed by 30 cycles of 94.1°C for 30 s, 52.1°C for 40 s, 72.1°C for 30 s. A final extension was performed at 72 .1°C for 7 min. As IAC a 566 bp fragment of the 16S rRNA gene was used and amplified with universal (907R) and bacterial (341F) primers (Muyzer et al., 1997). The volume of the reaction mixture was 50 mL containing 4.0 mM of each dNTP, 2 U Taq Polymerase, 4 mM MgCl₂, primers SdiA1, SdiA2, 341F, 907R (25 pmol each), 1 PCR buffer, 1.0 mg mL⁻¹ of BSA, and template DNA (4 mL). The cycling program consisted of denaturation at 94 °C for 5 min, followed by 30 cycles of 94.1°C for the 30s, 52.1°C for 40 s, 72.1°C for 90 s. A final extension was performed at 72.1°C for 10 minutes PCR amplification was conducted in a primus 96 plus Thermal Cycler (MWG Biotech AG, Ebersberg, Germany). PCR products were resolved by electrophoresis in 1.5-2% (w/v)

agarose gels and visualized under UV light after ethidium bromide staining. The DNA sequence of PCR fragment was obtained from a pure culture of *Salmonella typhi* and was determined by the dideoxy method (Sanger et al., 1977). Sequencing was carried out by Macrogen Inc. (Korea). To confirm that the sequence is a part of the *sdiA* gene, a search of the GenBank DNA database was conducted by using the BLAST algorithm (Altschul et al., 1990). The similarity of over 99% to the *sdiA* gene of *Salmonella enterica* strains showed that the set of primers described here amplifies exclusively the 274 bp fragment of the target gene.

Outcome parameters- Outcome parameters were based upon Positivity of *S.typhi* carrier state in chronic cholecystitis, Cholelithiasis and altered expression of p53 & in Chronic cholecystitis, Cholelithiasis cases by IHC

RESULTS

Among 100 patients, a total of 14 patients were male and eighty-five patients were female. The majority of the histology was Cholecystitis with Cholelithiasis, sixty-four in number. And thirty-six patients were of Chronic cholecystitis.

Table 1: Gender and Histology Association

Gender	Histology		Total
	Cholecystitis with Cholelithiasis	Chronic Cholecystitis	
Male	9	6	14
Female	55	30	85
Total	64	36	100

Among the 100 samples, 10% samples were found positive of IHC for p53 gene expression, nine samples were weakly positive and one was moderately positive. (Table-1)

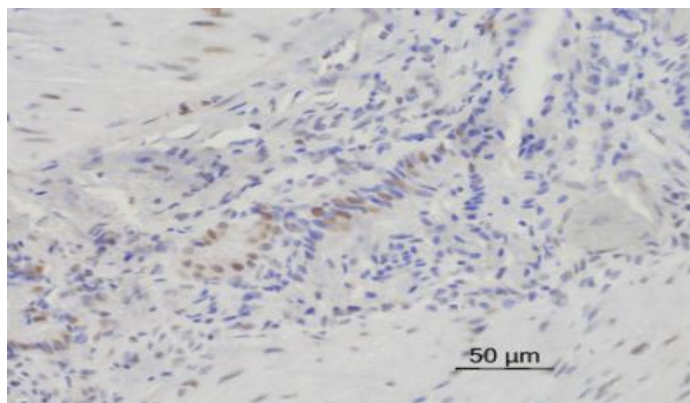


Figure1- IHC for p53 Gene (Weakly Positive)

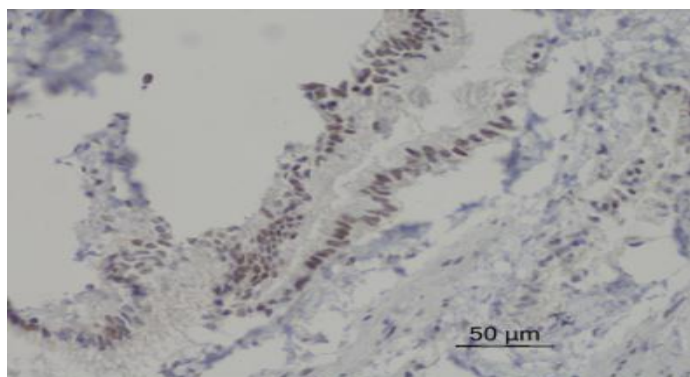


Figure2- IHC for p53 Gene (Moderately Positive)

PCR	Association between PCR and P53 status	Positive	Negative	Total number & Percentage
Positive	Count % within PCR	5 71.4%	2 28.6%	7 100.0%
Negative	Count % within PCR	5 5.4%	88 94.6%	93 100.0%
Total	Count % within PCR	10 10.0%	90 90.0%	100 100.0%

Salmonella Typhi carrier state was detected in seven (7) samples demonstrated by PCR. Among the Salmonella typhi carrier state, five (5) cases were p53 positive by Immunohistochemistry.

DISCUSSION:

Seven samples were positive for S. Typhi chronic carrier state (Six in Cholecystitis with Cholelythiasis samples, one in Chronic Cholecystitis sample); in percentage, it is seven percent (7%), which is much higher than the overall prevalence of S. Typhi carrier state which is only ~2.5% in the overall population as per present epidemiological data states [18]. From the above findings, we find a strong relation between p53 gene mutation and Cholecystitis & Cholelithiasis. Among the one hundred(100) samples, ten (10%) samples were found positive for IHC for p53 gene expression. Through IHC study, grades of p53 positivity were found. (nine samples were weakly positive and one was moderately positive). Gall-bladder cancer is usually associated with chronic cholecystitis and gallstone disease. P53 gene mutation is observed in a high proportion of gallbladder cancers as it can be accurately detected with conventional immunohistochemical techniques[19, 20]. IHC data also suggest that approximately one-third of the proximal tumors were found to express weak p53immunopositivity, whereas moderate immunopositivity and a higher rate were observed in tumors of the lower mid-region. Finally, moderate and marked p53 immunopositivity was observed in tumors of the ampulla and

Grade of p53 positivity among IHC positive subjects	Frequency	Percent
Weakly positive	9	90.0
Moderately positive	1	10.0
Total	10	100.0

Table 2 Seven samples (7%) were positive for S. Typhi chronic carrier state (Six in Cholecystitis with Cholelythiasis samples, one in Chronic Cholecystitis sample); which is much higher than the overall prevalence of S. Typhi carrier state which is only 2.5% as per present epidemiological data states.

gallbladder. In tumors of the lower mid-region of the ampulla and the gallbladder, a significantly higher p53 positivity was noted [21]. Also through the PCR analysis, it is clear that Salmonella typhi carrier state was detected in seven (7) samples demonstrated by PCR. Among the Salmonella typhi carrier state, five (5) cases were positive for p53 positive by Immunohistochemistry. And in percentage within the PCR is 71.4% which is moderately high. Previous data support that the alteration of p53 gene expression and S.Typhi carrier state lead to Carcinoma Gall Bladder [22]. Alteration of IHC expression of p53 gene in S.Typhi carrier state in Gall Bladder of Cholelithiasis & Chronic Cholecystitis patients are much high and there is a statistically significant correlation we established.

CONCLUSION:

From the above result and discussion, we may come to a point that gall bladder carcinoma/cancer is usually associated with chronic cholecystitis and cholelithiasis. P53 plays an integral part in cholecystitis and cholelithiasis. P53 gene mutation and change in codon play an important role in the above-mentioned diseases. At the onset of the study, we thought that p53 and KRAS genes would be a suitable marker to study and correlate the efficacy of S.typhi carrier state in chronic cholecystitis and cholelithiasis patients. But after the study performed we got the data that p53 showed altered expression in S.typhi carrier state in chronic cholecystitis and cholelithiasis patients, whereas IHC expression for KRAS was failed. And from the above study point, it got clear that there is no correlation between K-ras mutation and clinicopathological factors of gall bladder carcinoma. Altered p53 gene expression is a major factor here. And there is huge scope for this if we can perform the blood sample analysis and other indicators to show and present the relationship between those two.

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A CASE REPORT OF TUBEROUS SCLEROSIS COMPLEX

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ABSTRACT

Tuberous sclerosis complex (TSC) is a rare genetic disorder inherited as an autosomal dominant pattern with the prevalence of one in 6000 live birth, affecting both sexes and all ethnic groups. Tuberous sclerosis complex is characterized by the growth of numerous benign tumors in many parts of the body, including the brain, heart, lungs, eyes, kidneys, skin and other organs, leading to significant health problems like seizures, intellectual disability, autism or developmental delay. Tuberous sclerosis has no cure, but treatment such as medical, educational, and occupational therapy can help in relieving symptoms. Here we present a case of a young female patient with tuberous sclerosis with various clinical and radiological features who exhibited multiple hamartomas of various organ systems.

Key words: TSC, autosomal dominant, tumors, intellectual disability, autism

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INTRODUCTION

Tuberous Sclerosis or Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder characterized by the growth of numerous benign tumours in many parts of the body including the brain, heart, lungs, eyes, kidneys, skin and other organs. TSC is caused by mutations of two genes, TSC1 and TSC2, which encode for the proteins hamartin and tuberin respectively. These proteins act as tumour growth suppressors which are involved in cell proliferation and differentiation.¹ The estimated prevalence of TSC in general population is one in 95,136 and those <6 years of age is one in 14,608.² Genetic studies have shown

that two thirds the disease results from a new dominant mutation either in the TSC1 gene on chromosome 9q343 or the TSC2 gene on chromosome 16p13, with the latter accounting for an estimated 78% of cases and do not have any affected parents.³ About 80-90% of patients present with seizures in early first year of life which varies from subtle focal seizures, infantile spasms, to generalized tonic clonic seizures.⁴ Definite TSC is diagnosed when either 2 major features (out of a total of 11) or one major feature with 2 minor features (out of a total of 9) are present. However approximately 50% of patients who fulfil the diagnostic criteria have normal intellectual ability

and 15% among them remain free from seizures.⁵ Here we present a case of a young female with background history of seizures and multiple hamartomas involving various organs diagnosed as TSC using 2012 TSC diagnostic criteria.⁶

CASE REPORT

A 30 year old female, presented to the outpatient department of KIMS hospital with the history of fever since 4-5 days with no other localising symptoms. She was a known case of seizure disorder since 8 years of age receiving oral medications for seizures. On physical examination, there were multiple nodular skin lesions over face - adenoma sebaceum (fig.1), depigmented spots over back-ash leaf macules, nails showed periungual fibromas- kenyuontumour (fig.2) and a big nodular lesion over the left side of the scalp (4×5 cm) was seen (fig. 3).



Figure 1: Multiple nodular skin lesions over face-adenoma sebaceum



Figure 2: Periungual fibroma- kenyuon tumor



Figure 3: Nodular lesion on scalp

Investigations

Complete blood picture showed Hb: 8.5, total count 3000, platelet count of 65000 and peripheral smear showed pancytopenia. Other investigations like liver function tests, renal function tests, serum electrolytes remained normal. USG abdomen showed multiple renal cysts, with 2 angiomyolipomas (size 1cm × 2cm).

Visual examination: Vision of 6/12 not improving with the pin hole. Fundoscopy showed multiple white hypo-pigmented lesions suggestive of non calcified retinal astrocytic hamartoma (fig.4).

CT brain showed subependymal tumours, (size of about 1× 2cm). Biopsy of the lesion over the scalp was taken which showed fibrous tissues (hamartoma) on histopathological examination.

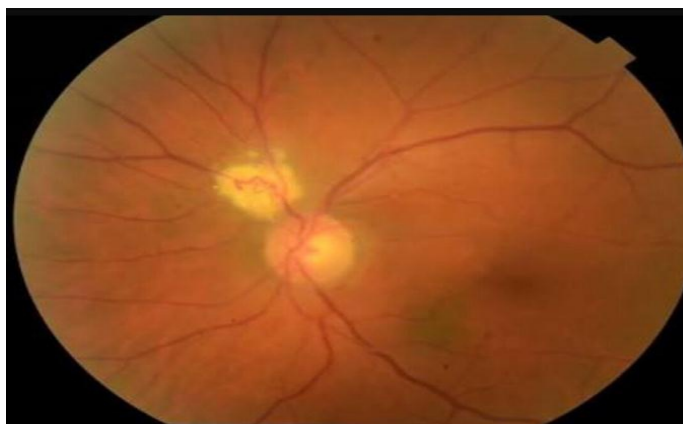


Figure 4: Non calcified retinal astrocytic hamartoma

The International Tuberous Sclerosis Complex Consensus Conference held in 2012, came with a new diagnostic criteria for diagnosis of tuberous sclerosis.⁶ Definite diagnosis includes two major features or one major feature with 2 minor features. Possible diagnosis includes either one major feature or 2 minor features. According to the above mentioned criteria our patient presented with more than 2 major criteria and was considered as TSC.

Major criteria

1. Hypomelanotic macules (3) at least 5-mm diameter
2. Angiofibromas (3) or fibrous cephalic plaque
3. Ungual fibromas (2)
4. Shagreen patch
5. Multiple retinal hamartomas
6. Cortical dysplasias
7. Subependymal nodules
8. Subependymal giant cell astrocytoma
9. Cardiac rhabdomyoma
10. Lymphangiomyomatosis
11. Angiomyolipomas (>2)

Minor criteria

1. "Confetti" skin lesions
2. Dental enamel pits (>3)
3. Intraoral fibromas (>2)
4. Retinal achromic patch
5. Multiple renal cysts
6. Nonrenal hamartomas

Based on the above clinical findings and investigations, a final diagnosis of tuberous sclerosis was confirmed.

TSC is a lifelong condition and there is no definitive treatment for the disease. Patient was started on antiepileptics and was on continuous

monitoring of symptoms. Family planning and genetic counseling was done.

DISCUSSION

Tuberous Sclerosis is an important genetic disorder, which affects the patient and the family in various ways.⁷ Various research projects are being done around the globe, to study more about genes involved and also the possible treatment strategies. Now, due to ongoing research, understanding of pathogenesis of disease, multiple drug therapies are available for certain manifestations of the disease.⁸ With available medications, symptomatic control can be done. Children affected with tuberous sclerosis can be offered schooling and occupational therapy. TSC presents with varied ophthalmic features. The retinal lesions more commonly include astrocytic hamartomas and non retinal lesions include coloboma, angiofibroma of the eyelid and papilledema (secondary to hydrocephalus).⁹ Different surgeries such as dermabrasion and laser treatment can be offered to patients with skin lesions.¹⁰

CONCLUSION

Early diagnosis is very important. Radiology is important in diagnosis and detecting complex nature of the disease. After detailed clinical and radiological evaluation, monitoring of symptoms, family planning, and genetic counselling can decrease the rate of morbidity and mortality.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information

to be reported in the journal. The patient(s) understand(s) that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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A RARE CASE OF PERINEAL LEIOMYOMA IN A POSTMENOPAUSAL WOMAN

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ABSTRACT

Leiomyomas are benign smooth muscle tumors. It usually originates from the smooth muscle of the uterus, but it can also arise from extrauterine tissue. Following menopause, leiomyomas tend to regress. Extrauterine leiomyoma is extremely rare and can mimic malignancies, making it difficult to diagnose. The histopathology of the lesion may be helpful in this case. Only two cases of perineal leiomyoma in postmenopausal women have been documented in the literature. We present a case that describes a 55-year-old woman who experienced perineal mass for 3 years. She underwent surgical excision, with histopathological findings consistent with leiomyoma. The patient was followed up and did not experience any pain or recurrence.

Keywords- Leiomyoma, perineum, postmenopausal

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INTRODUCTION

Leiomyomas are benign, soft tissue tumors that originate from smooth muscle and account for 3.8% of all benign soft tissue tumors. The most frequent type of leiomyoma is uterine leiomyoma. Extrauterine leiomyoma is rare, It usually arises in the genitourinary tract (in the vulva, ovaries, urethra, and urinary bladder but it can develop in any anatomic region (1,4,6).

To date, there are only two cases of perineal leiomyoma have been reported in the literature. One patient was in the postmenopausal age group and the other was a virgin 35 yr female (2, 3).

The present case describes a postmenopausal woman who presented with a perineal mass associated with pain, which was histopathologically diagnosed as a perineal leiomyoma

CASE REPORT

A 55-year-old married woman (gravida 12, para 12) was admitted to the Department of Surgery, Mayo institute of medical science, in December 2021 with perineal swelling for the last 3 years associated with pain for 1 year. The patient attained menopause 15 years back. She belonged to an urban area and had lower-middle-class socioeconomic status as per the modified Prasad Classification.

During local examination identified palpable, movable, firm to hard mass ms 6x4cm, 1-2 cm away from the anal verge, between 2-3 O'clock in the perineum.

Sonologist reported it as moderate size, well defined mass measuring 4.3 cm X 2.7cm X 3.6 cm with well-defined margin, homogenous echotexture suggestive of abscess/infective etiology.

The patient was advised for Fine Needle Aspiration Cytology, which did not yield any information regarding the swelling even on multiple attempts. Only hemorrhage was seen on microscopic examination. She was advised US-guided FNAC by the department of pathology but the surgeon decided to operate.

A solid and well-demarcated mass was removed (Fig 1) and it was sent for histopathological examination.

Gross examination revealed a round well-circumscribed solid mass measuring 5.0 x 5.0 x 3 cms. The cut section was homogenous grey white with focal hemorrhagic areas (Fig 2).

The microscopic examination confirmed the diagnosis of a soft tissue benign tumor composed of proliferated smooth muscle cells arranged in interlacing and whirling pattern with proliferated thin-walled new blood vessels. It was concluded that the mass was leiomyoma (Fig3,4). The patient was discharged from the hospital without any complications. On follow-up, there were no further problems or recurrence noted.



Fig 1: single grey-brown firm tissue piece measuring 5.0x5.0x3.0 cm.



Fig 2: homogenous grey white with occasional hemorrhage

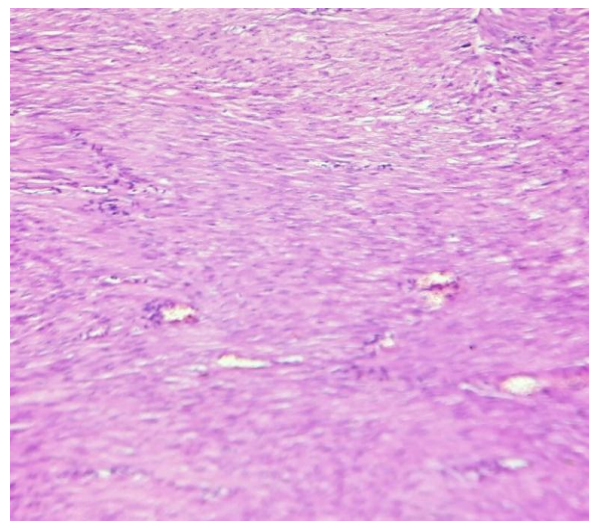


Fig 3: Tumor composed of interlacing fascicles of spindle-shaped cells. Blood vessels are proliferated and dilated.

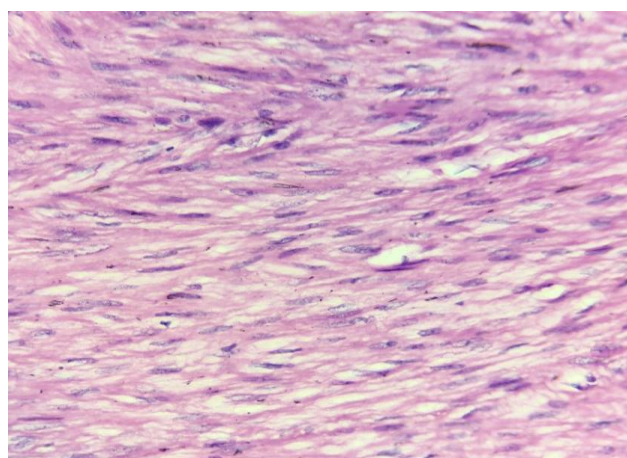


Fig 4: Tumor cells show uniform elongated nuclei with eosinophilic cytoplasm.

DISCUSSION

The differential diagnosis of perineal swelling is Bartholin cyst, Abscess, hematoma, and neoplastic etiology which may be leiomyoma, angiomyxoma, or leiomyosarcoma.

Leiomyomas occur infrequently outside the uterus. The clinical symptoms and imaging features depend on the location of the lesion and its growth pattern (4, 6). These tumors are composed mainly of smooth muscle cells and contain varying amounts of fibrous connective tissue. Leiomyomas are rarely found in postmenopausal women because their growth is estrogen-dependent(5). Leiomyoma develops most commonly in reproductive age group females and generally regresses following menopause. (2)

The earlier study describes a postmenopausal woman who presented with tenderness, lumbosacral radiating pain, and a perineal mass which was eventually diagnosed as a hormone-dependent perineal leiomyoma(2).

Another patient who was 35 yrs virgin female presented with mass in the perineum which was gradually increasing in size. It was painless, approximately 6 cm in size, and the right perineum (3)

The present study was done on a postmenopausal woman who presented with a perineal mass associated with pain. It was removed surgically. Later the diagnosis was confirmed histopathologically to be a perineal leiomyoma.

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